Frequently asked questions (FAQ) about various products

WaterShield™

We have a problem with our incubator. Microbes keep growing inside it. Could you suggest how to clean it and control this problem?

1. Remove all racks and trays and clean them for ex. with a dishwasher.
2. Switch off the incubator and let it cool down. Spray abundantly the interior of the incubator with Mycoplasma Off™ or wipe it off with Mycoplasma Off™ Wipes. Let it air dry. Keep the door open for evaporation preferably overnight or for at least 1 hour. If your incubator is equipped with a heat inactivation procedure, start this procedure instead. Keep the racks and trays inside. Do not forget to remove the CO_2 sensor if necessary.
3. If your incubator is equipped with a copper inlay, do not polish the interior. Copper oxide is bacteriostatic. Polishing will remove this bacteriostatic coating.
4. Reload the incubator. Use sterile aqua dest. for the pan and add WaterShield™.
5. For general operation, use plastic trays underneath your culture flasks to avoid spills during loading/unloading.
6. Use culture flasks with vent filters and keep the caps tight instead of standard flasks with open caps.

According to your datasheet, the treated water is safe for up to 4 weeks. Could you provide a data which shows that the treated water is safe for 4 weeks?

Yes, our data clearly show that antibiotic action of WaterShield™ (fungi and bacteria) is maintained for at least 4 weeks from addition to the incubator water pan. All data are summarized in a Technical Note, which can be downloaded from the product webpage.

You claim that WaterShield™ supplemented to the water pans of cell culture incubators is safe for cells because its ingredients do not evaporate. Could you provide data showing that WaterShield™ in incubators is not toxic to cells?

- Yes, our data demonstrate the lack of cytotoxic effects on cells grown in incubators containing WaterShield™
- All data are summarized in a Technical Note, which can be downloaded from the product webpage.

ZellShield®

Does ZellShield® contain some aminoglycosides?

The following ingredients are included in the product ZellShield®: Macrolide and polyene, lincosamide and fluoroquinolones.

What is the difference between ZellShield® and Mynox® and Mynox® Gold reagents? We have a cell culture infected with mycoplasma and would like to know which product best treats mycoplasma-contaminated cells?

ZellShield® cannot be used for curing an already contaminated cell line. You need to treat your cells with Mynox® or Mynox® Gold. If your cells are clean or you harvest cells from natural sources with a high risk of contamination (environment, students media), ZellShield® should be added to the medium to prevent mycoplasma growth.

Mycoplasma Off™

According to your website, Mycoplasma Off™ and Mycoplasma Off™ Wipes are effective against some kinds of viruses as well as mycoplasma. Which viruses can be inactivated?

Yes, Mycoplasma Off™ is active against naked viruses and also spores. The ingredient glutaraldehyde is responsible for removal of the spores and naked virus as well as of fungi. Surface Disinfection with concentrated product:
Hospital prophylaxis: 1 min. virucidal according to RKI, BG 01/2004 incl. HBV/HCV/HIV: 30 s.virucidal: 30 min. adenovirus: 5 min. polyomavirus (SV 40): 15 min

Can we use Mycoplasma Off™ Wipes for cleaning laminar air flow hood and CO_2 incubator surfaces (shelves, interior etc)?

Yes! For this purpose, you can use also the Mycoplasma Off™ spray. Both products are alcohol-based, non-corrosive and non-carcinogenic but should not be used on hard, nonporous surfaces or on alcohol sensitive surfaces like acrylic and fiberglass. Just make sure to do the cleaning (especially when using the spray) only after your cell samples have been removed from the incubator.

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Is Mycoplasma Off™ suitable for microscope disinfection?
For disinfection of smaller equipment with electric/electronic components we recommend using Mycoplasma Off™ Wipes. Especially for devices with sensitive elements like microscopes, the wipes allow a more controlled/safe treatment than the spray and are a much better choice to clean the device thoroughly (wheels, switches etc.). However, you could try to moisten a clean paper towel with Mycoplasma Off™ and wipe the microscope with it. Cleaning of delicate components like objectives/lenses/filters with Mycoplasma Off™ is definitely not recommended. We also suggest spot testing to check for possible discoloration.

