CELL CULTURE MYCOPLASMAS

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INTRODUCTION

Cell cultures have become indispensable tools for biological and medical research; In industrial biotechnology they are increasingly used for the production of biologically active pharmaceuticals. Any handling of cell cultures always poses the risk of contaminations, either with eukaryotic cells from other cell cultures or, more frequently, with microbiological organisms including fungi, yeasts, and bacteria. Concerning bacterial infections, mycoplasma contaminations are of particular importance, because they do not conspicuously overgrow the human or animal cell cultures and can only be detected applying special assays. Additionally, they are resistant to many commonly used antibiotics. Thus, contaminated cell cultures accumulated over the past decades and had lead to an average infection rate of ca. 25% of cell cultures all over the world. According to our experience there is a tremendous variation between independent cell culture laboratories and it appears that often either all cell cultures of a laboratory are infected with the same mycoplasma species or none at all. This indicates that a contaminated cell cultures might represent the main source of infection (1-4). As many cell lines are interchanged among laboratories, there is a constant danger of importing mycoplasma with new cell lines and spreading them among the mycoplasma-free cell lines.

Accumulated evidence indicates that mycoplasma infections are intimately connected with the cell culture techniques applied in the different laboratories. Therefore, not only the rigorous testing for contaminations and eradication of mycoplasma is important, but also the prevention of mycoplasma infections within the possibilities of routine cell culture is of utmost importance to prevent spread of infections. In this article we will discuss the incidence and sources of mycoplasma contaminations, the species most commonly detected in cell cultures, the effects of mycoplasma on the function and activity of infected cells, various detection assays with special consideration of the most reliable methods, and the elimination of mycoplasma contaminations from cell cultures with particular emphasis on antibiotic treatment. For information on the systematic and biology of the individual species we refer to the sections addressing these specific topics and the mycoplasma species in combination with their natural hosts.

PREVALENCE OF MYCOPLASMA CONTAMINATIONS

To investigate the effects of mycoplasma on eukaryotic cells, in 1956 Robinson et al. infected their cell cultures with mycoplasma. During this study, they found that the

uninoculated original cell cultures were already contaminated with mycoplasma. This was the first report on the detection of mycoplasma in cell cultures (5). In the aftermath, many human and animal cell cultures all over the world were found to be contaminated with mycoplasma. Some extensive studies in the United States during the 1960s to 1980s, investigating thousands of samples, resulted in a prevalence of about 15% of infected cell cultures. The studies included not only continuous cell lines, but also primary and short term cultures. Studies in other countries found similar or even higher prevalences of contamination. Some investigators determined an infection rate of more than 80% (6). One of the reasons for the diverging values for infections is the simultaneous investigation of primary, early passage, and continuous cell cultures. Usually, primary and early passage cultures are less frequently contaminated than continuous cell cultures. As shown in Table 1, the prevalences are ca. 1% for primary cultures (1), 5% for early passage cultures, and lie in the range of 15% to 35% for continuous cell cultures (7). This increase of infections with the number of passages indicates that the contaminations usually do not originate from the donor of the cells, but are introduced during cell propagation.

This notion is substantiated by the finding that mycoplasmas from different hosts are found in continuous cell cultures. Whereas many of the species specific mycoplasma strains can be detected in primary cell cultures, cultures of later passages contain mycoplasma species which are naturally not associated with the donor species. Although more than 20 different species were isolated from cell cultures, by far the majority of contaminations is caused by only half a dozen mycoplasma species: *M. arginini*, *M. fermentans*, *M. hominis*, *M. hyorhinis*, *M. orale*, and *Acholeplasma laidlawii*. These mycoplasma species account for more than 95% of all infections of continuous cell lines (Table 1). Similar to the overall contaminations, the individual percentages of these six species vary strongly between the different studies (8). The unequal distribution of the mycoplasma species indicates that the virulence of the different species might be diverse, and that certain culture conditions might be optimal for the above mentioned mycoplasma species. The latter finding and the limited number of multiple infections (~10%) might also suggest that an unknown interaction between the different mycoplasma species and/or the host cells exists which is independent from the host cell species. The close interaction between mycoplasma and host cells is further supported by the finding that the titers and effects seen with different mycoplasma species and different host cells are highly diverse.

