MycoSEQ *Mycoplasma* Detection Assay

- Accurate actionable results in less than 5 hours
- High-confidence detection of >90 *Mycoplasma* species at sensitivity as low as 10 colony-forming units (CFU) or genome copies (GC) per mL
- Proprietary Applied Biosystems™ MycoSEQ™ Discriminatory Positive/Extraction Control
- A regulatory-accepted rapid lot-release testing of mycoplasmas for multiple biotherapeutic modalities
- Ideal for in-process testing as part of a microbial risk mitigation strategy

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 mycoplasmas is typically performed.

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

Cell culture manufacturing process

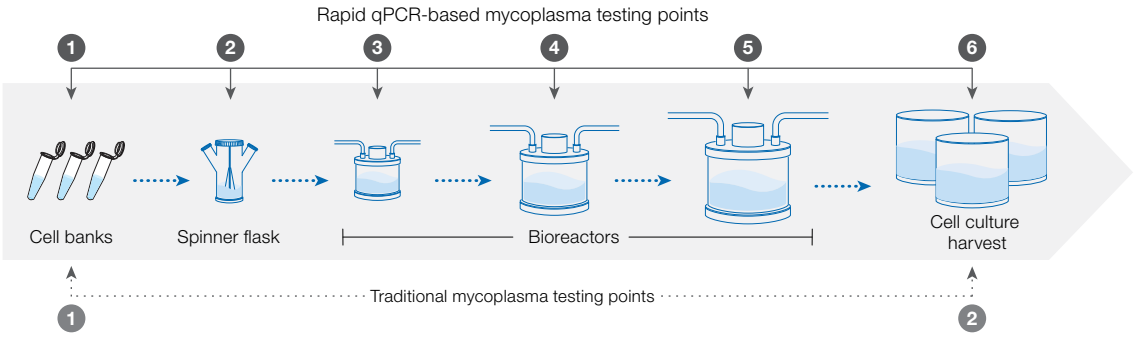

Figure 1. Sampling points for mycoplasmas. Rapid qPCR-based testing for mycoplasma infection can be conducted throughout the cell culture manufacturing process, from inoculation through harvest.

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MycoSEQ *Mycoplasma* Detection Assay

The Applied Biosystems™ MycoSEQ™ *Mycoplasma* Detection Assay is a real-time PCR assay designed and validated to meet the requirement of European Pharmacopoeia section 2.6.7. The assay design utilizes a proprietary bioinformatics pipeline to detect >90 *Mycoplasma* species with high specificity and no cross-reactivity with closely related bacterial species. Its sensitivity has been demonstrated by internal and external validation methods to detect 10 CFU/mL, or the genomic equivalent of 10 GC/mL, as recommended by regulatory guidance. As results are delivered in less than 5 hours, the MycoSEQ assay is ideal for in-process testing as part of an effective risk mitigation strategy and enables the earliest possible detection of a contamination event, reducing economic risk and accelerating production timelines.

Components of the MycoSEQ *Mycoplasma* Detection Assay include:

- Applied Biosystems™ *Power* SYBR™ Green Master Mix
- Assay mix
- Inhibition control
- MycoSEQ Discriminatory 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 typically deliver results in less than 5 hours (Figure 2). This rapid time-to-results allows 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 for 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, and draws on multiple parameters—threshold cycle (C_t), melting temperature (T_m), and derivative value—for interpretation of results. 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.

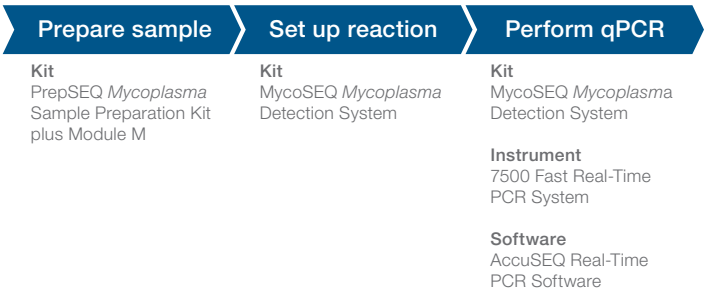

Figure 2. Easy workflow. Results are typically delivered in less than 5 hours, allowing for in-process testing.

