BioReliance

Biologics Safety Testing Services

Pinnacle Q-PCR™ Mycoplasma and Spiroplasma Detection Assays (EP 2.6.7)

Introduction

Nucleic acid amplification (NAT) methods for the detection of mycoplasma and spiroplasma species using real time Q-PCR can offer rapid turnaround to result. The Pinnacle Q-PCR™ platform developed at BioReliance couples state of the art bioinformatics for optimal assay design with automated sample preparation and assay set up. These features make the Pinnacle Q-PCR™ Mycoplasma detection assay an ideal next-generation test to augment current microbiological methods.

Mycoplasma testing is governed by regulatory guidelines EP, USP, PTC, and JP. Recently the use of NAT test methods for the detection of mycoplasma has been described within the FDA guidance for Industry and WHO technical series report series as well as European, United States and Japanese Pharmacopoeia. These reports have provided support for NAT as a suitable alternate to conventional test methods when appropriately validated.

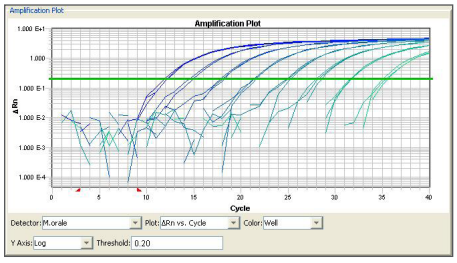
BioReliance has developed an NAT based test method that couples automated DNA extraction with automated real time Q-PCR detection to provide a rapid and validated test for the detection of Mycoplasma and Spiroplasma species.

Assay method for detection of Mycoplasma and Spiroplasma species using Pinnacle Q-PCR™

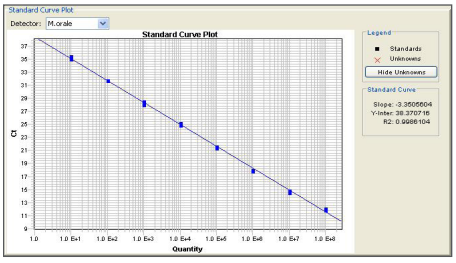
The efficiency of the extraction system is critical for the ability to support the introduction of a real-time Q-PCR assay for detection of Mycoplasma and Spiroplasma species which meets the European Pharmacopoeia regulatory guidelines (EP 2.6.7). The extraction system developed for this assay utilizes increased volumes of test material, followed by steps to concentrate the Mycoplasma/Spiroplasma species, then processing on an automated nucleic acid purification platform. Further purification of the nucleic acid renders the material suitable for evaluation in the Pinnacle Q-PCR™ system.

Fully automated liquid handling systems then prepare the 384 well PCR plates. All amplification and analysis is carried out on fully validated real-time PCR amplification and detection equipment.

Nucleic acid amplification technology is then used to exponentially increase the amount of target sequence that may be present in the DNA extract. The test system employs real time PCR technology to detect the presence of target sequences within a test sample.



Amplification plot of *Mycoplasma orale* plasmid positive control spanning a concentration of 1×10^8 to 1×10^1 copies per reaction.



Standard curve plot of cycle threshold vs concentration which illustrates a linear relationship over the concentration range of 1×10^1 to 1×10^8 copies per reaction.

Rapid real-time PCR (Q-PCR) based method for detection of Mycoplasma and Spiroplasma species

Pinnacle Q-PCR™ System incorporates advanced assay design with automation to ensure sensitivity and reproducibility

Fully validated, regulatory compliant test method

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The test employs three independent PCRs

- Detection of mycoplasma
- Detection of spiroplasma
- Detection of EPC (Extraction Positive Control) internal control

Parameter	BioReliance Pinnacle Q-PCR™	Conventional methods
Method	Real-time PCR	Agar and broth amplification; indicator detection system
Endpoint	Fluorescence	Mycoplasma colony count
Appropriateness	In Process Testing, Raw Materials, Cell Bank Screening, Investigations	Designed for Lot Release Testing. Also used for Raw Material Testing.
Sensitivity	10 cfu/ml†	10-100 cfu/ml
Assay Duration	5 hours	28 days
Specificity	High	High
Validation	Yes	Yes
Range of Species	102 species*	Viable
Robustness	Yes	Yes
Scalability	Yes	Yes
Regulatory	ICH Guidelines EP/USP compliance	EP/USP/PTC compliant

*Validated against *Mycoplasma. orale, Mycoplasma hyorhinis, Mycoplasma synoviae, Mycoplasma fermentans, Mycoplasma arginini, Mycoplasma pneumoniae, Acholeplasma laidlawii, Spiroplasma citri.*

† Exceeds the 100cfu/ml requirement for non-cultivable (indicator cell culture method) *Mycoplasma hyorhinis.*

Ordering Information

Assay Number	Assay Description	Regulatory Compliance	Sample Requirements
300200GMP.BUK	Rapid Pinnacle Q-PCR™ Mycoplasma Detection Assay (EP 2.6.7) (3 day turn-around time)	EP/USP, GMP	2 × 2 ml or 2 vials 5 × 10^5 cells/ml
300201GMP.BUK	Pinnacle Q-PCR™ Mycoplasma Detection Assay (EP 2.6.7) (7 day turn-around time)		
300202GMP.BUK	Rapid Pinnacle Q-PCR™ Mycoplasma and Spiroplasma Detection Assay (3 day turn-around time)	EP/USP, GMP	4 × 2ml or 4 vials 5 × 10^5 cells/ml
300203GMP.BUK	Pinnacle Q-PCR™ Mycoplasma and Spiroplasma Detection Assay (7 day turn-around time)		

References

World Health Organization (2010). Recommendations for the evaluation of animal cell cultures as substrates for the manufacture of biological medicinal products and for the characterization of cell banks. Proposed replacement of TRS 878, Annex 1. Adopted October 2010.

European Pharmacopoeia, Chapter 2.6.7. Mycoplasmas. 01/2008:20607.

United States Pharmacopoeia 34, General chapter 63, Mycoplasma Tests August 1, 2011.

Japanese Pharmacopoeia XV, 14. Mycoplasma testing for Cell Substrates used for the Production of Biotechnological/Biological Products. Supplement II. October 1, 2009.

PDA Technical Report No. 50, Alternative Methods for Mycoplasma testing. 2010.

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