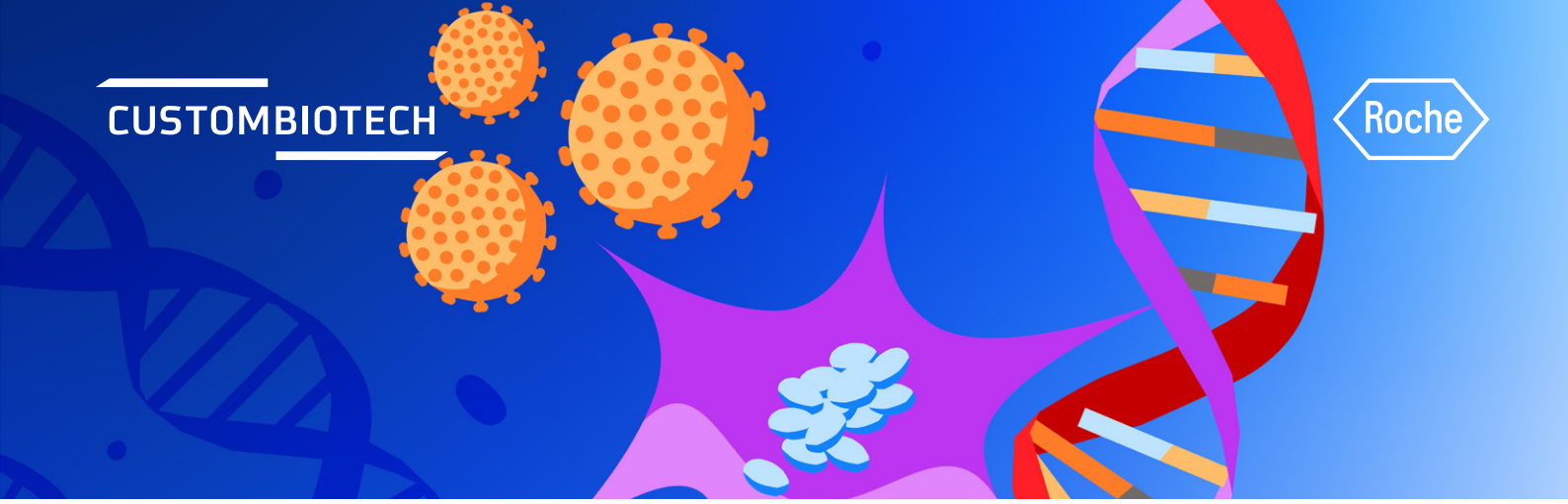

Technical Report

Mycoplasma testing of bio-pharmaceuticals and ATMP: Current regulations, challenges and trends

From traditional culture methods to automated high-throughput NAT-based methods

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4th updated edition

Preface

Mycoplasma contamination in the manufacturing process of 'classical' biopharmaceutical products (also known as biologics or large molecules) and of cell-based medicinal products (also known as cell therapy products or advanced-therapy medicinal products, ATMP) poses a potential health risk to patients. Mycoplasmas can affect virtually every cell culture parameter with often only minor visible effects, creating an uncontrollable environment that is undesirable in the pharma-ceutical and cell therapy industry. Therefore, regulatory agencies require manufacturers to test their biopharmaceutical products and ATMP to ensure the absence of mycoplasmas in released products. Most regulatory agencies have issued guidelines that provide protocols for mycoplasma testing, and some give recommendations for the validation of rapid NAT (nucleic acid amplification techniques) testing methods. These recommendations for rapid mycoplasma testing, however, are not harmonized, making establishment of such tests a challenge for manufacturing companies.

This document aims to provide a guideline with recommendations on how to validate and implement the Roche CustomBiotech MycoTOOL Mycoplasma Real-Time PCR (polymerase chain reaction) Assay (MycoTOOL qPCR – http://go.roche.com/mycotool) in combination with the Roche MagNA Pure 96 or MagNA Pure 24 Sample Preparation System as a rapid, automated, NAT-based mycoplasma testing method for biopharmaceutical products and ATMP.

Content Overview

1. A brief introduction about mycoplasmas is given. Furthermore, the relevance of mycoplasmas in biopharmaceutical and ATMP manufacturing processes is discussed.
2. An overview, and examples, of existing non-compendial and compendial mycoplasma testing methods are provided. The two compendial methods, the Culture Method and the Indicator Cell Culture Method are handled in more detail, and the rapid NAT-based method is introduced as a compendial testing method.
3. Regulatory aspects to be considered during the implementation of a rapid mycoplasma testing method are addressed. Regulatory guidelines such as the European Pharmacopoeia (EP 2.6.7.)[1], the United States Pharmacopeia (USP <63>)[2], and the Japanese Pharmacopoeia (JP 17th Ed.)[3], are discussed.
4. The fourth section addresses the potential design of a product-specific validation and implementation project and the recommended step-by-step approach. It touches upon each step in the process of implementation, from supplier due diligence and conducting a feasibility and validation study, to performing routine testing.
5. A summary and outlook are provided in the last section addressing long-term prospects offered by NAT-based rapid mycoplasma testing methods. The competitive advantages of these rapid methods when implemented as early warning systems and for lot release testing, as well as the revolutionary opportunities offered by these alternative testing systems for the biopharmaceutical and cell therapy industry, are discussed.

About us

Roche CustomBiotech
Leveraging the know-how of Roche Diagnostics and Roche Pharmaceuticals, CustomBiotech delivers high quality raw materials, instrumentation and services for biopharmaceutical, cell therapy, or in vitro diagnostics companies, customized to your unique quality and regulatory needs.

For more information:
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Mycosafe® Consulting
With her internationally recognized mycoplasma expertise and more than 40 years of experience in mycoplasma detection, prevention and control, Prof. Dr. Renate Rosengarten serves since many years as an independent mycoplasma expert, opinion leader and consultant to biopharma, biotech and cell therapy companies concerning all mycoplasma-related questions.

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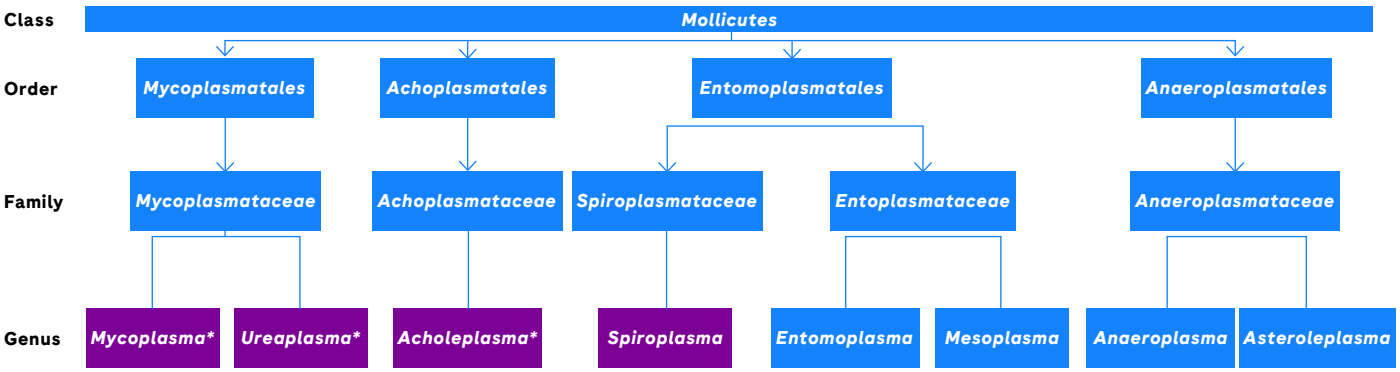
Introduction

The term 'mycoplasmas' is often used as a trivial name for all members of the bacterial class *Mollicutes* (lat. mollis = „soft", cutis = „skin") (Fig. 01). *Mollicutes* are characterized by the lack of a cell wall and a small genome size (0.5 –2.2 megabase pair) with low GC (guanine-cytosine) content (20–40 mol%). Due to their small genome, mycoplasmas are host-dependent and live as commensals or infectious agents in or on a variety of hosts, including humans, other vertebrates, plants, and insects. These microorganisms can multiply under aerobic or anaerobic conditions. They have a pleomorphic cell morphology, with the exception of spiroplasmas, which have a spiral shape, and some mycoplasmas of the genus *Mycoplasma*, which have a flask-like shape due to a terminal (tip) structure *(Mycoplasma gallisepticum, Mycoplasma pneumoniae).* Depending on species, mycoplasmas can grow in liquid media, either as single cells *(Mycoplasma arthritidis)* or in aggregates *(Acholeplasma laidlawii, Mycoplasma pneumoniae, Mycoplasma fermentans).*[4] The lack of a cell wall makes mycoplasmas resistant to cell wall-targeting antibiotics such as penicillin. Furthermore, some mycoplasmas can form biofilms on solid surfaces in liquid media, such as glass or plastic surfaces, which provides another level of resistance, namely to disinfecting agents and environmental stress conditions.[5] The first mycoplasma species was cultured at the Institut Pasteur in 1896; it was isolated from cattle with pleuropneumonia and much later described as *Mycoplasma mycoides* subsp. *mycoides* SC (small colony type).[6]



01
Taxonomy of the bacterial class *Mollicutes*. The red boxes indicate genera with relevant species in biopharmaceutical manufacturing processes.
*Genera containing mycoplasma species that are prevalent in humans. Source: Authors, 2017.

Due to their genome reduction, mycoplasmas lack several metabolic pathways either completely or partially, forcing them to acquire necessary nutrients (amino acids, nucleobases, and fatty acids) from the environment and to exert a parasitic life style.[7]

For a long time, mycoplasmas were largely underestimated as pathogens. For that reason, there was a lack of suitable molecular diagnostic approaches. This initial situation has changed considerably in recent years, and there has been greater acceptance and improvement of culture-based and molecular methods for mycoplasma detection.[8]

Mycoplasmas in Cell Cultures

In addition to their growing clinical significance mycoplasmas have gained great attention in the context of cell cultures. As they naturally reside in plant and animal tissues, every cell culture medium containing plant- or animal-derived supple-ments is prone to contamination by mycoplasmas. Due to their small size of only 0.1–0.8 µm on average (Fig. 02), and their variable shape resulting from the missing cell wall, mycoplasmas can pass through standard sterilizing filters and enter cell cultures with culture media or raw material-derived additives.

