

Nucleic Acid Techniques – Mollicute Screening Assay

The analytical method of Nucleic Acid Techniques (NAT) is utilized in a variety of both cell and gene product applications for in-process testing during drug product manufacturing and as a rapid supplement to compendial release testing methods.¹

MicroSafe Services from Millipore provides a validated method for NAT using qualitative real-time polymerase chain reaction (QPCR) for the rapid detection of mollicutes, of which mycoplasma is a member.² This rapid and accurate molecular based method for in-process testing is being driven by Process Analytical Technology (PAT), which calls for the manufacturer to audit its production line in real time at multiple points.³ The benefits of this initiative and method enable the manufacturer to make any necessary decisions and adjustments earlier in its process based on rapid test results, reducing the risk of contamination at the final product testing stage—which is extremely costly and ultimately delays the release of drug product.

What Products Should be Tested?

Our method for rapid mollicute detection is dependent on the product submitted to be tested. Please contact us so we can determine whether the method can be validated for your product.

- *Mycoplasma gallisepticum*
- *Mycoplasma hyorhinis*
- *Mycoplasma orale*
- *Mycoplasma synoviae*

These primers show no cross-reaction with the bacterial strains *Clostridium sporogenes*, *Lactobacillus casei* and *Salmonella typhimurium*, all of which demonstrate close phylogenetic relationships to mycoplasmas.

The method shows a limit of detection of 1 CFU/mL for all strains tested, except for *Mycoplasma fermentans*, which is 10 CFU/mL.

The assay is robust in its capacity to remain unaffected by small changes in primers, amount of DNA, storage of the QPCR reaction for up to 4 hours, or performance by different operators on different days.

Study Design

The rapid detection of mollicutes is performed using universal primers and SYBR Green I as the fluorescent dye in the QPCR assay.

The specificity of the primers is directed but not limited to:

- *Acholeplasma laidlawii*
- *Mycoplasma arginini*
- *Mycoplasma fermentans*

Your
partner for
critical testing in
microbiology

¹ USP reference: General Chapters: <1046> Cell And Gene Therapy Products — Analytical Methodologies

² EP 2.6.21 "Nucleic Acid Amplification Techniques"

³ Process Analytical Technology Guidance (PAT): A Framework for Innovative Pharmaceutical Development, Manufacturing and Quality Assurance (09/04).

When a positive QPCR signal is obtained, the identity of the micro-organism can be determined by DNA sequencing using the Applied Biosystems MicroSeq® Microbial Identification System (Figure 1).

Assay Duration: To be determined based on the sample provided by the customer and the method itself, once the sample is validated.

Detection of Viable Mycoplasma:
4 day culture test

Sample Size: Dependent on customer supplied product

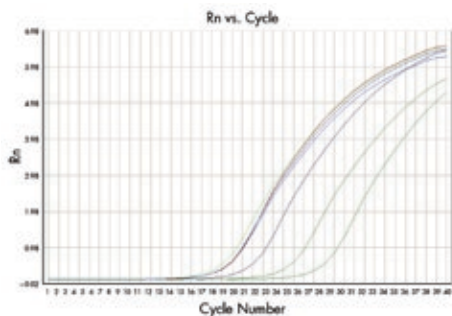


Figure 1. An example of a positive QPCR signal.

Study Design of the Bacterial Identification

MicroSafe Services from Millipore uses the MicroSeq rRNA Gene Kit to identify thousands of bacterial species based on the sequences of their 16S ribosomal RNA rRNA genes. Genomic DNA extracted from bacteria is amplified using PCR and subsequently sequenced.

Regulatory Compliance

- EP 2.6.21 “Nucleic Acid Amplification Techniques”

Final Report

MicroSafe Services from Millipore can provide each client with the following final reports:

- GLP report that includes all raw data, protocol methods and final result.
- GMP report that includes a certificate of results.

Custom Assays

We understand that no production plant is identical and that every process is unique. Using a combination of practical experience and a scientific approach, we will customize mollicute screening methods to meet your specific requirements including scientifically sound data that will be accepted by regulatory authorities around the globe.

Your Partner in Microbiology

MicroSafe Services from Millipore delivers a complete range of critical testing and consultancy services for the development and production of biopharmaceuticals. Leaders in the field of microbiology including *in vitro* virology testing, our staff of accomplished scientists, engineers and microbiologists will help you meet your specific critical testing requirements throughout every phase of drug production. All testing is conducted under GMP and/or GLP certifications as well as our own internal quality system.

SAFETY RELIABILITY QUALITY CUSTOMER RELATIONSHIP FEEDBACK & SUPPORT

Let Us Be Your Critical Service Provider

For more information on our critical support services, please contact Millipore at:

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