Although mycoplasmas are found in or on almost all organisms as natural hosts, nothing has been yet published on the infection of plant cell cultures. This apparent discrepancy might be due to the different tissues used for the establishment of the cell lines, because in plant cell

culture only the calli of the plants are used. Furthermore, the media for the propagation of the plant cell cultures are clearly chemically defined and usually no extracts of plants or other organisms are used as supplements.

Table 1: Prevalence, Most Common Species, and Sources of Mycoplasma Contamination in Cell Cultures

Prevalence	
15-35%	continuous cell lines
5%	early passage cell cultures
1%	primary cell cultures
Most common species	
20-40%	*M. orale* (human)
10-40%	*M. hyorhinis* (swine)
20-30%	*M. arginini* (bovine)
10-20%	*M. fermentans* (human)
10-20%	*M. hominis* (human)
5-20%	*A. laidlawii* (bovine)
Sources	
Cross-contamination from infected cultures (most common source)	
Laboratory personnel	
Culture reagents (e.g. bovine serum)	
Original tissue isolate (<1%)	

The exact source of the mycoplasma infections is not fully understood, because mycoplasmas are almost ubiquitously prevalent in or on most organisms. Most likely, a number of different sources is responsible for the contaminations with mycoplasmas, because most of the predominant mycoplasma species in cell cultures are usually associated with human, bovine or swine. The human species *M. orale*, *M. fermentans*, and *M. hominis* account for more than half of all mycoplasma infections and are found physiologically in the human oropharyngeal tract. *M. orale* is with 20 – 40% of all mycoplasma infections the most common contaminant. These contaminants indicate that the mycoplasma cells are transfered from the technician to the cell culture. Another group of frequent mycoplasmas in cell cultures originate from bovine: *M. arginini* and *A. laidlawii*. The

source for these species which account for about 40% seems to be the fetal or newborn bovine serum (FBS, NBS). FBS and NBS is collected in slaughterhouses and an undetected contamination with mycoplasma is likely. Nowadays, the FBS and NBS lots are commonly stringently tested for mycoplasma contaminations. But this was not performed more than a decade ago; furthermore it cannot be ruled out that low mycoplasma titers in huge lots of FBS/NBS remain undisclosedwhen relatively small aliquots are tested.

Investigating many cell cultures from different laboratories all over the world, we found that in laboratories with contaminated cells, most or all cultures from this laboratory are positive and infected with the same mycoplasma strain. Additionally, we found more than 15% of leukemia-lymphoma cell cultures to be cross-contaminated with other cell cultures or to be false cell lines (9). We suggest the same reason for both types of contaminations. Thus, mycoplasma infected cell cultures are themselves the single most important source for further spreading of the contamination (Table 1). As mycoplasmas are transmitted by droplets it is most likely, that inadequate cell culture technique leads to spreading by using laboratory equipment, media, or reagents that have been contaminated by previous use in processing mycoplasma-infected cells. Some relevant steps to prevent contamination of cell cultures have been summarized elsewhere (7).

Another source for contaminations may be the liquid nitrogen, where the cells are stored. Mycoplasmas were shown to survive in liquid nitrogen even without cryopreservation. Once introduced into the nitrogen, mycoplasmas may persist in the tank for an indefinite time, not proliferating, but being able to contaminate cell cultures stored in the liquid phase of the nitrogen. Although we estimate the probability of such a contamination rather low and we never realized a de novo contamination after storage in liquid nitrogen, we recommend storing the ampoules in the gaseous phase of the nitrogen to precautionary avoid contamination.

