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Strategies to Minimize Contamination in the Cell Culture Lab

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The Cost of Cell Culture Contamination

Contamination can be exorbitantly expensive, but there are plenty of steps that can be taken to curb it.

Lauren Tanabe

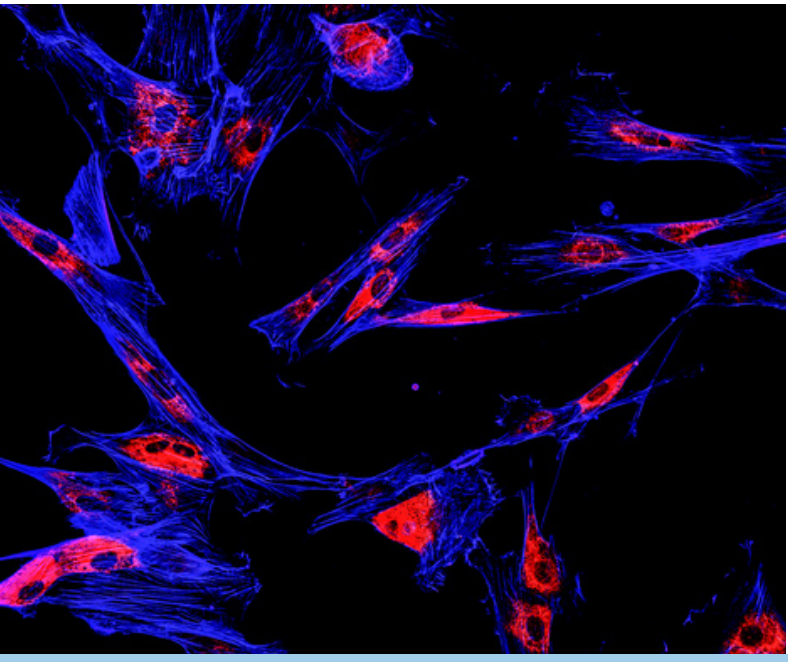

Image: Among the most notorious misidentified cell lines was MDA-435, a "breast cancer" cell line that turned out to be melanoma-derived. Image from Dreamstime.

Cell culture is expensive. But the cost of contamination of cell culture can be exorbitant. Sometimes the offenders are overt, causing media to turn opaque and milky or cells to suddenly become bespeckled, and you can cut your losses and start fresh. Other times, mycoplasma can run amok for months, invisible and odious, causing experiments to go haywire. A few years ago, researchers estimated that mycoplasma had the potential to affect hundreds of millions of dollars of NIH-funded research. And then there is contamination hiding in plain sight—misidentified cell lines, able to jeopardize years of experiments. (The most notorious may have been MDA-435, a "breast cancer" cell line that turned out to be melanoma-derived.)

Today, most journals require cell lines to be validated using STR (short-tandem repeat) or SNP (single nucleotide polymorphism) analyses for verifying cell-line identity. And many cell-line distributors assure authentication. But in the day to day, how can researchers protect cells (and future experiments) from the myriad offenders searching for a warm and humid place to call home?

You know the saying: An ounce of prevention is worth a pound of cure. This is especially true for working with cells, which must be inspected daily and tested for mycoplasma regularly. And lines should be validated prior to beginning any experi-

ments (especially if they happen to come from the lab down the hall). Contamination will happen on occasion, but there are plenty of steps that can be taken to curb it.

Know your enemy

While mycoplasma detection and cell-line validation require some extra effort, simply looking at your cells under the microscope everyday will help to weed out other biological contaminants. According to Yana Li, laboratory manager of the eukaryotic tissue culture facility at the Johns Hopkins University School of Medicine, these include bacteria, molds, yeasts, and viruses. Yi says that chemical contaminants such as impurities in the media, sera, endotoxins, and detergents can also be detrimental.

Use the medium as an indicator, paying attention to any rapid color changes (indicating a swift shift in pH, if phenol red is used) or increase in opacity. Familiarize yourself with what contaminated cultures look like and watch for bright particles in between cells (oftentimes yeast). Also, any changes in cell morphology—such as formation of cytoplasmic vacuolation, formation of nuclear granules, or reduced proliferation—are indicative of unhealthy cultures (and potential contamination).