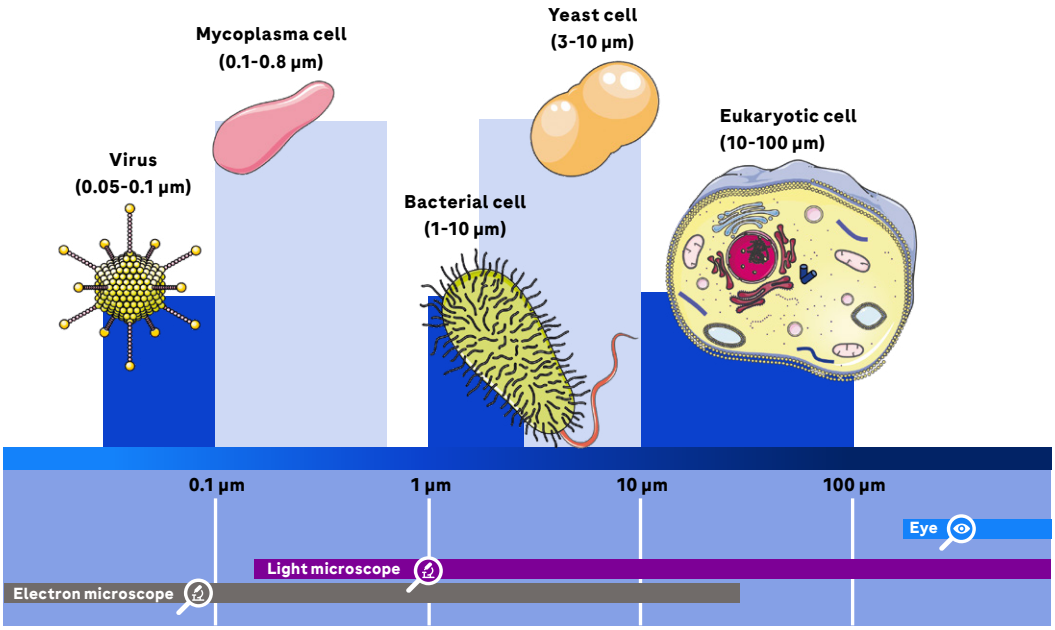
The two most common sources of contamination are laboratory personnel and already contaminated cell cultures, from which the contaminant is passed on by cross-contamination.[9] Since mycoplasmas are not visible with standard light microscopy setups and usually barely affect the obvious state of the cell culture, they often remain undetected. Nevertheless, they impact cell growth and metabolism, and consequently, the therapeutic proteins expressed by host cells.

Biopharmaceuticals

Biopharmaceuticals (also known as biologics) are the 'classical' medicinal products manufactured in and extracted from biological sources such as bacteria, yeast, mammalian cell lines, or mammals. Vaccines, purified blood components and recombinant proteins fall into this category. They can consist of nucleic acids, proteins, sugars and complex combinations of these and are either identical or similar to molecules naturally occurring in the human body.

In contrast to chemically synthesized drugs (often referred to as small molecules), biopharmaceuticals are much larger with a molecular weight 100 times that of small molecules. Biopharmaceutical manufacturing in mammalian cell lines typically involves the development of a genetically engineered eukaryotic cell line (such as CHO or HEK293) to express the biopharmaceutical, and subsequent harvesting, purification, and drug formulation. Also refer to Fig. 03.

Due to the complex manufacturing process, biopharmaceuticals face unique manufacturing and product release challenges. Firstly, cell lines may be contaminated with mycoplasmas, requiring mycoplasma testing prior to lot release. Secondly, they are sterilized by filtering, which has the potential risk that mycoplasmas or viruses pass through the filter. Thirdly, cell culture contamination may be introduced by raw materials. This is why mycoplasma testing methods, especially early warning systems (also known as in-process control), are essential to detect a contamination as fast and as early as possible.



02
Relative size of different microorganisms. Source: Authors, 2017. This figure is a graphical illustration by the authors of this Technical Report and provided under the terms of the Creative Commons Public License CC BY 3.0 (http://creativecommons.org/licenses/by/3.0/), and can be used under the terms of such license notwithstanding any rights that may exist with respect to the document it is embedded in.

Despite the negative effects of mycoplasma contamination, cell cultures are rarely monitored, even though testing for mycoplasma contamination is a necessary quality control procedure. Studies have shown a contamination rate of about 5–35% of existing cell lines available worldwide.[10, 11, 12] The only meaningful safety precaution to maintain mycoplasma-free cell cultures is to regularly test for mycoplasmas.

Only well-established routine mycoplasma testing during the ongoing process can minimize the risk of a concealed contamination that can lead to serious problems.[13] Because the testing procedure and subsequent results interpretation require solid training and experience, mycoplasma testing cannot always be carried out in-house. Outsourcing mycoplasma testing to a trustworthy and experienced contract research organization (CRO) is an alternative that brings several advantages. Firstly, qualified results can be obtained in the shortest time possible. Secondly, an in-house testing facility does not need to be maintained, which frees resources to concentrate on the core business. Last but not least, the potential risk of mycoplasma contamination associated with introducing necessary positive controls into a facility are avoided.

Mycoplasmas in Manufacturing Processes of Biopharmaceuticals and ATMP

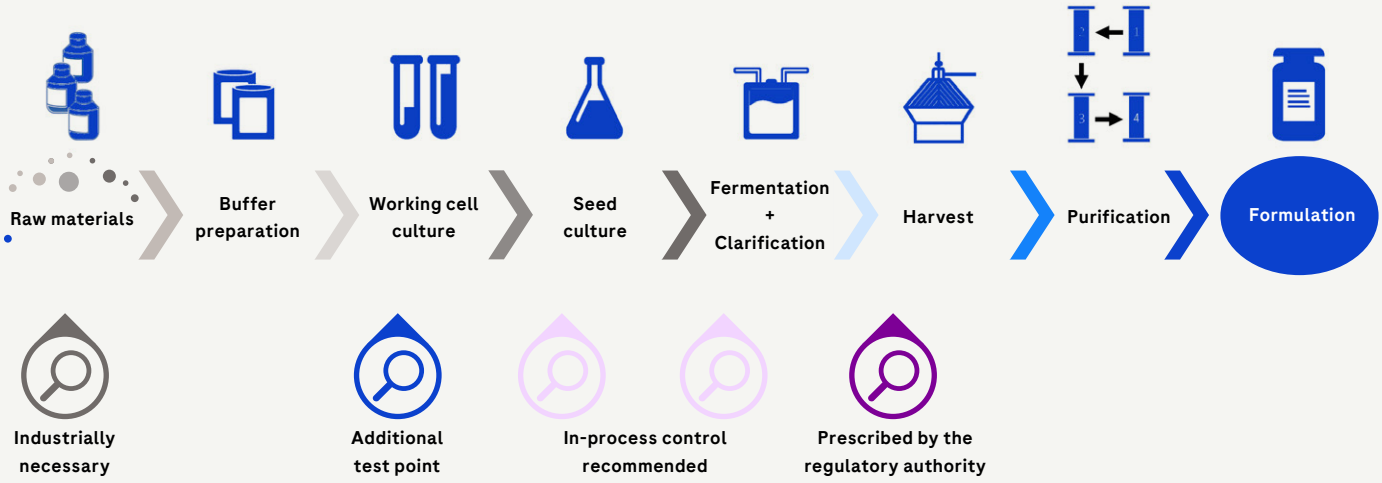
Especially in the biopharmaceutical and cell therapy industry, the effects of mycoplasma contamination are devastating, as entire production batches must be discarded and the manufacturing plant must stop production.[14] International regulatory authorities have published guidelines to demonstrate that biological products intended for preventive or therapeutic clinical use and prepared in cell culture substrates must be free of mycoplasmas to ensure product safety, purity and potency.

Therefore, early detection of mycoplasmas is essential for smooth processes in manufacturing of biopharmaceutical and cell therapy products. Fig. 03 depicts common testing points in the manufacturing process of ‘classical’ biopharmaceuticals. Given these multiple check points, numerous different methods for mycoplasma testing have been developed and will be covered in the following section.

ATMP

Advanced therapy medicinal products (ATMP) are a new class of therapeutics that are based on genes (gene therapy), somatic cells (cell therapy), or tissue (tissue engineering). These advanced therapies herald novel treatments of a number of diseases and thus, a huge potential for patients is expected. Autologous cell therapy, for instance, typically involves cell dissociation from an individual patient, cell culture outside the human body, and subsequent injection of cells back into the patient.

Compared to the ‘classical’ large molecule biopharmaceuticals, cell therapy products face additional manufacturing and release challenges. Firstly, most of them cannot be sterilized at all. Secondly, storage may be challenging as cell therapy products sometimes face a short shelf life and need to be injected into patients immediately. Thirdly, the batch size often consists of one dose rate, and volumes are usually very small. In such cases, rapid mycoplasma testing methods are favorable over the conventional culture-based mycoplasma testing methods because they require smaller volumes and release manufacturing batches much faster than conventional mycoplasma testing methods. Different from the ‘classical’ biopharmaceuticals, there is also the need to evaluate the possible risk in terms of entry and growth of uncommon mycoplasma species during the manufacturing process due to new cell types and production conditions.



03
Testing points for mycoplasma contamination in the manufacturing process of biopharmaceuticals. After raw materials have been tested for contamination (grey loop), and solutions like buffer and media have been applied to working cultures, it is necessary to check for contamination (blue loop), as mycoplasma contamination can also be introduced by the cell line and lab staff. It is also recommended to carry out in-process controls during the seed culture and the actual fermentation (light purple loops). The final and prescribed test point is the endpoint of the fermentation, the harvest (purple loop). Once the mycoplasma-free state of the harvest has been proven, further test points are usually no longer necessary since the purification of the products is carried out without living organisms. Source: Roche CustomBiotech, 2017.