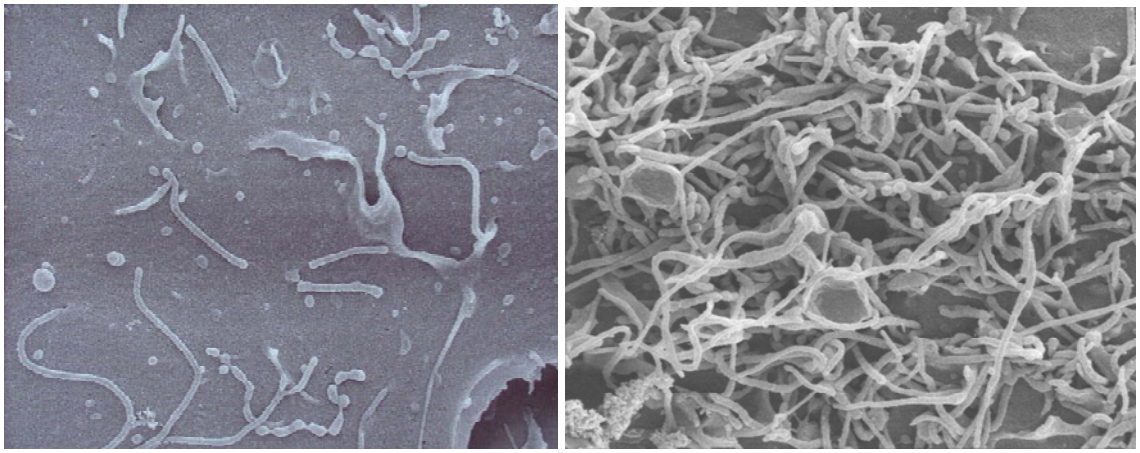
EFFECTS OF MYCOPLASMA CONTAMINATIONS

The contamination of cell cultures with mycoplasma cannot be regarded as a harmless infection with commensalic organisms that has no influence on the eukaryotic cells or on experimental results. Figure 1 documents the infection of a cell culture with mycoplasma to demonstrate the appearance and the possible intensity of the infection. The most remarkable effect, though only in relatively few cases observed, is the loss of the cell culture due to overwhelming growth by the microorganisms and irreversible deterioration of the eukaryotic

cells. Until now, no consistent effects which can be observed throughout all contaminated cell cultures were described. The manifestation of the effects can be quite variable and does not affect the various cells in the same manner and to the same degree, but rather depends on the mycoplasma species, the cell line and the culture conditions. However, a multitude of effects were described for infected cell cultures and a variety shall be mentioned in the following.

Figure 1: HELA cell line infected with *M. fermentans.* Scanning electron micrograph of critical point-dried cell cultures infected with infected cell line grown on coverslips. Note the impressive penetration of the mycoplasma cells into the eukaryotic cell surface (A) and the huge number of agglomerated spaghetti-like mycoplasma cells in certain areas of the eukaryotic cell surface (B). Original magnification 10,000x. (Micrographs by courtesy of Dr. M. Rohde, GBF – German Research Centre for Biotechnology, Braunschweig, Germany)

A **B**



One of the main reasons for the more or less severe cytopathic effects on cell cultures is the consumption of nutrients and basic components of the cellular metabolism, e.g. nucleic acid precursors, amino acids, vitamins, lipids, cholesterole etc. by the mycoplasmas. Due to their low metabolic capabilities, their unefficient energy gain, and the high number of mycoplasmas in the cell culture, those compounds can be used up rapidly. The non-oxidative degradation of the compounds also leads to an alteration of the pH value in the culture medium. The pH can be decreased by the formation of acids by mycoplasmas using the fermentative metabolic pathways. On the other hand, arginine-hydrolyzing mycoplasma (e.g. *M. arginini*, *M. hominis*) can increase the pH value due to the production of ammonia, which is also a highly toxic agent inhibiting cell growth. Additionally, activity of mycoplasmal arginine deiminase as well as mycoplasmal uptake and depletion of the growth medium were shown to inhibit cell proliferation and to induce apoptosis in cell lines (10, 11). As visible

effects, the cells show an abnormal growth rate, a decreased viability, adherent cells sometimes detach from the cell culture vessel surface, and granules are formed in the cells. The depletion of arginine might also be a reason for chromosomal aberrations, because this basic amino acid is a major component of the histones in the nucleus.

Another cause of chromosomal and genetic alterations and growth inhibition might be the competition of mycoplasma and eukaryotic cells for nucleic acid precursors. Chromosome breakage, multiple translocation events, and numerical chromosome changes were described in various cell cultures infected with different mycoplasma species (12). Eukaryotic DNAs and RNAs are degraded by exo- and endonucleases, which are produced and exported by mycoplasmas. Sokolova et al. showed for different lymphocyte and epithelial tumor cell lines that inhibition of proliferation and increased cell death, accompanied by DNA fragmentation and the morphological features of apoptosis was caused by mycoplasma infections (13). Similar DNA fragmentation and loss of chromosomal DNA was also observed by Rawadi et al. in *M. fermentans*-infected monocytic cell lines. The cytocidal effect was assigned to a nonlipid-associated protein fraction (14).