Mynox® and Mynox® Gold

Mycoplasma is still detectable after treatment of cells with Mynox®.
Following the Mynox® treatment, persisting mycoplasma contamination in cell cultures should be assessed after 4 passages at sufficiently high cell density. Mynox®'s eradication mechanism causes mycoplasma lysis. Thus, free mycoplasma DNA remains in the supernatant after treatment.
In cell cultures at adequate cell density, extracellular DNAses will hydrolyze such free DNA until it becomes undetectable. However, we are aware of different factors that might interfere with the efficiency of Mynox®. A crucial factor is the FCS concentration. FCS contains cholesterol and other target molecules for Mynox®. Hence, we strongly advise to follow our recommendations on FCS concentrations (maximum final 5 %). Another crucial aspect for efficient mycoplasma elimination is the cell concentration. If elimination of mycoplasma did not occur with the first treatment, we suggest lowering cell concentration and/or increasing the incubation time with Mynox®.
Prior to Mynox® treatment, make sure your cell suspension does not contain cell clumps. Extended trypsinization will greatly help avoiding the formation of cell clumps. In case of adherent cells, it is highly recommended to use Petri dishes for the treatment. This will prevent re-exposure of the cell suspension to aerosols possibly formed during pipetting of the cell suspension into the Mynox® solution. Such mycoplasma-containing aerosols could stick to the surface of the vessel not being exposed to Mynox®. These contaminated aerosols could re-contaminate the cell culture later on. Therefore, it is very important to transfer the cells directly into the Mynox® suspension and not vice versa.
If the mycoplasma titer at the beginning of a treatment is extremely high it might be necessary to treat the cells a second time with Mynox®. In that case, it is important to provide enough time for recovery (two days/check with the microscope) to the cells after the first treatment.

When can I be certain that mycoplasmas are permanently eliminated?
You can detect mycoplasma at an early stage with the highly sensitive Venor®GeM Mycoplasma Detection Kit to exclude persisting contamination. If a few mycoplasma particles survive the treatment with Mynox®, they will grow to detectable titers within four passages.

Do I have to remove standard antibiotics when treating with Mynox®?
No, standard antibiotics like Penicillin/Streptomycin can be maintained during the treatment with Mynox®. As a basic principle, we do not recommend routine use of antibiotics. Pen/Strep mainly affects germs of the mouth and facial cavity and was introduced at times when laboratory staff used to perform mouth-pipetting. Antibiotics can affect the cellular metabolism and thus the results of the experiments (compare with: Kuhlmann, Cytotechnology 19:95-105,1996 „The prophylactic use of antibiotics in cell culture"). Bacterial contaminations can interfere latently and remain unrecognized. With Onar® Bacteria, Minerva Biolabs provides a sensitive PCR Detection Kit for bacterial contamination (Cat. No. 12-1025).

Mynox® was ineffective in eliminating mycoplasma from virus suspensions.
The cell suspension must be free of cellular debris before treatment. Before the supernatant can be effectively treated, cellular debris should be eliminated by centrifugation (1.000 rpm, 5 min) and the pellet discarded. Such debris as well as cell membrane fragments will competitively bind Mynox®, thus decreasing the effective concentration of the elimination reagent.

At which confluence % can cell cultures be treated with Mynox® Gold?
For a successful treatment, the preparation of single cells is a necessity. We usually recommend to start the elimination procedure at 40 % to 60 % confluence. In these conditions, single cells can be obtained more easily.

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Is Mynox® effective against bacteria, fungi, or chlamydia?
No, Mynox® is only effective against mycoplasma.

Can primary cells be treated with Mynox®?
Yes, primary cells can be treated with Mynox®. However, we recommend a 10-fold increase in the cell concentration. (Note that an overall decrease in the elimination efficiency of Mynox® should subsequently be expected).

Can trypsin be a possible source of contamination in cell cultures?
Trypsin is derived from swine sources and believed to be a source of Mycoplasma hyorhinis contamination. However, Mycoplasma hyorhinis is lysed at room temperatures by trypsin within minutes.

Can mycoplasma contamination be visible to the naked eye?
No, mycoplasma can only be observed by electron microscopy. For highly sensitive detection of mycoplasma contamination, we recommend the Venor®GeM Mycoplasma Detection Kit.