Mycoplasma species frequently or potentially detected as contaminants in cell cultures and in manufacturing of biopharmaceuticals are listed in Table 01. For ATMP, additional mycoplasma species might be product-relevant, depending on the starting material. A mycoplasma species spectrum analysis is therefore recommended. The effects of contamination in the manufacturing process can lead not only to reduced product quality, but also to lower expression levels and consequently reduced production yields. In addition, poor quality and contamination with mycoplasmas may trigger serious side effects in patients.[15, 16]

Mycoplasma species	Primary isolation source (relevant for products where raw materials of the following origins are used)	Frequent cell culture contaminant based on published reports	Potential contamination source
Acholeplasma laidlawii	Bovine, porcine, avian, plant	Yes	Other cell line, bovine sera, nutrient broth powders
Mycoplasma arginini	Bovine, ovine, caprine, porcine	Yes	Other cell line, bovine sera
Mycoplasma bovis	Bovine	Yes	Other cell line, bovine sera
Mycoplasma fermentans	Human	Yes	Other cell line, personnel
Mycoplasma gallisepticum	Avian	No	Other cell line, embryonated eggs
Mycoplasma hyorhinis	Porcine	Yes	Other cell line, porcine trypsin
Mycoplasma orale	Human	Yes	Other cell line, personnel
Mycoplasma salivarium	Human	Yes	Other cell line, personnel
Mycoplasma synoviae	Avian	No	Other cell line, embryonated eggs
Spiroplasma citri	Plant	No	Other cell line

T01
Mycoplasma species which are frequently, occasionally or potentially detected in cell cultures and in biopharmaceutical processes.

Mycoplasma Testing Methods Overview

Detection of mycoplasmas presents a challenge for quality control of cell cultures and biopharmaceuticals because especially low-grade contaminations can only be identified through expertise and experience. Two conventional methods have been used for regulatory mycoplasma testing in recent decades, as they have proven to be sensitive and reliable: (i) the Culture Method (also referred to as agar and broth method) and (ii) the Indicator Cell Culture Method. Non-compendial methods, such as enzyme-based and immunology-based assays, are easy and fast to apply but most do not reach the level of sensitivity of the culture-based methods. Therefore, pharmacopoeial monographs do not consider these tests acceptable substitutes for regulatory testing. The necessity for fast yet sensitive and robust detection of mycoplasmas has increased over the past years, because the prolonged conventional Culture Method can delay pharmaceutical product release, which is often associated with high costs. The EP guidelines permit substitution of the conventional culture methods by NAT if they achieve equivalent sensitivity as the traditional methods and prove to be as robust and specific.

This section briefly summarizes the non-compendial and compendial methods used for mycoplasma detection. NAT-based methods are described in more detail, with special focus on the MycoTOOL qPCR. This test method has passed all necessary validation criteria formulated by the EP mycoplasma NAT validation guideline to substitute both the Indicator Cell Culture Method and the Culture Method.

Non-compendial Testing Methods

Non-compendial tests for mycoplasma detection often lack the sensitivity to detect the level of contamination in a sample that is required by regulatory monographs. Moreover, results are sometimes difficult to interpret if a contamination is at low level. These tests are prone to giving false-negative results.

Direct DNA Staining

Direct staining of cultures with a DNA (Deoxyribonucleic acid)-specific fluorescent dye is sensitive, but not recommended for the purpose of detecting mycoplasma contaminations. Although the test reliably detects heavily contaminated cultures, interpretation of low-grade contaminations is often difficult because DNA from the cell culture may give rise to small points of fluorescence that can mimic mycoplasmas.[17]

Enzyme-based Method

Enzyme-based assays are selective biochemical tests that exploit the activity of mycoplasma enzymes. A prerequisite for such a test to be applicable for routine mycoplasma testing is that the enzymatic activity measured must ideally be ubiquitous among mycoplasmas, but missing in the eukaryotic cell matrix. An example of an enzymatic assay is the luciferase-based mycoplasma detection assay.[18, 19] Although the assay is fast (< 20 min), relatively easy to handle (two luminescence readings), and the interpretation of results is easy, no such test has yet been shown
to reach the limit of detection that is required from a compendial test (≤ 10 CFU (colony forming units)/ml). Most mycoplasma species are detected only at a high titer of 10^4 to 10^5 CFU/ml.[20]

Mycoplasma PCR-ELISA

An application that combines PCR with a subsequent ELISA (enzyme-linked immunosorbent assay) is the mycoplasma PCR-ELISA, a photometric enzyme immunoassay that detects PCR-amplified mycoplasma DNA in cell culture.[21] During the PCR reaction, digoxigenin-labeled nucleotides are incorporated into the amplicons, allowing their detection in a subsequent ELISA assay. The mycoplasma PCR-ELISA test is claimed to have a detection sensitivity of 1–3 mycoplasma "particles" for particular mycoplasma species (e.g., *M. fermentans* and *A. laidlawii*).[22] However, since for others the Limit of Detection (LOD) was 1000 "particles" per ml sample, the test does not fulfill the requirements of the EP regulatory guideline as compendial test for mycoplasma detection.

Compendial Testing Methods

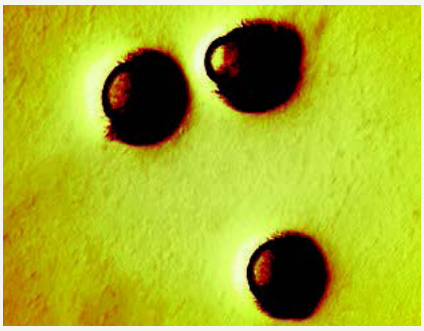
These methods are, on one hand, based on conventional microbiological culture procedures using liquid media and agar media and, on the other hand, based on rapid molecular techniques. The methods and their advantages and disadvantages are summarized in this section and Table 02.

Method	Advantages	Disadvantages
Culture Method	• Sensitive • Detects 0.1 CFU/ml • Detection of 'real' contaminations caused by viable multiplying mycoplasma cells	• Up to 28 days incubation period • Requires more than one growth medium for the cultivation of different mycoplasma species • Risk of false-negative results: highly fastidious *M. hyorhinis* cultivar alpha strains and ureaplasmas are not detected if standard mycoplasma culture media are used
Indicator Cell Culture Method	• Inexpensive	• Subjective interpretation that can be biased • Not mycoplasma-specific • Less sensitive
NAT-based Method	• Sensitive • Detects ≤ 10 CFU/ml • Full automation possible • High-throughput testing • Application as early warning system	• Strongly dependent on quality and efficiency of sample preparation and DNA extraction • Risk of false-negative results due to incomplete mycoplasma species coverage or PCR inhibition depending on the method • Requires DNA extraction kit and costly equipment instruments • Requires validation to substitute Culture and Indicator Cell Culture Methods

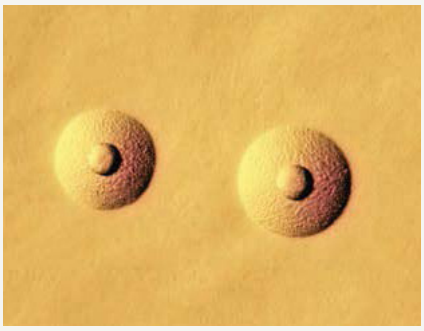
T02
Advantages and disadvantages of compendial mycoplasma detection methods.

Culture Method

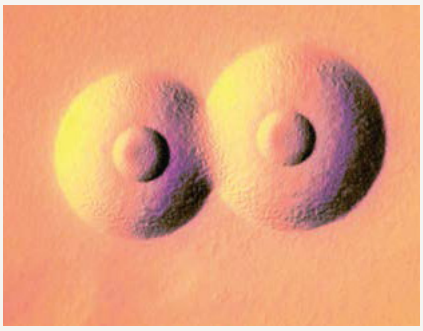
Traditional culture methods were used well before today's molecular techniques and are still found in regulatory and compendial protocols throughout the world (formulated in the EP, USP and JP regulatory guidelines). The Culture Method is based on the targeted cultivation of mycoplasmas in culture media that promote mycoplasma growth. A sample to be tested is inoculated into the liquid mycoplasma culture media and onto agar media, and mycoplasma growth is promoted by micro- aerophilic incubation conditions, such as 36 ± 1°C, 5.5 ± 0.5% CO2, 3 ± 1% O2 and 90 ± 5% relative humidity. Subcultivation from the liquid cultures onto agar plates is carried out up to 21 days after the initial inoculation. On the agar medium, mycoplasmas develop microscopic colonies (< 100–400 µm diameter). Mycoplasma colony morphologies can vary from the typical fried-egg shape to a more irregular colony shape, which in Spiroplasma citri is caused by the formation of satellite colonies due to the motility of the spiroplasma cells (Fig. 04). As mycoplasma colonies can be very small, colony counting under the microscope requires some experience. A schematic illustration of the Culture Method is shown in Fig. 05.



