

Analytical sensitivity and specificity of MYCOPLASMACHECK in detecting the WHO International Mycoplasma Standard and other mycoplasma DNA

Introduction

Mycoplasma is a genus of bacteria that belongs to the class of Mollicutes. Mycoplasma are common cell culture contaminants that adversely affect cell line properties, leading to erroneous, non-reproducible results. Since mycoplasma can easily escape detection by conventional methods, 35% of the world's cell lines are estimated to be affected by mycoplasma contamination¹.

The MYCOPLASMACHECK testing service offers fast, easy testing for mycoplasma contamination from cell culture with reliable detection of all mycoplasma species mentioned in the European Pharmacopoeia (E.P.) 2.6.7: *M. arginini*, *M. fermentans*, *M. orale*, *M. hyorhinis*, *M. hominis*, *M. genitalium*, *M. salivarium*, *M. synoviae*, *M. pirum*, *M. gallisepticum*, *M. pneumoniae*, *M. yeatsii*, *Spiroplasma citri* and *Acholeplasma laidlawii*. The integrated performance control readily identifies PCR inhibitors that typically lead to false-negative findings.

Here, the performance parameters of the qPCR-based assay in detecting the World Health Organisation (WHO) standard or mycoplasma DNA from cell culture media, are reported.

MYCOPLASMACHECK detects 1 IU of WHO International Standard for mycoplasma DNA

The WHO Mycoplasma Standard (8293/13) is used globally to standardise nucleic acid technique-based assays for mycoplasma detection². The Standard consists of *Mycoplasma fermentans* with a potency of 200,000 international units (IU) per ml. Dilutions of the WHO Standard were prepared in DMEM medium and subjected to MYCOPLASMACHECK. A detection limit of 1 IU per PCR was established (Fig. 1).

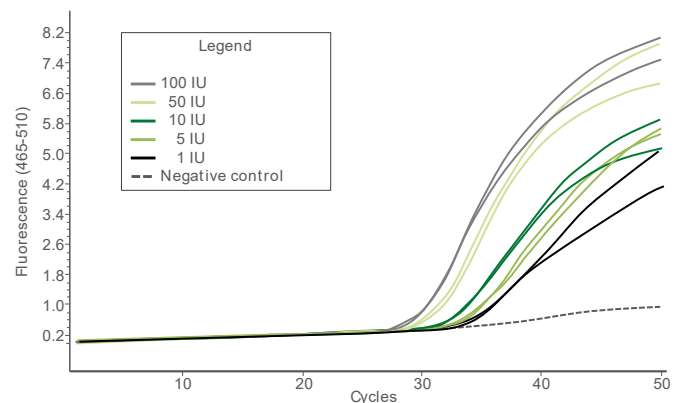


Figure 1. Amplification plots produced by MYCOPLASMACHECK from a dilution series of the WHO International Standard for mycoplasma DNA (in duplicates). Concentrations of 1 IU were successfully detected.

MYCOPLASMACHECK detects low amounts of mycoplasma DNA in various cell culture media

MYCOPLASMACHECK was performed on tenfold dilution series of plasmids containing mycoplasma target sequences prepared in a DMEM+10% Fetal Calf Serum (FCS) or Roswell Park Memorial Institute (RPMI) medium. A detection limit of 10 copies per PCR was determined for all Mollicutes species listed in Table 1.

Mollicute species	Detection limit (copies/PCR)
<i>M. fermentans</i>	10
<i>M. hominis</i>	10
<i>M. pneumoniae</i>	10
<i>Spiroplasma citri</i>	10
<i>A. laidlawii</i>	10

Table 1: Detection limit of artificial DNA from common mycoplasma contaminants spiked and diluted into various media.

¹ Drexler HG, Uphoff CC (2002). *Cytotechnology* 39: 75-90.
² Nübling CM et al. (2015). *Appl Environ Microbiol*, 81(17): 5694-702.