Proteinase K

How many mg of proteinase are provided?
Each vial of Proteinase K (Cat. No 56-0002) contains 11 mg enzyme at ca. 30 U/mg, yielding a concentration of 20 mg/ml after resuspension in 550 µl of the included rehydration buffer.
I have a problem with the isolation of Mycoplasma DNA with Venor®GeM Sample Preparation Kit. Sometimes there is problem with the columns clogging up due to the specific nature of the sample. Sometimes white flakes (probably of organic origin) appear in the sample and remain in supernatant after centrifugation, thereby clogging the columns.
Clogging of the columns is sometimes observed with samples containing higher amounts of proteins (e.g. vaccines, high serum concentrations). Even if proteins may not be the main component, they could be involved in the formation of complexes between proteins and other ingredients. We recommend to try a proteinase K treatment according to the description of the manual of the Venor®GeM sample preparation kit (optional step prior to the 70 °C incubation step).

What incubation conditions do you recommend? The DNA extraction kit (Venor®GeM Sample Preparation Kit) recommends adding proteinase K then continue with the extraction (10 min, 70 °C incubation). Does this treatment scheme give the enzyme enough time to work? Wouldn´t the enzyme denaturate at such temperature?
The incubation with proteinase K in our extensively tested protocol is performed simultaneously with the sample lysis (at 70 °C for 10 min). The idea is to combine lysis and proteinase K treatment in a single step, so to keep the protocol as short and yet as efficient as possible. Our data and quality control show that such treatment is very effective with relatively complex matrices, suggesting negligible denaturation with short incubation times (and enzyme excess!). With much more complex matrices like tissues, for instance, we would recommend to separate extraction/lysis step and proteinase K treatment, and perform the latter at the optimum temperature for the enzyme 50-56 °C.

Venor®GeM Sample Preparation Kit

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We will perform mycoplasma testing for EP-compliance. Therefore, we plan to extract mycoplasma DNA from cell supernatants with Venor®GeM Sample Preparation kit. However, we need to freeze some supernatants at -20 ºC. What do you recommend: extracting DNA from all the samples and freeze them or treat the supernatant at 95 ºC (stabilization step) and freeze them? For how long we can keep them frozen?
We strongly recommend to heat-inactivate the samples directly after sampling. If you go for freezing instead, DNases will be active during the thawing phase degrading low copy numbers of mycoplasmal DNA. After heat-inactivation, you are save and could even store the samples for one week at 2-8 °C and handle them without restrictions at RT. Both versions are equally useful: heat-inactivation or DNA extraction, both immediately after taking the sample.
With both versions you can store the samples at <-18 °C for at least 1 year.

Venor®GeM Product Line (for conventional PCR)

Applies to all Venor®GeM Mycoplasma Detection Kits
How many Mycoplasma species can be detected with the kit?
Based on primer-genome sequence alignments, our kits can detect at least 110 species belonging to the Mollicutes class (Acholeplasma, Ureaplasma, Spiroplasma, Mycoplasma) of about 165 species which are mentioned in the literature, so far. For many – less common – species, however, there is no reliable data or sequence information. Thus, we cannot make any statement about the kit specificity for these species.
Importantly, we can detect all species mentioned in publications as significant cell culture contaminants and many others.

Applies to all Venor®GeM Mycoplasma Detection Kits
What is the function of the Internal Control DNA (or Internal Amplification Control)?
The Internal Control represents a second amplification reaction taking place in parallel to the target amplification (mycoplasma) in the same PCR reaction tube. The appearance of the internal control amplicon indicates that the conditions are PCR-permissive (no inhibition of the PCR). The Internal Control DNA is therefore a useful validation tool that helps ruling out false negatives. In the Venor®GeM kits, the internal control competes weakly with the target amplification reaction. As a result, a strong amplification (e.g. strong contamination) of the target DNA may lead to failure of the internal control amplification. A missing internal control is therefore a perfectly normal scenario in case of heavily contaminated samples or very efficient PCR amplifications. Consequently, the results of the internal control become irrelevant for positive PCR reactions.