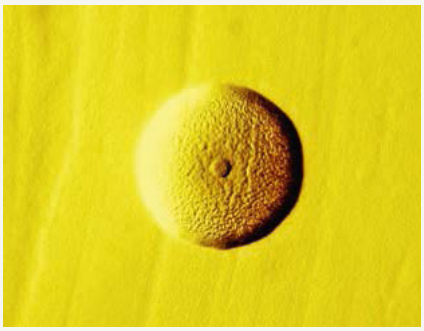
A. laidlawii
BRP EDQM Y0000693



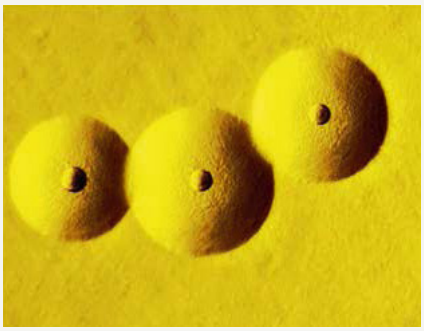
M. arthritidis
PG6^{T} NCTC 10162 ATCC *19611*



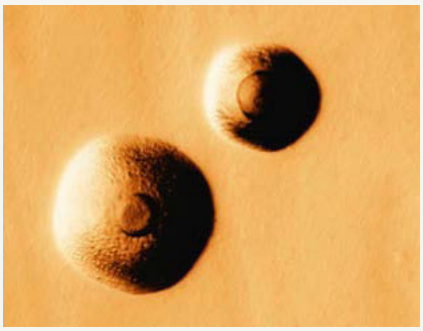
M. fermentans
BRP EDQM Y0000692



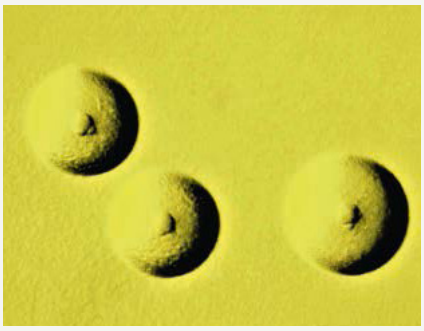
M. gallisepticum
MEVET A70



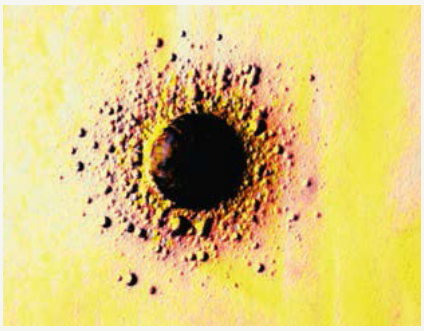
M. hyarhinis
3131



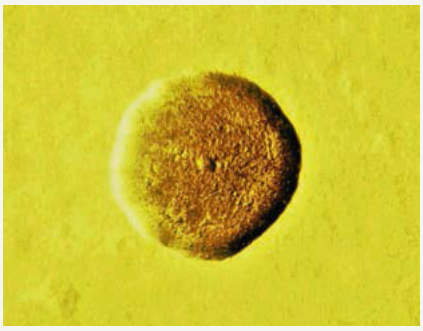
M. orale
BRP EDQM Y0000691



M. pneumoniae
5167



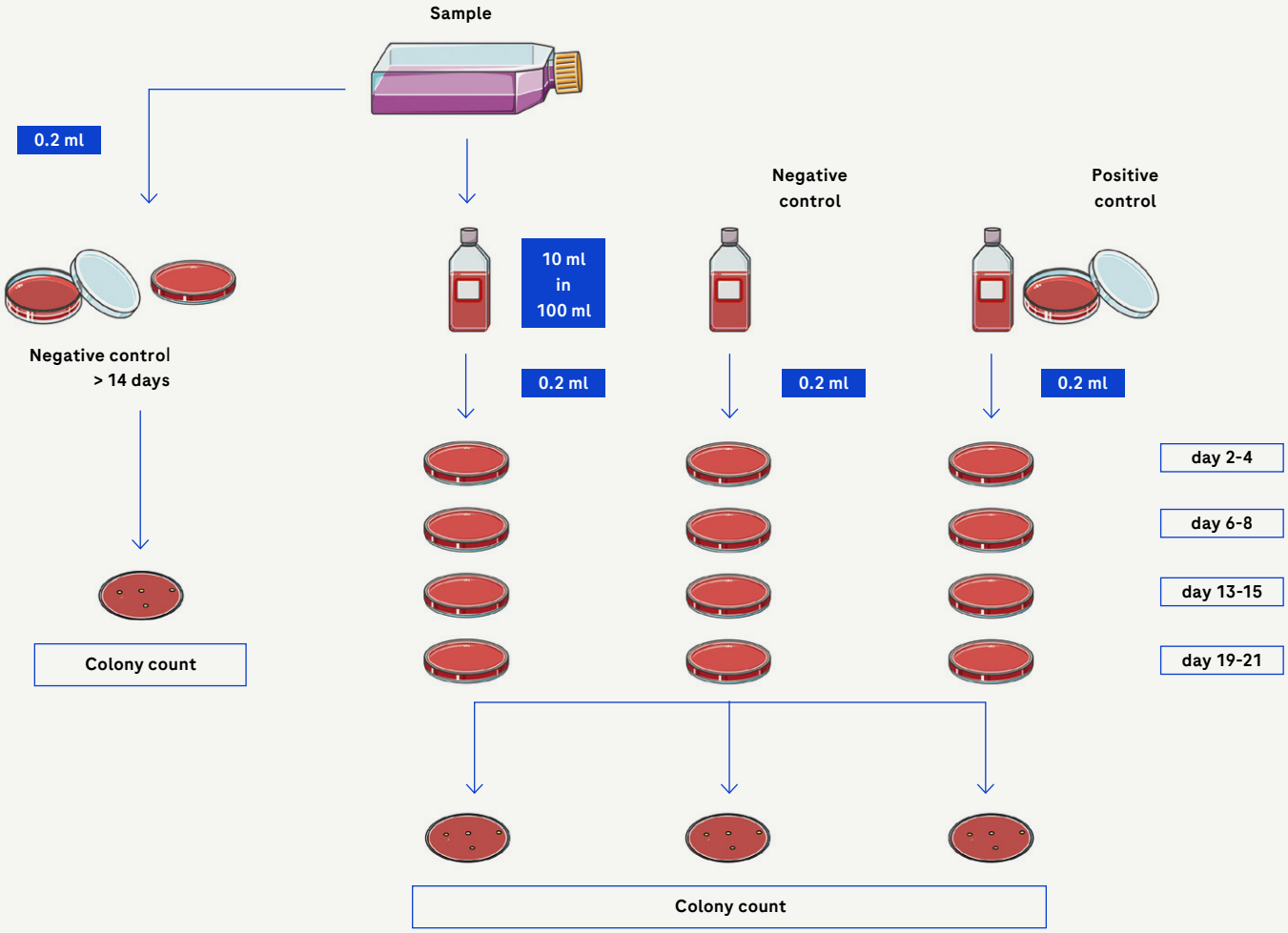
S. citri
R8-A2^{T} NCTC 10164 ATCC 27556



M. hyorhinis cultivar alpha
DBS 1050 ATCC 29052

04
Colonies of mycoplasma reference strains, including selected pharmacopoeia type and reference field strains, and the highly fastidious 'non-cultivable' cell-culture-adapted pharmacopoeia cultivar α reference strain, grown on different mycoplasma agar media.

The relatively large sample volume (10 ml) and the long incubation period (28 days in total) render the Culture Method one of the most sensitive tests, with a theoretical and experimentally proven detection limit of 0.1 CFU/ml, corresponding to 1 CFU/10 ml sample. The method fulfills the EP 2.6.7 requirement of detecting ≤ 10 CFU/ml, which is why this method is still the reference method in regulatory documents worldwide. The Culture Method has, however, a few disadvantages. The predominant drawbacks come from the lengthy cultivation period of 28 days. This time factor poses major challenges for many companies, including product release delays that entail higher storage costs, as well as increased personnel costs for logistics during testing of raw materials, the cell line, and the process controls in the up- and down-streaming process. Another limitation is that the Culture Method requires usage of several different growth media. Not all mycoplasma species grow in the same standard mycoplasma culture medium.



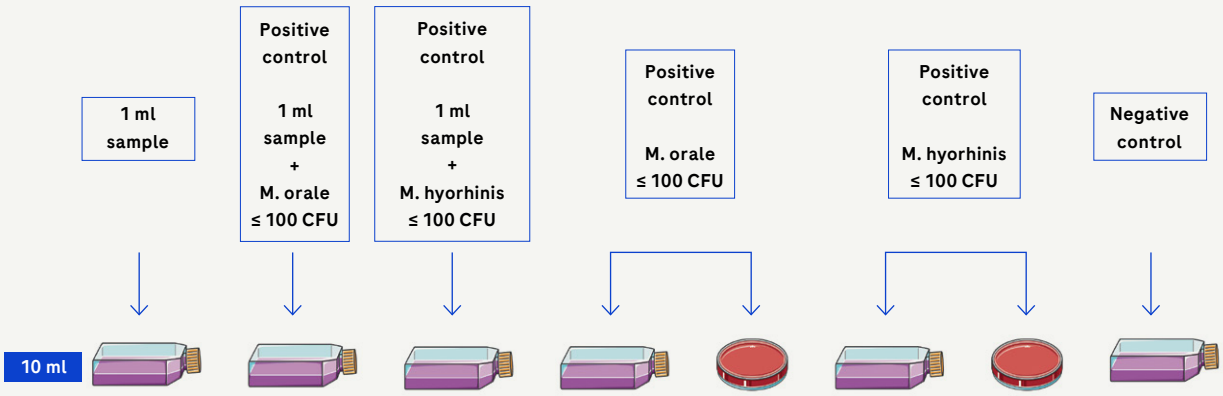
05

Schematic illustration of the Culture Method according to EP 2.6.7. Aliquots of 200 µl from a sample to be tested for the absence of mycoplasma contamination are plated onto mycoplasma agar medium and 10 ml are inoculated into 100 ml liquid growth medium. Uninoculated media serve as negative controls, and media inoculated with ≤ 100 CFU serve as positive controls. The liquid medium is incubated for 20–21 days. On days 2–4, 6–8, 13–15 and 19–21 after inoculation, 200 µl of the medium inoculated with the sample, and the negative and positive controls are plated onto agar medium. The inoculated agar media are incubated for not less than 14 days, except those corresponding to the 20–21 day subculture, which are incubated for 7 days. Source: Authors, 2017. This figure is a graphical illustration by the authors of this Technical Report and provided under the terms of the Creative Commons Public License CC BY 3.0 (http://creativecommons.org/licenses/by/3.0/), and can be used under the terms of such license notwithstanding any rights that may exist with respect to the document it is embedded in.