One of the nucleotide-transforming enzymes is the uridine phosphorylase which inactivates the artificial bromodeoxyuridine (BrdU). BrdU is toxic for eukaryotic cells and added as thymidine analogue for the selection of cells with a thymidine kinase (TK) defect. Cells with normal TK activity phosphorylate and incorporate BrdU and will die. Cells with a TK defect which are used for cell fusion experiments grow in the presence of BrdU. In the presence of mycoplasmas, BrdU is degraded and the eukaryotic cells survive even though they do not possess a TK defect.

Mycoplasmal proteins alter a number of eukaryotic properties in different manners. Rawadi et al. showed that heat-inactivated mycoplasma particles induced the inflammatory cytokines interleukin 1 (IL-1), IL-6, and tumor necrosis factor in monocytes and THP-1 cells (14). *M. fermentans* also induced IL-10 in human monocytes. The secretion of immunoglobulins was altered in B-cells, as well as the expression of various colony-stimulating activities (e.g. granulocyte-monocyte colony stimulating factor) and the induction of interferon expression (1).

Another example for the detrimental effects of mycoplasma contaminations is the impact on virus propagation in cell cultures. The virus production can be decreased by suppression of metabolism and growth of the cells connected with partially severe cytopathic effects, and arginine depletion by arginine oxidizing mycoplasmas. Decreased yields can be found with

arginine requiring viruses, such as Herpes simplex, vaccinia, adeno-viruses and several others. Increased virus yields can be obtained due to interferon-α inhibition, leading to diminished cell resistance. On the other hand, interferon activity can also be induced or stimulated by mycoplasma infection. For example *Acholeplasma* species lipoglycans have endotoxin-like activities that induce interferon activity leading to resistance against some viruses in vitro or in vivo (1).

The few examples out of the nearly endless array of possible effects of mycoplasma infections on cell cultures can only give a percursory idea of the very complex relationship between mycoplasma and eukaryotic cells. Thus, any experimental result from mycoplasma-infected cell cultures may rise prima vista substantial doubts.

DETECTION OF MYCOPLASMA CONTAMINATION

As seen in the previous chapter, mycoplasma infections of cell cultures can be highly diverse and no universal effect can be observed which may serve as an indicator for a contamination. Thus, special techniques were developed to detect mycoplasma in cell cultures. During the pre-PCR era many methods were developed based on microbiological culture, e.g. staining techniques, electron microscopy, biochemical and immunological tests, and recently some hybridization assays. The various techniques are summarized in Table 2. Many of the assays are relatively elaborate and time consuming, applicable only to a portion of the contaminating mycoplasmas, exhibit a low sensitivity, or the interpretation is subjective and fault-prone, or special equipment is necessary.

One of the first and still one of the officially approved (European Pharmacopeia) (15) assays is the microbiological culture method. In this test, an aliquot of the cell culture supernatant is added to rich liquid mycoplasma medium, cultivated for a few days and subsequently transferred to agar plates with the same medium components. The plates are incubated for up to two weeks aerobically at 37°C. In case of positive samples, typical small colonies (ca. 100 – 400 µm in diameter) often with a “fried eggs” appearance comprising a dense center and a brighter corona appear on the agar plates (see Figure 2). Preparation and components of the media to grow mycoplasma are described in detail elsewhere (8). Applying the described media, the test is sensitive, reliable, and robust for monitoring cell culture contaminations. Nevertheless, some strains of *M. hyorhinis* grow poorly or not at all on those media. We found that a certain number of *M. hyorhinis* strains grow indeed on the media, but in a number of cases the growth is not supported.

Figure 2: Mycoplasma colonies on agar. *A. laidlawii*; original magnification 100x. Note the dense growth and even confluence of colonies indicative of a high mycoplasma titer. The colonies show the tell-tale “fried-egg” appearance.

