

Q&A ATCC® Excellence in Research Webinar “Mycoplasma detection – protect your continuous cell lines”

General Questions

1. Will we be able to download the presentation?

This presentation will be available to watch on demand [here](#).

2. Can bleach and IPA destroy mycoplasma?

Bleach, ethanol and or Sporicidin® are commonly used to disinfect surfaces that may have been exposed to mycoplasma contamination. Neither bleach nor IPA (isopropyl alcohol) should be used on the cell cultures, those should be discarded in a biosafety waste bag and autoclaved.

The inner surfaces of biosafety cabinets should be disinfected with 70% ethanol or Sporicidin® disinfectant solution. After the inner surfaces have dried, leave the UV light on overnight before using the biosafety cabinet.

To disinfect an incubator, remove the shelves, shelf racks, humidity pan, and any other removable metal components. Autoclave all of the metal components before reinstalling them into the incubator. Disinfect the interior cabinet of the incubator with 70% ethanol and allow it to dry completely before reinstalling the racks and shelves. If your incubator has an automated cleaning cycle, run the decontamination cycle.

Bleach should not be used routinely to disinfect the hood or the incubator since bleach will corrode the metal. However, it is acceptable for decontaminating the water bath. ATCC typically uses a 1:10 dilution (10%) of regular household bleach to disinfect a water bath. Wipe all surfaces of the water bath, including the lid and rim with the dilute bleach and allow it to sit for several minutes. Rinse the water bath with sterile distilled water after disinfection. Refill the water bath with sterile distilled water before use.

3. Does Plasmocin eradicate mycoplasma contamination?

If your cell culture is found to be contaminated with mycoplasma, it is best to discard the culture and start over. Curing cell lines of mycoplasma contamination is time consuming and does not always work. ATCC has not specifically used Plasmocin to attempt to cure a mycoplasma contamination but we have used a few other antibiotics and have had success with those. The following are the two most common antibiotics used to treat mycoplasma contamination and specifications on their use:

BM Cyclin

There are three options for using BM cyclin:

1. Add 4 µl BM cyclin per ml of culture medium for 3 days
2. Add 4 µl BM cyclin per ml of culture medium for 4 days
3. Add 4 µl of BM cyclin per ml of culture medium for 3 weeks total

Ciprofloxacin

Use 10 µg ciprofloxacin per ml of culture medium for 1-2 weeks

When curing a cell line of mycoplasma, the cells were cultured for 1 to 2 weeks in the presence of BM Cyclin (Roche) or Ciprofloxacin, and then cultured without antibiotic for 1 to 2 months. At this point, the line was retested to make sure that the culture was clean. Periodic retesting was necessary to make sure that the contaminant did not reappear. Since many antibiotics may be toxic to cells, a selected population that no longer exhibits qualities of the parental line may result. It may be necessary to examine the cured culture to assure that it retains properties sufficiently similar to the original line.

4. Does the ATCC Universal Mycoplasma Detection kit does the kit include positive & internal control?

The kit does come with a positive control which is purified plasmid containing a portion of the *Mycoplasma arginine* genome.

5. How often are primary cultures contaminated by mycoplasma?

ATCC thoroughly tests primary cells at ATCC before distribution to confirm that the primary cells are free of mycoplasma contamination. ATCC uses two methods of testing for mycoplasma - the Hoechst DNA stain and direct culture method.

The ATCC primary cells are free of contamination and the results are reported on the Certificate of Analysis for the specific lot. If you are isolating the primary cells yourself, they will need to be tested for mycoplasma contamination.

6. How do you biobank cells?

Initiate and grow the cells to an exponential stage of growth, freeze down an initial lot for seed and a working stock. Keep track of the number of cells/vial, population doublings and passage number. Refer to the following page on our website for guides and printed information on maintaining cell lines:

http://www.atcc.org/en/Documents/Learning_Center/Resources_for_Cell_Biology.aspx

In particular, the ATCC Animal Cell Culture Guide found on the page above, is particularly useful for instructions on how to grow, preserve and test cells.