Applies to all Venor®GeM Mycoplasma Detection Kits
I would like to freeze the supernatant after the inactivation step at 95°C and use it later on. At which temperature and for how long can I store such samples?
Based on our experience, samples can be safely stored at -20 °C for at least 1 year. One can also store the inactivated supernatants at RT for 5 days or between +2 °C and +8 °C for 14 days.

Applies to all Venor®GeM Mycoplasma Detection Kits
Our assays with Venor®GeM qEP have always worked well. However, this time the positive control has failed. The kit was stored correctly as advised in the protocol after rehydration etc. What could have gone wrong? Which recommendations do you have to ensure a good performance of the positive control?
The Positive Control is provided as one vial. After rehydration, this reagent is stable for frequent thawing/freezing cycles as long as you are using DNase-free pipet tips and working in a clean working area. However, aliquoting the rehydrated Positive Control might help.
We recommend to aliquot into PCR tubes as they are usually clean, DNase-free and low-binding.

Applies to all Venor®GeM Mycoplasma Detection Kits
Could you please let me know if culturing the cells 48 h without antibiotics is enough before testing for mycoplasma using a Venor®GeM Mycoplasma Detection Kit?
If your antibiotics do not display activity against mycoplasma, the test can be performed without any prior antibiotics-free culture and will provide reliable information about mycoplasma contamination.

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In case of mycoplasma-active antibiotics, we know that minute amounts of most mycoplasma species will grow up to easily detectable levels within 5 days of antibiotics-free culture. Fast growing mycoplasma species might be detectable after 48 h. In summary, waiting 5 days for the culture to grow in absence of such antibiotics will give you reliable results. Please keep in mind that PCR is the most sensitive method. If you are using other techniques, you may need to culture and delay testing even longer.

Applies to all Venor®GeM Mycoplasma Detection Kits
The manual of Venor®GeM Classic says that the use of Penicillin and/or Streptomycin in the culture does not affect the test. However, we use an antibiotic-antimycotic solution, which apart from Penicillin and Streptomycin contains Amphotericin B. Should we culture our cells in the absence of such antibiotic-antimycotic solution before performing the mycoplasma test?
Amphotericin B is not active against mycoplasma and should not affect the mycoplasma test.
Cultures supplemented with antibiotic-antimycotic solution can be directly tested with the Venor®GeM Classic kit.

Applies to all Venor®GeM Mycoplasma Detection Kits
We use Gentamycin in our media and I was wondering if that can interfere with the test. You state that penicillin and streptomycin do not significantly alter the sensitivity of the test. Will Gentamycin interfere?
Whereas the PCR assay itself will not be affected by gentamicin, the ability of mycoplasma to grow in your sample obviously will. Gentamicin is active against mycoplasma and unfortunately leads to the development of resistant strains rather quickly. Therefore, we generally recommend to test cultures, if possible, after cultivation for 1 week without antibiotic additives.

Applies to all Venor®GeM Mycoplasma Detection Kits
We would like to test cells with Venor®GeM Advance directly after thawing (stored frozen in liquid nitrogen) without prior culture. Is that possible?
Direct testing of thawed samples or cryo samples with our PCR detection methods (any kit) is generally not possible. The reason is the high concentrations of FBS and DMSO usually contained in the freezing media. Both these substances inhibit the PCR reaction. For such samples DNA extraction prior to PCR is necessary.

Applies only to Venor®GeM Classic
My PCR performed according to the protocol did not detect any bands (neither positive control nor internal DNA control). The reagents were all freshly resuspended and not repeatedly frozen and thawed. I used a Hot-Start Taq Polymerase, which performed well in other PCR reactions.
What went wrong?

- For our Venor®GeM Classic,we recommend our Hot-Start DNA Taq polymerase.
- Using a polymerase which performs well in other PCR setups does not necessarily guarantee the success of this particular PCR assay.
- The buffer provided with the kit is essential for the primers to work properly.
- Not all polymerases are performing well with the kit buffer and conditions.
- Getting no amplification at all usually indicates the incompatibility of the enzyme with the kit.