A second detection method recommended by the European Pharmacopeia (15) is the DNA fluorochrome staining (4´,6-diamidino-2´-phenylindole-dihydrochloride [DAPI] and Hoechst 33258 stain). This assay is relatively easy and rapid to perform (8). But the results are sometimes difficult to interprete and some experience is definitely necessary. Especially when the cell culture is not in a good condition, mis-interpretations are frequent. The sensitivity and specificity of the direct DNA staining procedure can be highly increased by use of indicator cell lines. In this indirect DNA staining method, supernatant from the cell culture to be tested is added to a mycoplasma-free adherent cell culture (e.g. Vero B4, NIH-3T3 or 3T6 cell lines). The cells are grown in vessels containing sterile cover slips. After growth for several days to approximately half-confluency, the cover slips are washed and stained with the fluorochrome. Mycoplasma infections can be detected very efficiently, but again, the test is relatively-time consuming and mycoplasmas are cultured in the laboratory, which may lead to further spread of contaminations.

Nowadays, a number of assays are available, which can detect almost all mycoplasma contaminations within at most two days, including one or more incubation steps over several hours. These techniques are all indirect tests, which determine or visualize mycoplasmal components or enzyme activities. One of the most prevalent assays for the detection of mycoplasma contaminations is the polymerase chain reaction (PCR) technique. The test is easy to perform, sensitive, specific, fast, reliable, and cost effective. Most of the 16S rRNA sequences of mycoplasma are known and can be used to create primers for the amplification of specific DNA fragments. The primer design defines the specificity of the PCR reaction.

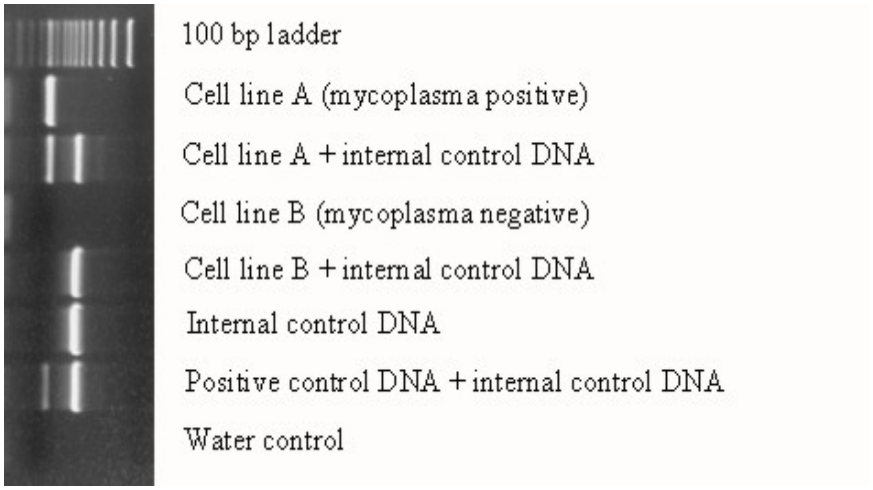
Oligonucleotides from variable 16S rRNA regions are usually specific for a limited number of mycoplasma species. Sequences from the 16S-23S intergenic regions can be used for the detection of single mycoplasma species. For the detection of mycoplasma in cell cultures, the specificity of the primers needs to be broad enough to detect Mycoplasma as well as Acholeplasma species. On the other hand, the specificity should be narrow enough to exclude amplification of sequences from other common bacteria, which might be contaminations of the PCR reagents.

However, some more important general aspects should be considered when performing this technique (16). 1) The sensitivity of the procedure makes it susceptible to contaminations with the target DNA which is present in high amounts after the first amplification of mycoplasma-specific DNA. Therefore, extreme care has to be taken to prevent carry-over of target DNA fragments. This is especially the case when a nested PCR is performed. 2) The PCR should be performed with extracted DNA and not with a crude lysate of the cell culture supernatant, because the cell culture components might contain inhibitors of the Taq polymerase. 3) The use of antibiotics in cell culture should be minimized and the cell cultures should be cultured without antibiotics for several passages or at least two weeks to allow the mycoplasmas to grow to detectable amounts or to ensure that no residual mycoplasmal DNA is left in the culture medium. 4) It is of note that a positive result of the PCR does not necessarily indicate viable contaminants, especially after a mycoplasma elimination procedure using antibiotics against mollicutes. Thus, the PCR method should be properly established and all assays should be performed with the utmost care.