7. Can mycoplasma be retained by a 0.2 micron filter?
Mycoplasma species can range in size from 0.15-0.3 micrometers, so a 0.2 micron filter may only catch a portion of mycoplasma contaminants, not all. It is best to use a small filter size, around 0.1 microns.
8. Does the ATCC PCR kit require supernatant or cells for mycoplasma detection?
The PCR kit (ATCC item #30-1012K) requires cells, not supernatant for detection of mycoplasma. See the product sheet instructions for complete directions on how to prepare the cells.
9. Can all species of mycoplasma be removed by filtration?
All Mycoplasma that are in suspension should be removed by filtration using a 0.1 µm filter. But Mycoplasma can adhere to cells and even though they may be removed from the media, they may still be present on the cells.
10. Is there an optimal temperature that samples should be stored at prior to testing, e.g. does the mycoplasma degrade at 4°C?
Cells should be incubated at optimal culturing temperature (37°C for mammal cells, generally) until tested. Shipping overnight at ambient temperature for testing is acceptable.
11. Should an antibiotic effective against mycoplasma be included in cell culture medium?
It is best to avoid the indiscriminant use of antibiotics as their overuse can lead to the emergence of antibiotic-resistant organisms. We recommend only using antibiotics in special applications for short durations.
12. Is your kit approved by the FDA for detection of mycoplasma in product production cell cultures?
Currently, the U.S. FDA guidelines for mycoplasma testing of cell cultures recognize only the fluorochrome DNA stain and the direct culture methods. Therefore, we continue to use these two methods as our primary quality control assays. However, our understanding is that regulatory agencies, such as the U.S. FDA and European equivalents, are considering PCR as a method for mycoplasma detection. We do not know where the regulatory agencies are in this process but suspect there will be an extensive validation period before PCR is added to the list of recommended methods. Now that PCR is becoming a more acceptable method for mycoplasma detection, particularly internationally, ATCC developed the UMDK to meet European Pharmacopeia 2.6.7 guidelines.

13. Mycoplasma contamination doesn't seem to be a concern in bacterial cultures, why?

Mycoplasma is less of a problem when culturing bacteria because it tends to be a fastidious, slow growing culture. However, it is prudent to watch for any form of contaminant when culturing bacteria.

14. What are the most common sources of mycoplasma for tissue culture?

Some of the various sources of cell culture contamination include laboratory personnel and equipment, cross contamination, and through contaminated culture reagents. Laboratory personnel are considered to be the primary source of *Mycoplasma orale*, *Mycoplasma fermentans*, *Mycoplasma salivarum*, and *Mycoplasma hominis*, which account for more than half of all mycoplasma contamination events in cell culture. These particular contaminants are commonly introduced into your cell culture due poor aseptic technique and culturing practices.

Mycoplasma contamination can also come from dusts and particles that are brought in from outside the laboratory on your skin, clothes, or shoes. These contaminants can then be spread throughout your lab by the use of equipment that either kicks-up dust or creates aerosols, such as pipetting devices, vacuum pumps, centrifuges, blenders, sonicators, or sources of vented heat such as radiators and freezers. Alternatively, these dusts and aerosols can also be spread directly to your cell culture due to poor culturing practices, including improper sealing of culture dishes, and mishandling of cell cultures.

In addition to laboratory personnel and equipment, mycoplasma contamination can also result from cross-contamination from other cell lines -- including newly acquired cell lines. One mechanism for these cross-contamination events to occur is through the aerosolized dispersion of contaminated cell cultures, which can either directly affect other cell cultures nearby or contaminate surrounding surfaces, equipment, or media that may come in contact with other cell lines. So, to avoid this type of situation, it is best to work with only one cell culture at a time and to prepare separate culture regents for each individual cell line. Further, all new cell lines that you acquire should be quarantined upon entering a lab until it is confirmed that they are free from mycoplasma contamination.

In addition to aerosolized dispersion, cross-contamination can occur if your biological safety cabinet has a non-existent or faulty laminar flow. As many of you may know, biological safety cabinets rely on a steady flow of air to maintain aseptic conditions. If this flow is disrupted in any way, it could lead to potential contamination events.

Lastly, a third means of mycoplasma contamination is through the use of contaminated culture regents. These reagents can become contaminated during the manufacturing process, through improper handling, through aerosolization when working with a contaminated cell line, through ineffective sterilization techniques, or, in the case of sera, through an infected donor. In fact, contaminated sera are major sources of contamination with *Mycoplasma arginini*, *Mycoplasma hyorhinsis*, and *Acholaepasma laidlawii*. So, for

these materials, is important to only use sterile culture reagents that have been tested for microbial contamination.

15. Are ATCC cell lines mycoplasma free, and what is the best method to treat mycoplasma?

ATCC thoroughly tests all cell cultures produced at ATCC before distribution to ensure that the cultures are free of mycoplasma contamination. ATCC uses both the direct culture method and the Hoechst stain to ensure that the ATCC cell cultures are mycoplasma-free.