As all mycoplasma species from E.P.2.6.7. share identical primer and probe sequences with at least one species from Table 1, we can deduce that these are detected with 10 copies per PCR as well. When in silico analysis is performed, more than 100 additional Mollicutes strains can be detected by our service without cross-reactivity with E.coli or eukaryotic cells.

MYCOPLASMACHECK was successfully validated

As a service that tests supernatants from various cell culture media, MYCOPLASMACHECK substantially reduces resources and eliminates the need for laborious sample handling (Fig.2). Highly consistent results in accordance with the latest international detection standards are provided directly in a personal website account. Outsourcing of quality control frees the researcher from maintaining cumbersome lab records by providing data certificates that can be directly compiled for any cell line from any testing period. Such a certificate is downloadable as a PDF file and can be readily used for manuscript or grant submissions.

Summary

Intensive evaluation of MYCOPLASMACHECK is carried out on a regular basis. A detection limit of 1 IU of the WHO International Standard or 10 copies of mycoplasma per PCR was demonstrated. The service can be conveniently ordered at any time with results made available in an online account 48 hours after sample receipt. Results of each test or combinations of several tests can be conveniently compiled into a data report. MYCOPLASMACHECK is an ideal service for outsourcing quality control of cell lines in regards to mycoplasma contamination.

Contact us

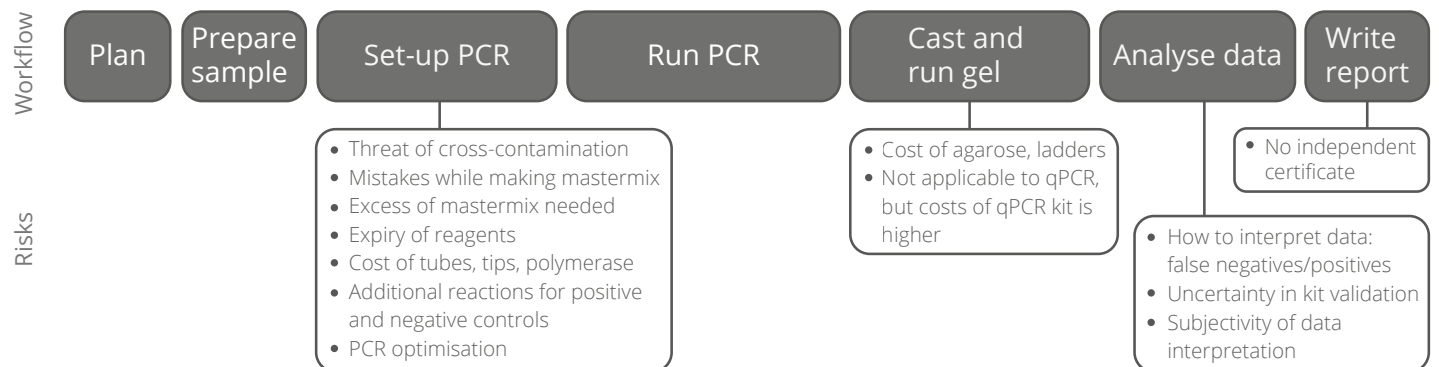
Ensure your cell lines are mycoplasma-free to prevent health risks in your lab and to guarantee the quality of products and data from your research. Try MYCOPLASMACHECK for yourself:

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In-house PCR testing for mycoplasma with hands-on time of 2.5 hours or more!



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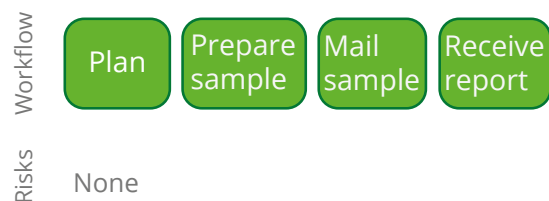


Figure 2. Comparison of the workflows of in-house PCR testing for mycoplasma versus the MYCOPLASMACHECK service in terms of risks, resources and efficiency.