The PCR can be performed with a single round of amplification or as nested PCR with two primer pairs. The second method increases the sensitivity and the specificity. But one of the drawbacks of the nested PCR is the possible generation of false positive results due to contamination with target DNA. For the routine cell culture technology, the PCR is satisfactory to detect mycoplasma contaminations, because the titer of the mycoplasmas in the cell cultures is sufficiently high to be detected by the PCR. Special conditions, e.g. after mycoplasma elimination procedures or for the detection of mycoplasma in cell culture products like FBS, the nested PCR might be of advantage. Another possibility to increase the sensitivity of the assay is to perform a reverse transcription PCR (RT-PCR) to detect ribosomal RNA which is more abundant in the cells than the rRNA-coding DNA. However, the latter option is clearly more labor-intensive. In summary, we would suggest to perform a single PCR with genomic DNA for routine cell culture and to test the cultures frequently for contaminations. Several PCR kits are commercially available, e.g. from ATCC, Minerva Biolabs, Roche, Stratagene, TaKaRa Bio, and detailed descriptions and positive and internal

control DNAs for the establishment of a PCR can be obtained from the DSMZ. A typical gel is shown in Figure 3.

Figure 3: PCR analysis of mycoplasma status in cell lines. Shown is an ethidium bromide-stained gel containing the reaction products following PCR amplification. Two paired PCR reactions were performed: one reaction containing an aliquot of the sample only and the second contained the sample under study plus a control DNA as internal standard. Note that cell line A is specifically positive for mycoplasma and also for the internal control whereas cell line B is specifically negative for mycoplasma being positive in the internal control.



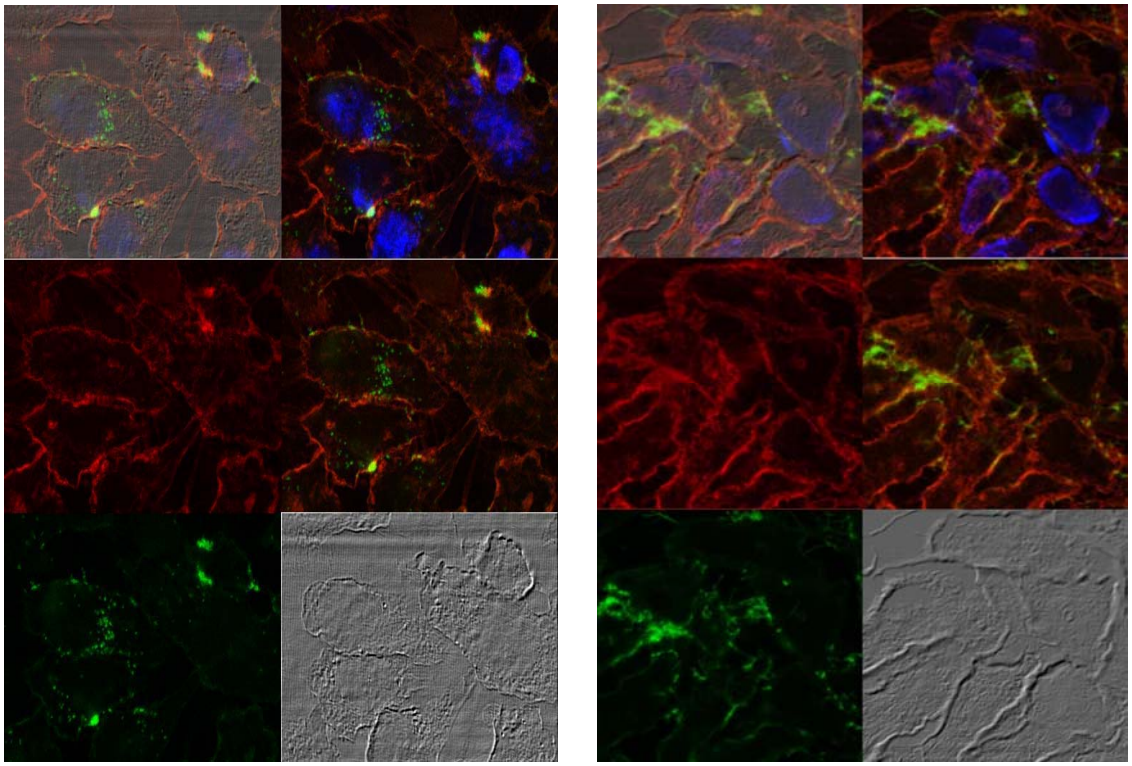
Laboratories that do not have access to a PCR machine need to employ other techniques. Beside the microbiological culture method and DNA fluorochrome staining several other techniques can be applied, some of them are available as kits. ELISA kits are available from Roche (but this assay does not detect *M. fermentans*) and Stratagene; these assays employ antisera or monoclonal antibodies against the different mycoplasma species. DNA-RNA hybridization assays use radioactively or fluorochrome labeled probes (GenProbe, San Diego, CA, USA). The kits are sensitive, specific, and straightforward. Results are obtained within several hours or a couple of days.