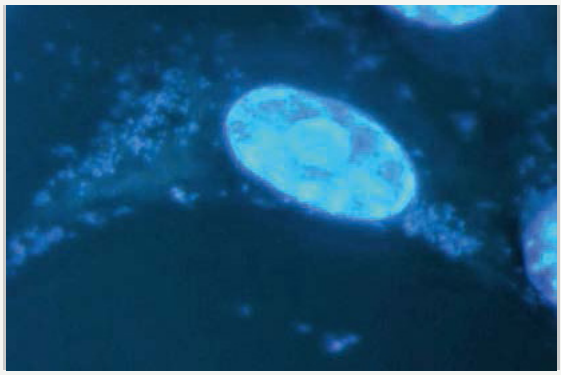
Thus, depending on the source of the sample, different mycoplasma culture media are used in parallel to increase the detection spectrum of possible contaminating mycoplasma species. For this reason, the use of at least two standard mycoplasma culture media is recommended in EP 2.6.7.: FRIIS medium for the detection of non-avian mycoplasmas, and FREY medium for the detection of the avian mycoplasma species *M. synoviae*. The perhaps greatest disadvantage of the Culture Method is, however, that highly fastidious mycoplasma strains, such as the *M. hyorhinis* cultivar alpha strains (with *M. hyorhinis* DBS 1050 as reference strain), do not grow in the standard culture media due to growth inhibition by certain peptones and yeast products.[23] Growth of these cell-culture adapted strains is dependent on their habitat, the cell culture. In order to detect these cultivar alpha strains, an additional test has to be performed in parallel, using the Indicator Cell Culture Method.

Indicator Cell Culture Method

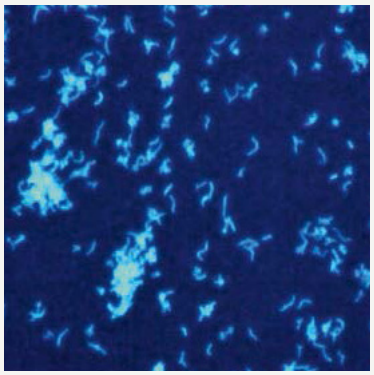
The Indicator Cell Culture Method is normally carried out with Vero or 3T3 cell lines, although the use of a production cell line that is equivalent in effectiveness for detecting mycoplasmas is also accepted by the EP regulatory guideline. The indicator cell culture is inoculated with the sample and incubated at 35–38°C until grown to confluence. For positive controls, the indicator cell line is also inoculated with the type strain CH19299^T of *M. orale* and the *M. hyorhinis* cultivar alpha reference strain DBS 1050 with and without the presence of the test sample. Before staining, the subculture is fixed with a suitable fixing solution and stained with a fluorescent dye that binds to DNA. The presence of mycoplasmas is characterized by a spherical fluorescence pattern on the cell surface and by strong fluorescence in the surrounding areas. Mitochondria in the cytoplasm are also stained, but are easily distinguished from mycoplasmas. The test is invalid if the positive controls do not show fluorescence typical for mycoplasmas or if the negative control shows fluorescence typical for mycoplasmas. A schematic illustration of the Indicator Culture Method is shown in Fig. 06.



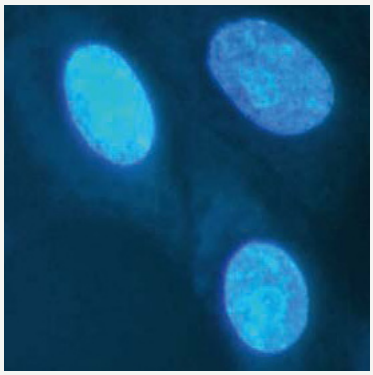
Fluorescence Microscopic Evaluation



Positive controls: Vero cells + *M. hyorhinis* DBS1050



M. orale CH19299^T enriched in Vero cell culture



Negative control: Vero cells uninoculated

06

Indicator Cell Culture Method according to EP 2.6.7. A reshly prepared Vero indicator cell culture is inoculated with 1 ml sample. Four positive controls are prepared. Two positive controls consist of Vero cells inoculated with 1 ml sample spiked with not more than 100 CFU *M. hyorhinis* cultivar alpha reference strains DBS 1050 and *M. orale* type strain CH19299^T, respectively. The other two positive controls are Vero cells inoculated with not more than 100 CFU *M. hyorhinis* DBS 1050 and *M. orale* CH19299^T without the sample. The two positive control strains are plated onto agar medium to check for viability. The negative control is a freshly prepared Vero cell culture that is left uninoculated. All cell cultures are incubated in a CO_2 incubator until the cell density of 100% is reached. The cell layer is then washed with buffer and trypsinated. The detached cells are resuspended in cell culture medium, transferred to chamber slide flasks and incubated in a CO_2 incubator until the cell density is approximately 50%. The cell layer is fixed twice with a freshly prepared fixing solution, air dried and stained with a Hoechst Stain method. The microscope slides are evaluated using a fluorescence microscope.

Both the Culture Method and the Indicator Cell Culture Method take a long time to results (up to 28 days for the Culture Method and 7 days for the Indicator Cell Culture Method), and carry the intrinsic risk of introducing a mycoplasma contamination into the facility due to the required handling of viable mycoplasma cells as positive control organisms.

NAT-based Methods

NAT-based methods include all tests based on nucleic acid detection, often performed by PCR.

PCR

PCR is a molecular biological method used in many areas such as food and environmental analysis, forensics, and medical diagnostics. The underlying principle is the specific amplification of DNA to a level that can be detected. This amplification is carried out by the enzyme DNA polymerase in repeated amplification cycles that are automated by thermocyclers. One cycle consists of three main steps:

1. Double-stranded DNA (dsDNA) is denatured into single-stranded DNA (ssDNA) by heat
2. PCR primers bind to specific ssDNA sites (e.g. to specific target genes)
3. DNA polymerase elongates the annealed primers according to the sequence of the ssDNA

Usually after 30–50 PCR cycles sufficient DNA is amplified for detection by gel electrophoresis and staining with fluorescent dyes.

PCR temperature protocols have been optimized with the goal to develop more sensitive, specific, or rapid PCR assays. Touchdown PCR (TD-PCR), for example, is very commonly used to make PCR assays more specific to a targeted gene. Primers bind with high specificity to a targeted DNA sequence at high annealing temperatures during the first few PCR cycles. This ensures the exclusive amplification of a specific DNA sequence. The annealing temperature is then gradually decreased to reach highest PCR efficiency. TD-PCR protocols reduce the amount of nonspecific DNA amplified by PCR.[24] MycoTOOL qPCR leverages TD-PCR for highly specific mycoplasma detection.

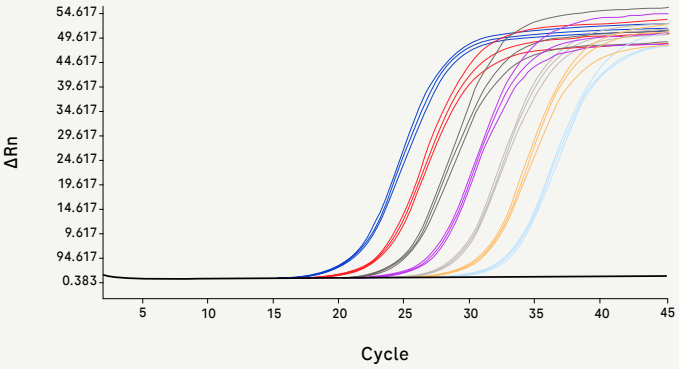
You will find a summary and more information about the history and evolution from the conventional PCR to the qPCR here: (https://diagnostics.roche.com/in/ en_gb/ article-listing/the-evolutionof-pcr.html)

In contrast to PCR, Real-Time PCR (qPCR) reports the amplification of DNA in real time. Thus, there is no need for post-PCR DNA detection such as gel electrophoresis. This reduces the risk of PCR contamination in the laboratory dramatically and facilitates the interpretation of end results. qPCR uses probes consisting of a fluorescent dye attached to a short DNA sequence (18–30 base pairs) that is added to the PCR. The probe is incorporated into the new strands of DNA produced in each amplification cycle. There are a variety of different probe designs on the market, but one of the most common ones are the hydrolysis probes used in MycoTOOL qPCR. This probe reports the amount of total DNA as fluorescence intensity after each PCR cycle. The fluorescent signal increases proportionally to the amplification of the target sequence. The fluorescence intensity is plotted over time and forms a typical sigmoid qPCR curve (see Fig. 07).

Both, PCR and qPCR are sensitive methods and thus prone to DNA contamination. The most common sources of contamination are the DNA template itself and amplified DNA from post-PCR reactions. DNA molecules may be spread around the lab via air conditioning systems or laboratory staff. The most effective strategy to eliminate these contamination sources is a unidirectional workflow from sample drawing to DNA detection in separate work areas or separate rooms.[25]

MycoTOOL Mycoplasma Real-Time PCR Kit (MycoTOOL qPCR)

The MycoTOOL Real-Time PCR Kit is a qPCR assay optimized for the detection of mycoplasmas in cell culture. It fulfills all EP 2.6.7. requirements for NAT-based assays for mycoplasma detection with respect to sensitivity (i.e., detection limit of ≤10 CFU), specificity, robustness and comparability. It does not require a mycoplasma enrichment or pre-incubation step. The kit uses primers and probes that are highly specific to the mycoplasma 16S ribosomal DNA gene. This allows the detection of more than 150 cultivable and non-cultivable mycoplasma species. It includes the most frequently occurring cell culture contaminants, namely *A. laidlawii, M. arginini, M. fermentans, M. hyorhinis, M. orale and M. salivarium*, as well as the human pathogenic mycoplasma species *M. pneumoniae* and *M. hominis*, the avian pathogenic mycoplasma species *M. gallisepticum* and *M. synoviae*, and the plant pathogenic mycoplasma species *S. citri* (Table 01).