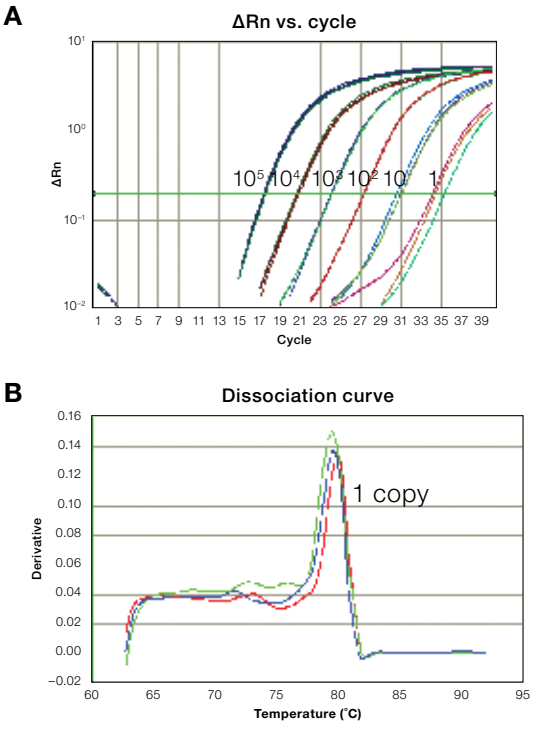

Figure 3. Sensitive detection of mycoplasmas. (A) Analysis of a 10-fold dilution series (10^5 to 1 GC/reaction) of purified *Mycoplasma arginini* DNA. **(B)** Melt curve analysis of the PCR reaction at 1 GC/reaction.

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 containing as many 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 mycoplasmas 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 harvest for vaccine manufacturing
- 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* and *Spiroplasma* species, and other European Pharmacopoeia species. Common isolated species recommended for testing and validation are in bold.

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

programin ranga pateikiama kartu su MycoSEQ rinkiniu

Discriminatory positive control

The MycoSEQ *Mycoplasma* Assay also includes the proprietary MycoSEQ 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 T_m of approximately 84°C, which is outside the range of amplicons generated from mycoplasmas with this assay (Figure 4). Thus, the T_m can be used to discriminate between a positive test result from a mycoplasma and the control DNA. This novel protocol design enables risk-free DNA spike control testing, minimizing the possibility of a false-positive result due to accidental cross-contamination of a test sample with the positive control DNA.

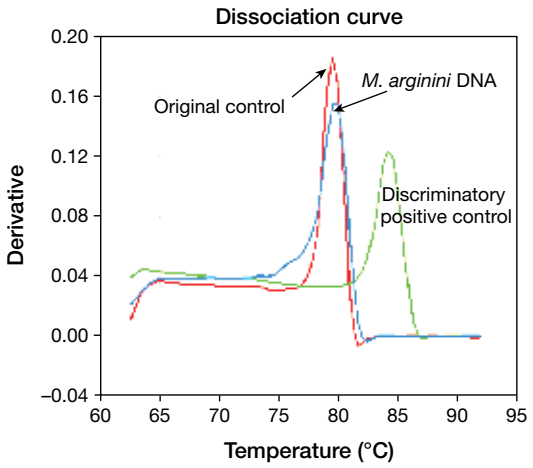

Figure 4. Melt curve analysis of control vs. mycoplasma DNA. The graph demonstrates the noticeable difference in melting temperatures to enable clear differentiation between valid mycoplasma DNA and the control sample.

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 values 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 EP section 2.6.7, ICH Q2 R1, and feedback gathered at the 2008 FDA-CBER Workshop on Rapid Mycoplasma Testing.

The study verified the limit of detection (LOD) with both genome copies (GCs) 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 demonstrated for the first time the sensitivity of a PCR-based test for mycoplasmas recovered from 10 mL samples of CHO cells.

Cell Culture Rapid Methods Program

The MycoSEQ *Mycoplasma* Detection Assay is an ideal choice for in-process testing, as part of an effective microbial risk mitigation strategy. When combined with the Applied Biosystems™ ViralSEQ™ Mouse Minute Virus Detection System, the MycoSEQ *Mycoplasma* Detection Assay provides streamlined detection of two common contaminants of mammalian cell culture–based biopharmaceutical manufacturing. This application sets high standards in workflow efficiency and product quality, combining one sample preparation step with real-time PCR–based assays for the detection of mycoplasmas and mouse minute virus (MMV) on one instrument platform.

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Ordering information

Product	Quantity	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
Sample preparation and automation		
PrepSEQ 1-2-3 Nucleic Acid Extraction Kit	100 preps	4452222
PrepSEQ *Mycoplasma* Nucleic Acid Extraction Kit	100 rxns	4443789
PrepSEQ Express Nucleic Acid Extraction Kit	52 isolations	4466351
AutoMate *Express* Nucleic Acid Extraction System	1 instrument	4467754
Real-Time PCR System		
Applied Biosystems 7500 Fast Real-Time PCR System, with notebook computer	1 instrument	4365464
Software		
AccuSEQ 2.1 Real-Time PCR Software	1 license	4443420

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