There are also newly developped assays based on fluorescence in situ hybridization (FISH) (17) and on ATP generation (Cambrex, UK) detected by fluorescence microscopy and luminometer, respectively. An example of an extended FISH method is shown in Figure 4 to demonstrate that the method can also be used for research purposes. Until now, no published data are available concerning the sensitivity, specificity, and the accuracy of both

assays applied in routine cell culture. But preliminary results are promising concerning the above mentioned parameters and in particular with regard to the speed of the assays. The FISH test takes about two to three hours and results from the luminescence test can be generated within 20 minutes.

Figure 4: Fluorescence in situ hybridization (FISH). FISH (green) combined with membrane staining (red) and nuclei staining (blue) of (a) HELA infected with *M. fermentans*, and (b) HELA infected with *M. orale* applying a confocal laser microscope. Note the localization of the mycoplasmas in the cytoplasma of the eukaryotic cells in (a) and the colocalization of the mycoplasmas and the eukaryotic cell membrane in (b).

a **b**



All described methods may fail when cell cultures are tested which were treated with antibiotics. In general, all treated cell lines should be cultured for at least two weeks without any antibiotics before the cells are retested. Both, false negative as well as false positive results may occur. PCR and other assays depending on the determination of DNA or RNA can produce false positive results, because residual DNA or RNA is detected, in the absence of viable mycoplasmas. False negative results are produced when the titers of the mycoplasmas are below the detection levels of the assays.

We recommend to perform two or even three independent assays for the detection of mycoplasma in cell lines which newly arrive in the laboratory. The cells should be kept isolated in a quarantine laboratory until all tests show that the cells are free from mycoplasma, if possible at all. During continuous culture one sensitive assay should be performed regularly to monitor the cell cultures.

Table 2: Selected Methods for Mycoplasma Detection

Microbiological culture	
	Growth in liquid medium
	Formation of typical small colonies on agar
Electron microscopy	
Biochemical assays	
	Detection of adenosine phosphorylase activity (6-MPDR assay)
	Enzymatic conversion of ADP to ATP detected by luciferase
	Chromatographic detection of conversion of radioactively labeled uridine to uracil by mycoplasmal uridine phosphorylase
Immunological assays	
	Immunofluorescence
	Enzyme linked immunosorbent assay (ELISA)
Molecular biological assays	
	Liquid hybridization assay
	Autoradiography (dot-blot) with mycoplasma specific probes
	Polymerase chain reaction (PCR), reverse transcription PCR
	PCR-ELISA
Microscopic detection assays	
	Direct DNA fluorescent staining (DAPI, Hoechst 33258)
	Indirect DNA fluorescent staining with indicator cell line
	Fluorescent in situ hybridization