Some cell cultures deposited to ATCC contain mycoplasma, but are considered valuable enough to be distributed despite the contamination. In such cases, the product description for the culture on the ATCC website and the product information sheet shipped with the culture both state that the cell culture is known to be infected with mycoplasma.

Cell cultures that are distributed as depositor stock are not tested for mycoplasma contamination. Many of these cultures were deposited in support of patent applications. ATCC is required by patent law to continue distributing these cultures.

If your cell culture is found to be contaminated with mycoplasma, it is best to discard the culture and start over. Treating cell lines of mycoplasma contamination is time consuming and does not always work.

When attempting to cure a cell line of mycoplasma contamination, first identify the contaminant and select a suitable antibiotic, preferably by testing the contaminating mycoplasma for its antibiotic sensitivity. Culture the cells for 1 to 2 weeks in the presence of the antibiotic, and then culture them without antibiotic for 1 to 2 months. Then retest the line to make sure that the culture is clean. A very sensitive testing method should be used. Periodic retesting is necessary to make sure that the contaminant does not reappear. Since many antibiotics may be toxic to cells, a selected population that no longer exhibits qualities of the parental line may result. It may be necessary to examine the cured culture to determine if it is sufficiently similar to the original line.

16. When receiving lines from ATCC, do we need to quarantine and test those as well?

Cells lines in the ATCC General Collection are screened for mycoplasma contamination and unless specifically noted will be free of mycoplasma contamination. Most cell culture laboratories will incorporate mycoplasma testing into their routine cell culture operations. We sell the Universal Mycoplasma Detection kit ATCC 30-1012K because we understand that many customers prefer a quick method to screen for mycoplasma contamination as they expand and culture the cells in their lab.

17. You mentioned every 6 - 12 weeks for testing continuous cultures. Is always being on the 12 week side of things sufficient? We use PCR based method every 12 weeks, and a biotin-tagged every 6 - 9 months, and keep coming up clean. Does this seem sufficient?

The frequency of mycoplasma testing is generally at the discretion of the lab. If there is no history of mycoplasma contamination in previous results, every 12 weeks should be fine. However, any new cell culture entering your lab should be meticulously examined for mycoplasma and other contaminants upon arrival, unless you are receiving cell cultures directly from ATCC. Each lot of cells from ATCC is tested for mycoplasma and the certificate of analysis with the results is available on our Web site.

18. We make our own medium, working up the DMEM with sodium bicarbonate and then filtering it. We then add the serum (not filtered in our lab). What is the chance that the serum we get from a reputable source is contaminated?

It is very rare, since most companies now triple-filter sterilize their sera using 0.1 µm filters and test for bovine viral diarrhea virus (BVDV). Most companies will also note which other viruses they have tested for in their sera.

19. If using a PCR method that doesn't use a touchdown cycle, how does this decrease sensitivity?

Not using touchdown PCR increases the chances of false positives and reduces the sensitivity of the kit. The kit uses universal primers specific to the conserved 16s rRNA coding region of the mycoplasma genome. The touchdown PCR protocol employs a high annealing temperature in the initial cycle that decreases with subsequent cycles. This protocol increases the likelihood of primers binding to the specific targets and reducing the likelihood that non-specific targets (i.e. cells or other bacteria like *E. coli*) will be amplified. Using this method, mycoplasma contamination is easily recognized as a distinct PCR product. More information on our Mycoplasma Detection Kit can be found on our website: specifics on the Mycoplasma Detection Kit, refer to http://www.atcc.org/en/Documents/Learning_Center/Resources_for_Cell_Biology.aspx and select the link to the Universal Mycoplasma Detection Kit under Application Notes.

20. I would like to try culturing for mycoplasma, but have never seen any instruction to do so. Can you point me to a place where I can learn how to do this (in combination with our current PCR detection method)?

We have some general instructions on culturing Mollicutes in our Bacteriology Culture Guide online (www.atcc-guides.org). However, direct culture can be difficult to accomplish, since many Mycoplasma species display fastidious behavior in culture (i.e., you're more likely to get a false negative). That's why the FDA recommends points-to-consider using compendial methods – or, two confirmatory methods. The easiest method to append to your current routine is Hoechst DNA staining.

21. Which solution do you recommend to clean equipment?

See question #2

22. Would centrifugation of the mycoplasma be a way to concentrate the mycoplasma for detection?

Yes, you can centrifuge mycoplasma to concentrate it. With our Mycoplasma Detection kit, we recommend centrifuging 1 ml of your sample (generally 10^4 to 10^5 cells) in a microcentrifuge at 13000 rpm for 3 minutes at 4°C.