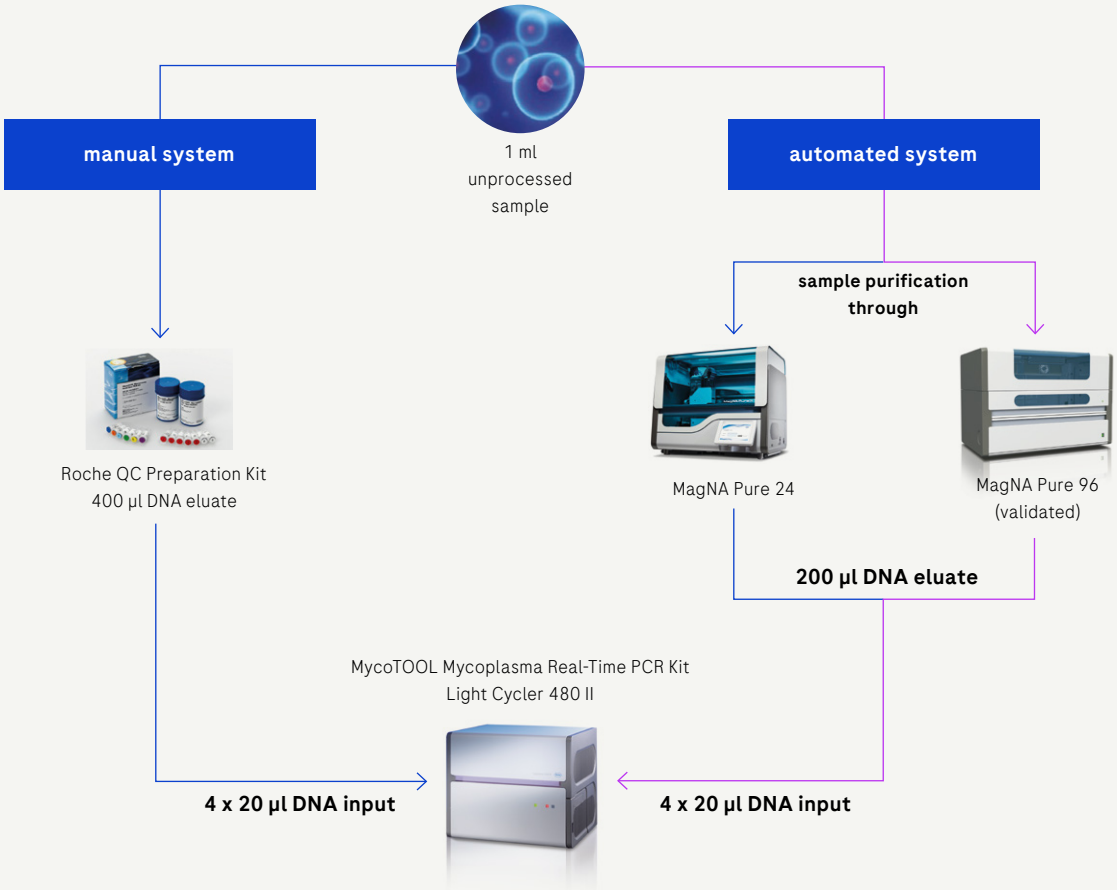
07

Example of mycoplasma testing by MycoTOOL qPCR analysis. In the first 15 cycles of the qPCR, the baseline describes the initial signal at which little change is seen in the fluorescence intensity. This signal can also be defined as background fluorescence of the reaction. The threshold cycle (Cq) is the cycle number at which the fluorescence signal of the sample exceeds the background signal. The lower the Cq value the higher the amount of DNA in the sample. Source: Roche CustomBiotech, 2017.

(https://go.roche.com/magnapure96)

(https://go.roche.com/magnapure24)

The MagNA Pure 24 and 96 instruments purify nucleic acids from a wide range of starting materials (e.g. whole blood, plasma, cell culture) using magnetic glass particle technology. For more information use the QR-Code for our MagNA Pure 96 and for our MagNA Pure 24 System.



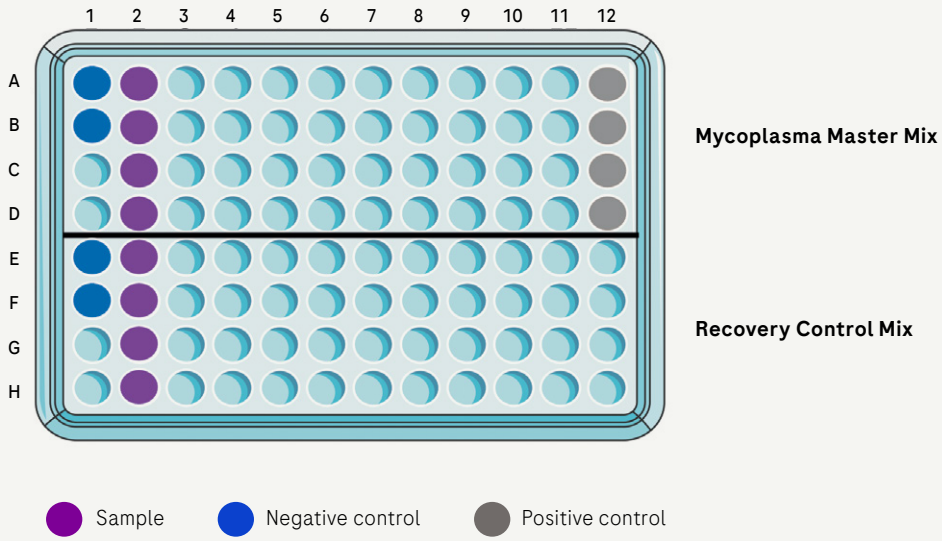
08

MycoTOOL qPCR workflow. Unprocessed sample (1 ml) with a cell density of $5x10^6$ cells/ml is prepared using a manual or automated workflow. For manual sample preparation, nucleic acids are isolated with the Roche QC Preparation Kit. With the automated nucleic acid isolation system, the sample DNA is purified with one of the MagNA Pure systems. Subsequently, a mycoplasma-specific qPCR reaction is performed on the LightCycler 480 II Real-Time PCR system. The automated workflow based on the MagNA Pure 96 and LightCycler 480 II system, shown as the purple marked procedure, has been fully validated by Roche Pharma Biotech and according to EP chapter 2.6.7. The generic validation information is available on request under confidential disclosure agreement.
Source: Roche CustomBiotech, 2017.

Automated DNA extraction may be conducted with either a MagNa Pure 24 or a MagNa Pure 96 instrument, followed by subsequent qPCR performed on the LightCycler 480 II realtime PCR instrument. Manual DNA extraction may be done with the Roche QC Preparation Kit for samples with cell densities up to $5x10^6$ cells/ml, or with MycoTOOL Mycoplasma Detection Prep Kit, High Cell Density for samples with a cell density range of 5×10^6 cells/ml to 1×10^8 cells/ml. Carrier DNA is available for analysis of cell-free samples and may be added to the biological sample prior to nucleic acid extraction and purification. The entire workflow from sampling to result takes 4 to 6 hours, depending on the level of automation and the number of samples. The complete workflow is depicted in Fig. 08.

MycoTOOL qPCR includes three controls to ensure validity of results. To verify the integrity of all reagents used during qPCR, a plasmid-based positive control is added to each experiment. False negative results are controlled by a H_2O negative control. The third control used is a plasmid-based recovery control (RC; also known as an exogenous internal control) that is added to each sample prior to DNA isolation. It is co-amplified using a second set of primers and probes in a separate vial. The RC verifies the integrity of the complete workflow, from DNA isolation to PCR. Because the RC is spiked into the sample as an exogenous control, MycoTOOL qPCR is not limited to a specific cell line and may be used across the spectrum of cell lines commonly used in biopharmaceutical manufacturing.

All amplification reactions are carried out in technical replicates (two or four for the negative control and positive control/RC - sample, respectively). Fig. 09 shows a typical pipetting scheme of a 96 well qPCR plate.



09

MycoTOOL qPCR pipetting scheme. Before DNA preparation with the MagNA Pure 24 or 96 instruments, a sample is spiked with a defined concentration of a RC plasmid. The DNA from 1 ml sample is eluted in 200 µl buffer. Four technical replicates are tested with 20 µl each. This corresponds to a volume of 40 % of the biological sample. Source: Authors, 2017. This figure is a graphical illustration by the authors of this Technical Report and provided under the terms of the Creative Commons Public License CC BY 3.0 (http://creativecommons.org/licenses/by/3.0/), and can be used under the terms of such license notwithstanding any rights that may exist with respect to the document it is embedded in.

Acceptance criteria for a plate to pass evaluation are: all negative controls must give a negative result, and the positive control reactions as well as all RC reactions of a sample must give a positive result.

Regulatory Overview

Since mycoplasma contamination evidently affects cell cultures, testing for mycoplasmas has increasingly become regulated by authorities. Today, mycoplasma testing in manufacturing of biopharmaceuticals and ATMP is controlled by law in almost all countries around the world. Regulatory authorities have published legally binding documents with national pharmacopoeias for mycoplasma testing. These documents define the methods used and products to be tested. However, recommended methods for mycoplasma testing and their detailed test protocols may differ among countries. In general, one must differentiate between conventional culturebased and alternative NAT-based mycoplasma testing methods. The traditional compendial methods, such as the Culture Method and the Indicator Cell Culture Method (see section 2.2.), are considered the long-standing gold standard and thus, are widely recommended in all pharmacopoeias. Although individual methodological steps may vary slightly from one national pharmacopoeia to the other, the protocols for these culture-based tests are largely harmonized across the countries. Please refer to Table 03 for a summary of regulatory authorities and pharmacopoeias containing regulatory guidelines relevant for mycoplasma testing in some countries.

The regulatory approval system

The health authorities are responsible for the scientific evaluation, supervision and safety monitoring of medicinal products developed by pharmaceutical companies. Regulatory approval ensures that all medicinal products available on the market are safe, effective and of high quality. Pharmaceutical approval and market authorization of medicines for humans and animals require manufacturers to meet official quality standards. This is controlled by different regulatory agencies and committees. The standards that manufacturers have to meet are defined and published in the pharmacopoeia. The pharmacopoeia lists all tests to be carried out on medicines, intermediates and raw materials, and is legally binding for a country or all member states of a union.