ERADICATION OF MYCOPLASMA CONTAMINATION

As mentioned above, mycoplasmas cannot be regarded as harmless bystander organisms in cell cultures. Thus, the best way to get rid of the infections is to autoclave the culture and to replace it with a new and uncontaminated culture. Unfortunately, the contaminated cell

culture may often be unique in some regards and may not be replaceable. In these cases, the mycoplasmas have to be eliminated without affecting the eukaryotic cells. Over the years, a number of elimination methods had been developped, applying physical, chemical, immunological and chemotherapeutic treatments. The treatments are not restricted to cell cultures only, but also for surfaces, cell culture media and supplements. Methods include heat treatment, filtration, exposure to detergents, culture in the presence of 6-methylpurine deoxyriboside, passage through nude mice, antibiotic treatment, and others (18). Regarding the treatment of cell cultures, many of the methods are laborious or not efficient. Additionally, some of the elimination methods had been investigated only in experimentally infected cell cultures. This might not necessarily reflect the complex nature of a chronically infected culture and the occurrence of intracellular mycoplasma also has to be considered. From our experience, treatment with several specific anti-mycoplasma antibiotics is the method of choice for infected cell cultures. Usually, the antibiotics are also active or even might be accumulated in the eukaryotic cells (19).

As mycoplasmas are very unusual bacteria in many respects, this is manifested also in the susceptibility against chemotherapeutic agents. Many of the commonly applied antibiotics are not effective against mycoplasma, due to the lack of the antibiotic target (e.g. penicillins, streptomycin, etc.). On the other hand, although not killing the mycoplasmas, some antibiotics might suppress their growth and thus mask the presence of the infectants. Beside the enforcement of strictly sterile cell culture technique and the development of resistances, this is one reason not to apply antibiotics prophylactically in routine cell culture.

Until now, three groups of agents were shown to be highly active against mycoplasmas: macrolides, tetracyclines, and quinolones (Table 3). Macrolides and tetracyclines both inhibit protein synthesis, but bind to different subunits of the ribosomes. The quinolones (also named fluoroquinolones) inhibit the bacterial gyrase, an enzyme which is essential for the DNA replication. Our own data show that several antibiotics from these groups can be applied in single or combination treatments (20). The quinolones tested in cell cultures are: ciprofloxacin (brand name Ciprobay 100, Bayer, Germany), enrofloxacin (Baytril, Bayer), sparfloxacin (Aventis Pharma, Ireland), and an unpublished quinolone reagent available as Mycoplasma Removal Agent (MRA, ICN, Eschwege, Germany). The macrolide Tiamulin and the tetracycline Minocycline are available as BM-Cyclin from Roche (Mannheim, Germany) and are applied subsequently in one treatment.

Table 3: Effective anti-mycoplasma antibiotics

Brand name	Generic name	Antibiotic category
BM-Cyclin	Tiamulin Minocycline	Macrolide Tetracycline
Ciprobay	Ciprofloxacin	Fluoroquinolone
Baytril	Enrofloxacin	Fluoroquinolone
Zagam	Sparfloxacin	Fluoroquinolone
MRA		Fluoroquinolone
Plasmocin		Tetracycline Fluoroquinolone

In our hands the curation efficiency of the antibiotic approaches varied between 66 and 85%, depending on the antibiotic used. But these numbers do not only reflect the killing of the mycoplasmas, but also include the loss of the culture, due to growth inhibition of the eukaryotic cells. The loss of cultures is frequently seen when the cells are heavily infected and already in a very bad condition (3-11% of treated cultures, depending on the antibiotic). In these cases the antibiotics might be the last hit to kill the eukaryotic cells. On the other hand, resistances against one antibiotic (7-24% of treated cultures, depending on the antibiotic) can be overcome by application of antibiotics from another group. Another combination product developed for the eradication of mycoplasma from cell cultures is Plasmocin (InvivoGen, San Diego, USA). It contains an unpublished antibiotic against protein synthesis (presumably one of the above mentioned) and a quinolone, which are used simultaneously. No published data are available for this treatment until now.

Pretreatment of heavily infected cultures with other methods, e.g. exposure to hyperimmune antimycoplasma serum, coculture with macrophages, or washing the cells with surfactin-containing solutions, might be helpful, because the bulk of the mycoplasmas can be eliminated.

The more recently developped membrane-active peptides, e.g. alamethicin, dermaseptin B2, gramicidin S, and surfactin, are highly efficient in pure mycoplasma cultures, but in the

presence of serum, the activities are decreased. Thus, the concentrations and treatment times required for the elimination of mycoplasmas from cell cultures are toxic to the eukaryotic cells (21).

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