MycoSEQ *Mycoplasma* Detection Assay

- Rapid time-to-results—typically less than 5 hours
- Detection of more than 90 *Mycoplasma* species
- Demonstrated sensitivity—detects less than 10 copies/reaction
- Proven specificity
- Applied Biosystems™ PrepSEQ™ sample preparation for high-efficiency DNA recovery
- Proprietary Applied Biosystems™ MycoSEQ™ 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 testing 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.

Cell culture manufacturing process

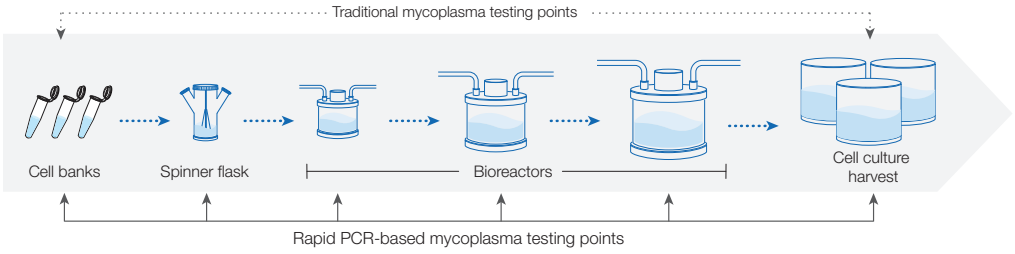

Figure 1. Sampling points for mycoplasma. Rapid PCR-based testing for mycoplasma infection can be conducted throughout the cell culture manufacturing process, from inoculation through harvest.

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MycoSEQ *Mycoplasma* Detection Assay

The Applied Biosystems™ MycoSEQ™ *Mycoplasma* Detection Assay is 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 MycoSEQ system delivers rapid time-to-results, typically in less than 5 hours, allowing for the early detection of a contamination event. This supports in-process monitoring for the presence of mycoplasma during cell culture manufacturing, providing protection against the spread of contamination into downstream equipment, processes, and media.

Components of the MycoSEQ *Mycoplasma* Detection Assay include:

- Applied Biosystems™ *Power* SYBR™ Green Master Mix
- Assay mix
- Inhibition control
- Positive control
- Optimized PrepSEQ sample preparation kit
- 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 typically in less than 5 hours (Figure 2). This rapid time-to-results allows the early 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 interpretation for objective test results.

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

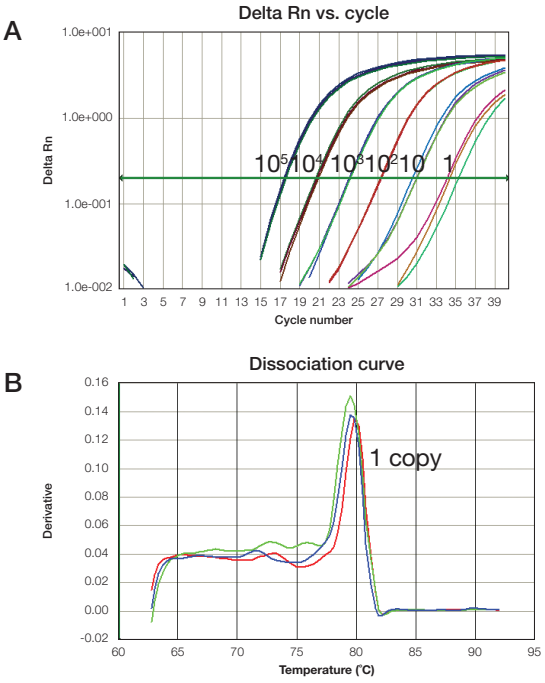

Figure 3. Sensitive detection of mycoplasma. (A) Analysis of a 10-fold dilution series of purified *M. arginini* DNA—from 100,000 genome copies to 1 genome copy/reaction. **(B)** Melt curve analysis of the PCR reaction at 1 genome copy/reaction.

PrepSEQ *Mycoplasma* Sample Preparation Kit with Module M

Provided with the MycoSEQ assay, the Applied Biosystems™ 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 Applied Biosystems™ PrepSEQ™ 1-2-3 kit uses 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.

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
- Bioassay cell lines
- Stem cell cultures
- Lymphocyte proliferation cultures for autologous transplantation
- Cell and tissue therapy cultures
- Serum
- Cell culture media

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*

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, minimizing the possibility of a false-positive result due to accidental cross-contamination of a test sample with the positive-control DNA.

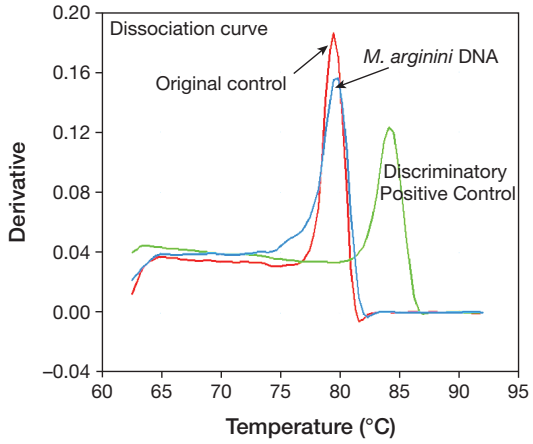

Figure 4. Melt curve analysis of control vs. mycoplasma DNA. The graph demonstrates the noticeable difference in melting point temperatures to enable clear differentiation between valid mycoplasma DNA and control sample.

AccuSEQ real-time PCR software for automated mycoplasma data analysis

Automated presence or absence results from MycoSEQ mycoplasma detection can be generated using Applied Biosystems™ AccuSEQ™ Real-Time PCR Detection 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.

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 genome copy (GC) to colony forming unit (CFU) ratio (GC/CFU) for all 10 *Mycoplasma* species tested, and clearly demonstrated 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 seeks to set high 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 minute virus of mice (MMV) on one instrument platform.

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Ordering information

Product	Size	Cat. No.
MycoSEQ *Mycoplasma* Detection Assay		
MycoSEQ *Mycoplasma* Detection Assay with Discriminatory Positive Control Includes PrepSEQ Sample Preparation	100 rxns	4460626
MycoSEQ *Mycoplasma* Detection Assay with Discriminatory Positive Control	100 rxns	4460623
AccuSEQ Real-Time PCR Detection Software v1.0 with *Mycoplasma* Software Analysis Module v1.0		
AccuSEQ Real-Time PCR Stand-alone Software	1 license	4443420
Related products		
Applied Biosystems 7500 Fast Real-Time PCR System, with Notebook Computer	1 instrument	4365464

Find out more at **thermofisher.com/mycoseq**

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