	Europe[26]	UK	USA	Japan	China	Brazil	Argentina
Health Authority	European Medicines Agency (EMA)	Medicines and Healthcare Products Regulatory Agency (MHRA)	Food and Drug Administration (FDA)	inistry of Health, Labour and Welfare (MHLW)	Ministry of Health (MOH)	Brazilian Ministry of Health	Argentine Ministry of Health
Regulatory Agency for Pharmaceutical Approval	Committee for Medicinal products for Human Use (CHMP)	National Approval by MHRA Centralized Approval by EMA	Food and Drug Administration (FDA)	Pharmaceutical and Medical Devices Agency (PMDA)	Chinese Food and Drug Administration (CFDA)	National Sanitary Surveillance Agency (ANVISA)	Drug Regulatory Authority (ANMAT)
Publisher Pharmacopoeia	European Directorate for the Quality of Medicines (EDQM)	British Pharmacopoeia Commission	US Pharmacopeial Convention	Pharmaceutical and Medical Devices Agency (PMDA)	Chinese Pharmcopoeia Commission (ChPC)	National Sanitary Surveillance Agency (ANVISA)	Drug Regulatory Authority (ANMAT)
Pharmacopoeia	European Pharmacopoeia (EP)[27]	British Pharmacopoeia[28]	United States Pharmacopoeia (USP)[29]	Japanese Pharmacopoeia (JP)[30]	Chinese Pharmacopoeia (ChP)[31]	Brazilian Pharmacopoeia [32]	Argentine Pharmacopoeia [33]
NAT Acceptance for Mycoplasma Testing	✓	✓	✓	✓	?	Not described	Not described
Specification of NAT Validation Requirements	✓	✓		✓**	?	✗	✗

T03
Overview of the health authorities in the EU, the USA, Japan, China, Brazil and Argentina, their regulatory agencies, legally binding documents (pharmacopoeias), NAT acceptance for regulatory mycoplasma testing, and specification of NAT validation requirements.
* The USP mentions "by a procedure demonstrated to be comparable"
** comparable to EP 2.6.7.

The situation is very different for rapid mycoplasma testing methods, like NAT. Even though many national pharmacopoeias mention NAT as a valid mycoplasma testing method, there is little harmonization across countries regarding protocols or validation requirements. Some pharmacopoeias such as the EP and JP mention detailed validation guidelines, whereas others merely point out that NAT is a valid testing method after validation. However, all countries require an appropriate validation and comparison with conventional mycoplasma testing methods. The EP provides the most detailed NAT validation guideline of all pharmacopoeias in its chapter 2.6.7. Four requirements must be met by NAT-based mycoplasma testing methods: Limit of Detection, Specificity, Robustness and Comparability (Table 04). For full and generic validation of a NAT method, it is advisable to include additional parameters such as precision and crosscontamination.

Validation Requirements	EP 2.6.7.
Limit of Detection (LOD)	To define the detection limit, a positive cut-off point should be determined for each species (the chapter provides a list of mycoplasma species to be used as test organisms). For each strain, a minimum of three independent 10-fold dilution series should be tested, with a sufficient number of replicates at each dilution to give a total number of 24 test results for each dilution. The positive cut-off point is defined as the concentration of mycoplasmas that can be detected in 95 percent of test runs, thus in at least 23 test results.
Specificity	It is important to use PCR primers that are specific for a wide range of mycoplasmas. However, it is likely that PCR primers will also detect other bacterial species. This potential cross-detection should be documented by testing related bacterial genera such as gram-positive bacteria with close phylogenetic relation to mycoplasmas (the chapter provides a list of bacterial genera to be tested)
Robustness	The measure of the NAT method's capacity to remain unaffected by small but deliberate variations in method parameters and test method modifications needs to be demonstrated. The chapter provides examples of variations and test modifications that may be tested.
Comparability	The comparability should include a comparison of the LODs between NAT and the compendial methods. The chapter defines the following acceptance criteria: 1) Culture Method replacement by NAT: A detection limit of ≤ 10 CFU/ml needs to be demonstrated. 2) Indicator Cell Culture Method replacement by NAT: A detection limit of at least ≤100 CFU/ml needs to be demonstrated for each mycoplasma test species. 3) In both cases the NAT alternative method needs to be performed in parallel to both conventional methods to evaluate simultaneously the LOD of both methods using the same samples of CFU-calibrated mycoplasma test strains.

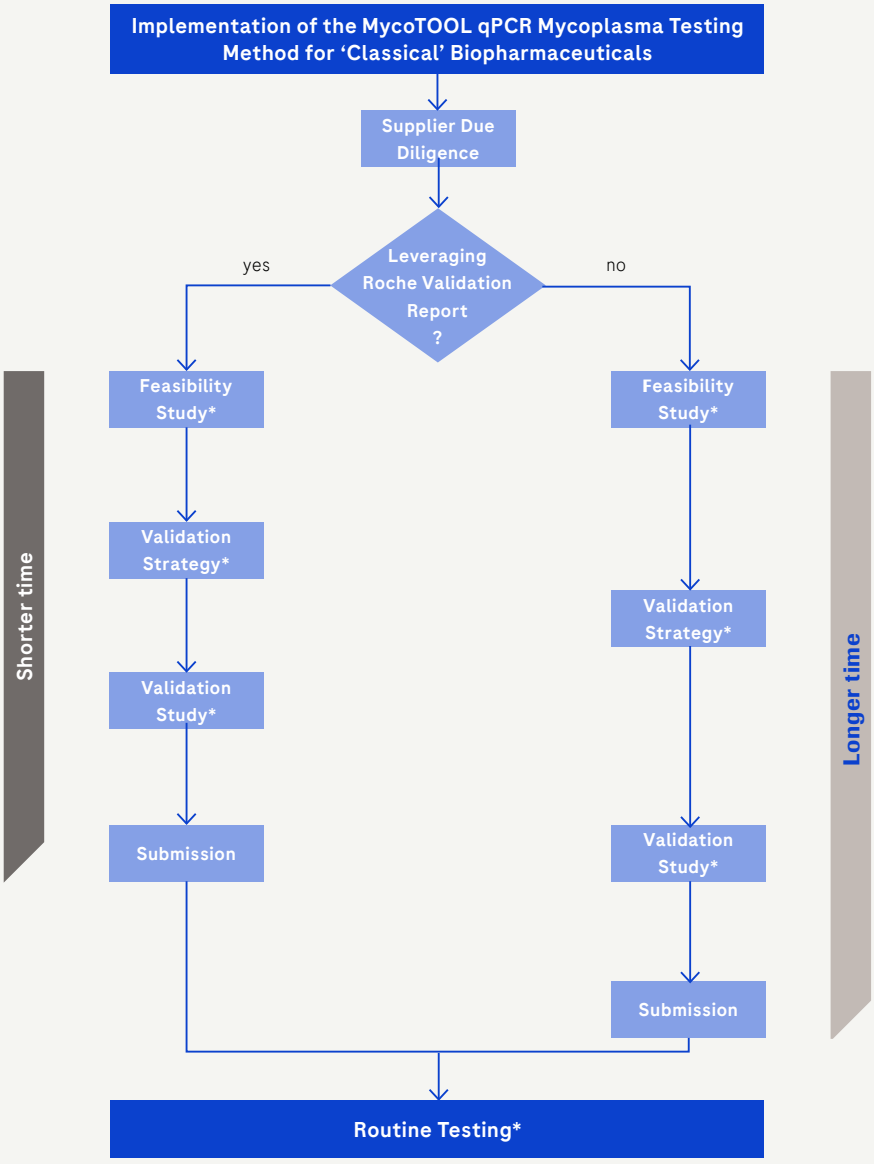
T04
Summary of validation requirements as detailed in EP 2.6.7. For more detailed information, refer to the EP mycoplasma NAT validation guideline directly.

There are other non-legally binding documents that may be considered as a reference for implementation of NAT mycoplasma testing methods:

(i) The Parenteral Drug Association (PDA) published a Technical Report named "Alternative Methods for Mycoplasma Testing" in 2010. It guides new users of rapid mycoplasma tests and describes assay procedure, assay validation, and potential applications for alternative mycoplasma testing methods.[34]
(ii) Roche Pharma uses the MycoTOOL qPCR assay to release new products to the market. The assay has been validated according to EP chapter 2.6.7., and the generic validation report is available on request under confidential disclosure agreement. A summary of the study design and results is available under http://go.roche.com/MycoTOOLqPCR. (iii) In addition to the USP, theme-specific guidelines are published by FDA departments. The CBER of the FDA published a guideline in 1993 called "Points to Consider in the Characterization of Cell Lines Used to Produce Biologicals" (PTC), and a "Guidance for Industry" in 2010 that provide additional information on mycoplasma testing. Neither is legally binding.[35, 36] (iv) Bioanalytical Method Validation is another non-legally binding document published by the FDA that helps gain an overview of best practices to validate an alternative method for mycoplasma testing.[37]

Step-by-Step Validation and Implementation of MycoTOOL qPCR

This section provides an overview of the processing steps and timelines to be considered in the product-specific validation and implementation of the MycoTOOL qPCR method for rapid mycoplasma testing of 'classical' biopharmaceuticals. Usually, a mycoplasma qPCR implementation project includes five steps: the supplier due diligence, the feasibility study, the development of a validation strategy, the performance of the validation study, and the submission. MycoTOOL qPCR is a method generically validated by Roche Pharma. The validation report that is available on request can be leveraged to save time during the implementation procedure. The complete workflow is depicted in Fig. 10 and described in detail below. For ATMP, a simplified validation approach that uses the entire panel of product-relevant mycoplasma species as test organisms and includes experimentally verified spikes at the regulatory required LOD of ≤ 10 CFU/mL might be sufficient.



*This service can be outsourced to a suitable CRO

10
Flowchart for the implementation of MycoTOOL qPCR. The left hand side of the flowchart depicts validation carried out by leveraging Roche's generic validation report (implementation for CHO manufacturing processes). Implementing MycoTOOL qPCR for a non-CHO process or changing the method used in Roche's generic validation requires revalidation of the MycoTOOL qPCR method. This process, presented on the right hand side of the flowchart, is more time consuming.