Applies only to Venor®GeM Classic, OneStep, and Advance
Some samples show a strong band for the gene of interest (mycoplasma) and no band for the internal control. Is my sample contaminated or is my PCR run invalid?
The appearance of the Internal control band is required for negative samples only. When your samples are strongly contaminated with mycoplasma, its DNA will be proportionally amplified. Under these conditions, the Internal Control band fades out due to reagents competition. Therefore, you can conclude that your PCR run is valid and unfortunately your samples heavily contaminated.

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Applies only to Venor®GeM Classic, OneStep and Advance
We used Venor®GeM Classic with the Taq Polymerase from Minerva Biolabs, as suggested in the product manual. We could not detect any band in the positive or negative control lanes (the internal control is not amplified). What could be the reason?

- Among the possible causes of a PCR reaction failure, we recommend considering the possibility that the PCR cycler in-use might have a technical problem. This is not a remote possibility. In order to evaluate the performance of the cycler, we suggest testing another cycler with the same PCR set-up or another PCR set-up with the same cycler.
- Rehydration of the positive control DNA was not correct (e.g. volume).
- The positive control was correctly rehydrated but too many freeze/thaw cycles were performed or DNA degradation due to contamination with DNase occurred.

Venor®GeM Product Line (for qPCR)

Applies only to Venor®GeM qOneStep and qEP
I need advice on which qPCR-based kit for Mycoplasma detection best fits my purposes. I would assume that labeled probes and primer pairs included in Venor®GeM qOneStep are identical to Venor®GeM qEP. Is it correct? If so, which differences are there between the two kits?
Venor®GeM qOneStep is a version of the assay for Version research use only to perform the screening of cell cultures for mycoplasma contaminations. The Internal Control for PCR performance is already included in the mix. The kit is not suitable for compliance testing according to EP 2.6.7.
Venor®GeM qEP is suitable for cell culture screening and also for quality control according to the EP 2.6.7. This product has been validated and the results of the validation study can be requested. In this case, the internal control is provided as a separate vials due to its possible use also during the DNA extraction procedure. For a quick overview of the differences between the products, please have a look at the table below.

	Venor®GeM qEP	**Venor®GeM qOneStep**
Recommended Use	For direct screening of cell cultures and biologicals. For EP 2.6.7/JP compliant release testing; Applicable in research and industry	Applicable in research for direct testing of cell cultures and cell culture derived biologicals.
Kit Components	Primer sets, nucleotides and polymerase/ Rehydration buffer / separate Internal amplification control / Positive control DNA / PCR grade water	Primer sets, nucleotides, internal amplification control and polymerase / Rehydration buffer / Positive control DNA / PCR grade water
Sample Volume per PCR	10 µl for EP/JP compliant testing / or 2 µl for screening	2 µl
EP/JP compliance	Yes, after appropriate sample matrix and process validation	No
Validation	Validation report available on request	No

Applies only to Venor®GeM Classic and qEP
Are these mycoplasma detection kits certified to be GMP-compliant?
Generally, in contrast to IVDs, mycoplasma detection kits are not certified or approved by any official body or governmental institution. The GMP-compliant use of the product requires a two-step validation process:

1. Product validation by the manufacturer (e.g. Minerva Biolabs)
Comprehensive validation reports are available on request for these assays.

2. Matrix validation by the user/customer:
The impact of lab-specific setup constraints and matrices on assay sensitivity and robustness must be assessed and validated by the customer. To this purpose, we offer 10CFU™ Sensitivity Standards containing inactivated and pre-titrated (10 CFUs)

mycoplasma particles for easy sample preparation, available for all EP/JP-relevant species. By simple addition of the typical sample matrix to the pre-titrated mycoplasma preparations, the customer can proceed to validate his/her own matrix and setup. We are glad to assist you during the planning and design phase of these processes. Did you know - for example - that it is not always necessary to test all mycoplasma species listed in JP/EP compendia?

Both validation reports (1. and 2.) are filed by the customer for approval of the entire manufacturing and downstream cleaning process. We actually do have a few customers around the globe, who went through this process and use our products for approved procedures.
As manufacturer, claiming to have obtained such an approval and advertising it as product compliance would be, therefore, incorrect. Unfortunately it can happen to find such incorrect claims in a few descriptions of competing products.

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