Supplier and CRO Due Diligence

This step may take several days to a few months. It involves a due diligence of marketed mycoplasma detection kits and CRO to carry out the study. Product specifications must be checked against testing requirements and the capabilities of the mycoplasma detection kit suppliers and CRO have to be assessed. Before moving forward with the validation, several points should be clarified:

- Does the supplier provide appropriate documentation with regards to design and manufacture of instruments and reagents?
- Does the supplier have change control systems in place?
- Does the supplier provide quality and supply agreements if required?
- Does the supplier deliver in-time and reliably?
- Are references of previous validation studies available?
- Do the supplier and CRO respond to questionnaires and allow physical audits at their testing facility?
- Does the supplier provide end-user training programs, on-site technical service, installation and operational qualification services, preventive maintenance service, and a technical support hotline?
- Does the CRO have sufficient experience with productspecific NAT validation studies and implementation of NAT-based routine testing?
- Does the CRO provide a validation report, protocols or similar documentation?
- Does the CRO provide solutions for technology transfers?

During this phase, it may also be required to prepare an economic assessment or financial justification (i.e., write a business case) to apply for budget internally. Thus, associated costs, such as one-time costs (requirements for laboratory space, instrumentation costs, installation costs, etc.) and operating costs (maintenance, cost per sample, etc.) should be clarified with the contract service provider during this step.

Roche Pharma Generic Validation Information

Roche Pharma conducted a full generic validation according to EP 2.6.7. (NAT validation guideline) for MycoTOOL qPCR with defined instrumentation (LightCycler® 480 Instrument II and MagNA Pure 96, see Figure 10) and specifically for processes using Chinese hamster ovary (CHO) cell lines. Typically, regulatory agencies such as FDA or EMA do not require users to fully revalidate MycoTOOL qPCR, as long as the main process, including instrumentation, is unchanged. Thus, a product-specific validation study using the unchanged workflow will require testing of fewer samples and hence less time. However, this option should be discussed with the relevant regulatory agency before taking a decision.

This validation information is available on request and under confidential disclosure agreement. Please contact your local CustomBiotech representatives.

(https://go.roche.com/cbcontact)

Feasibility Study
This step may take from one week to several months. It may be accomplished by using rental equipment on site or, more efficiently, by collaborating with a CRO who conducts the study. A feasibility study is a technical proof-of-concept testing prior to purchasing instrumentation and prior to investing in a validation study. It is done to uncover any technical incompatibilities between MycoTOOL qPCR and the product material intended to be tested. Since MycoTOOL qPCR was validated using CHO cells at a concentration of $5x10^6$ cells/ml, other cell lines and concentrations must be tested to examine product matrix effects, such as PCR inhibition. Another goal of the feasibility study is to assess if the required LOD can be achieved, and what adjustments to the MycoTOOL qPCR test protocol can help reach the target LOD. Such adjustments can be a larger PCR reaction volume or an increase in PCR cycles.

Validation Study
This step may take from two to several months. The validation study demonstrates that MycoTOOL qPCR is capable of consistently detecting mycoplasmas according to regulatory requirements. It may be performed internally using the respective instrumentation, or it may be outsourced to a CRO. The CRO executes the experiments defined during the validation strategy, and documents evidence in a validation report.

Validation Strategy
This step may take from a few weeks to several months. The validation strategy provides a roadmap for all experiments that will be conducted during the MycoTOOL qPCR validation study (e.g., LOD testing, robustness testing, specificity analysis). The strategy includes a timeline, responsible parties, planned experiments, and relevant acceptance criteria. The generic validation conducted by Roche can be taken into account. Usually, a few criteria of the generic validation must be re-confirmed (such as the LOD for a number of selected regulatory mandatory and product-relevant mycoplasma reference strains). Generally, using the generic validation report as basis for a validation study reduces the number of samples to be tested and the overall cost of the study. It is important to always discuss the strategy with the respective regulatory agency, especially if the Roche generic validation may streamline your project.

Submission & Routine Testing
After successful submission and approval of the validation report by the regulatory authority, routine testing may be carried out. Depending on the customer and the project, routine testing may be carried out by internal quality control experts or the complete process remains outsourced to a CRO.

Summary and Outlook

Automated NAT-based rapid methods for mycoplasma testing offer revolutionary opportunities and competitive advantages for the biopharmaceutical and cell therapy industry. Latest sample preparation and mycoplasma detection kit improvements and recent advances addressing product-specific validation challenges to meet the regulatory requirements will certainly push these new test systems towards being the superior long term alternative in comparison to compendial culture methods. Based on recent developments and trends, it is expected that in the next few years regulatory bodies like the EMA and the US FDA will increasingly approve routine mycoplasma testing of biopharmaceuticals and ATMP by rapid NAT-based methods, using commercial tools such as the Roche CustomBiotech MycoTOOL qPCR assay in combination with the Roche MagNA Pure Sample Preparation System. This guideline gives a general overview and specific recommendations on how to validate and implement MycoTOOL qPCR as a rapid automated NAT-based mycoplasma testing method for in-process control and lot release of biopharmaceutical products. For ATMP, risk assessments are recommended as basis for an appropriate product-specific MycoTOOL qPCR validation design to meet the regulatory requirements and risk-based mycoplasma safety concepts.

Current users of MycoTOOL qPCR appreciate the benefits of this highly automated analytical system. Easy handling, rapid workflow for same-day results, high-throughput sample processing, cost effectiveness, lack of product matrix and PCR inhibition effects even with “worst case products” of high cell densities, and the ability to test cell-free samples are convincing advantages of the system. Straightforward validation designs for this automated mycoplasma testing system can be individually tailored for any biopharmaceutical product and ATMP. The implementation of MycoTOOL qPCR as an early warning system for raw material testing, in-process control and for final lot release increases efficiency and product quality by reliably excluding the risks of mycoplasma contaminations during manufacturing stages.

A rise in the application of automated NAT-based MycoTOOL qPCR method for routine mycoplasma testing can be expected in the future as it has significant advantages compared to compendial culture methods, and customized product-specific validation plans are available from experienced experts and CRO. This will ultimately lead to a change in quality control and lot release standards throughout the biopharmaceutical and cell therapy industry and will certainly impact the market.

Glossary

Abbreviation	Definition
ADP	Adenosine diphosphate
ANMAT	Drug Regulatory Authority (Argentina)
ANVISA	National Sanitary Surveillance Agency (Brazil)
ATMP	Advanced Therapy Medicinal Product/s
ATP	Adenosine triphosphate
CBER	Center for Biologics Evaluation and Research (US FDA)
CDER	Center for Drug Evaluation and Research (US FDA)
CFU	Colony forming unit/s
CHO	Chinese hamster ovary (cells)
ChP	Chinese Pharmacopoeia
ChPC	Chinese Pharmacopoeia Commission
Cq	Threshold cycle
CRO	Contract Research Organization/s
CFDA	Chinese (State) FDA
CVM	Center for Veterinary Medicine (US FDA)
DNA	Deoxyribonucleic acid
dsDNA	Double-stranded DNA
EDQM	European Directorate for the Quality of Medicines
ELISA	Enzyme-linked immunosorbent assay
EMA	European Medicines Agency
EP	European Pharmacopoeia
EU	European Union
FDA	US Food and Drug Administration
G+C	Guanine-cytosine content
GMP	Good manufacturing practice

Abbreviation	Definition
HEK	Human embryonic kidney
ICH	International Committee on Harmonization
JP	Japanese Pharmacopoeia
LOD	Limit of detection
MHLW	Japanese Ministry of Health, Labor and Welfare
MHRA	Medicines and Healthcare Products Regulatory Agency (UK)
MOH	Chinese Ministry of Health
MycoTOOL qPCR	MycoTOOL Mycoplasma Real-Time PCR Assay/Kit
NAT	Nucleic acid amplification technique/s
PBS	Phosphate-buffered saline
PCR	Polymerase chain reaction
PDA	Parenteral Drug Association
PM DA	Pharmaceutical and Medical Devices Agency
PTC	Points to Consider (FDA/CBER Guideline 1993)
QC	Quality control
qPCR	Quantitative Real-time PCR
RC	Recovery control DNA
RNA	Ribonucleic acid
SC	Small colony type
ssDNA	Single-stranded DNA
TD-PCR	Touchdown PCR
UK	United Kingdom
US	United States (of America)
USP	United States Pharmacopeia

Appendix

Kits	Pack Size	Material No.	Description
MycoTOOL Mycoplasma Real-Time PCR Kit	1 kit (160 PCR reactions)	06495605001	For the testing of cell culture samples for the absence of mycoplasma with qPCR. MycoTOOL Real-Time PCR Kit makes Mycoplasma testing fast, easy and reliable.
MycoTOOL Mycoplasma Detection Amplification Kit	1 kit	05184240001	For the testing of cell culture samples for the absence of mycoplasma with PCR.
QC Sample Preparation Kit	1 kit	08146829001	The Kit is designed to manually extract and purify nucleic acids to be used with the MycoTOOL Mycoplasma Real-Time PCR Kit or MycoTOOL PCR Mycoplasma Detection Amplification Kit. The kit includes protocols for normal and high cell density cell culture samples. Additionally it can also be used for the Residual DNA CHO Kit and Residual DNA *E. coli* Kit. The kit does not include Triton.
MycoTOOL Control Plasmid	10 ng	05196132103	Plasmid used for validation of mycoplasma test method using MycoTOOL test.
Residual DNA *E. coli* Kit	1 kit (96 reactions)	07728735001	For the testing of cell culture samples for the absence of residual host cell DNA from *E. coli* bacteria.
Residual DNA CHO Kit	1 kit (96 reactions)	07427689001	For the testing of cell culture samples for the absence of residual Host Cell DNA from CHO cells.

Instruments	Pack Size	Material No.	Description
MagNA Pure 96 Instrument	1 instrument, control unit and accessories	06541089001	High-throughput robotic workstation for fully automated purification of nucleic acids from up to 96 samples.
MagNA Pure 24 Instrument	1 instrument, built-in control unit, and accessories	07290519001	High-throughput robotic workstation for fully automated purification of nucleic acids from up to 24 samples.
LightCycler® 480 Instrument II	1 instrument (96-well)	05015278001	Rapid high-throughput, plate-based Real-Time PCR amplification and detection instrument.

Regulatory Disclaimer
For use in quality control/manufacturing process only.
LightCycler® 480 Instrument II: For life science research only.
Not for use in diagnostic procedures.
MagNA Pure Systems: For *in vitro* diagnostics use.

References

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