"Always buy cell lines from reputable vendors who have characterized the cell lines and can certify that they are mycoplasma free," advises Peggy Keefe, research assistant and lab manager for the University of Oklahoma Stephenson School of Biomedical Engineering. And be careful with gifts of cells from other labs. "They may be free at the onset but will cost you much later." But, if you must use cells that come from another lab, quarantine them in a separate space until they've been tested and shown to be free from infection. Yi says, "Before conducting experiments, those cells should be authenticated."

Manage your workspace

"The specific requirements depend mainly on the type of research conducted. It's preferable to maintain a separate tissue culture room to designate an aseptic work area for cell culture work," advises Yi. Ideally, cell culture rooms should also have separate ventilation and air conditioning systems. Equipment should be set up away from doors and other high-traffic areas.

"Do a good 'footprint measure' of the cell culture room to arrange your equipment. Limit the distance between equipment, let's say the incubator where the cells are growing and the hood you are working in," says Yi.

Incubators should also be kept away from vibrations (such as from elevators or heavy equipment). If the space has windows, keep them closed at all times. Cell culture supplies should be kept close to where you are working to minimize air disruption.

Control your environment

Every surface and piece of equipment that comes into contact with cell plates must be thoroughly disinfected (70% ethanol or propanol works fine). This includes the microscope. Similarly, the biosafety cabinet (or flow hood) is meant to be a sterile sanctuary for cells. Ensure that the window sash is in the correct position, and wait several minutes until the airflow is established inside.

Keefe says that the biggest mistakes scientists make include not waiting long enough for the hood to warm up and overcrowding the workspace inside the hood. "Only put in the hood what you will be using. Don't use it for storage of pipet boxes, waste containers, etc." This balance is critical because every single item and every movement that occurs inside

of the hood can disrupt the laminar airflow and put cells at risk. When working, Keefe suggests using as much of the space as possible. "Move open bottles or flasks out of the way. You don't want to pass your hands over top of them, increasing the chance of contamination." And while having that bunsen burner on might feel like its offering some protection, in actuality it is also interfering with the airflow, so best to keep it off.

Zan Chen, research fellow in cell biology at Harvard University, says that oftentimes the vacuum system gets ignored and this is a mistake. "When the tubing gets old and [misshapen], the vacuum is no longer strong enough and sometimes the medium or buffer can backflush into cell culture dishes and cause contamination. It happened to us several times."

Likewise, care should also be taken with the incubator. Although newer incubators are equipped with antimicrobial copper surfaces and decontamination settings, regular maintenance (like changing the HEPA filter and refilling the water pan) will keep things running smoothly. Try to minimize the time that the door is open and be careful to clean up any spills quickly and thoroughly.

Keefe says that her lab decontaminates the incubators more often since the introduction of auto decontamination cycles. "With the auto-decontamination cycle, the incubator is sterile overnight and back in service. Before, you had to move the cells, autoclave the racks, wipe it down with isopropyl alcohol, then reassemble the racks, add water, and wait for the humidity to go back up." All of which took at least a day or two.

Li agrees that purchasing an incubator with a high temperature decontamination cycle is helpful. "Good equipment maintenance is essential for safe and efficient operations." But she also thinks that a great management team is necessary: "Ensuring that equipment is properly maintained, supplies are readily available, and managing emergency cell handling during weekends and holidays is critical to keeping tissue cultures healthy."

Check yourself

The most advanced antimicrobial equipment cannot save you from sloppy work or inexperience. This is because humans are covered in microbes. Particles from hair, skin, or clothing can blow into cultures, so precautions should be taken.

"Thorough training in tissue culture practices for anyone working in the tissue culture room will reduce the risk of contamination," says Keefe. Researchers should change into a cell culture designated lab coat upon entry to the cell culture space (and consider shoe covers as well). Wash and disinfect your hands and then glove up and disinfect again.

Try to minimize talking, and if you must speak, turn your head away from cells. One of the predominant species of mycoplasma comes from human mouths, "especially when [they are] laughing, which can overwhelm the air barrier," notes Keefe.

When contamination strikes

Despite best efforts, contamination will probably happen on occasion. Microbes are everywhere. But if cells are checked daily, mycoplasma is tested for regularly, and there are protocols in place for the maintenance and sanitizing of equipment, contamination can be downgraded from crisis to annoyance. When it does strike, there is little hope for the cells. Li says, "Once contaminated, most tissues or cells are only fit for the trash."

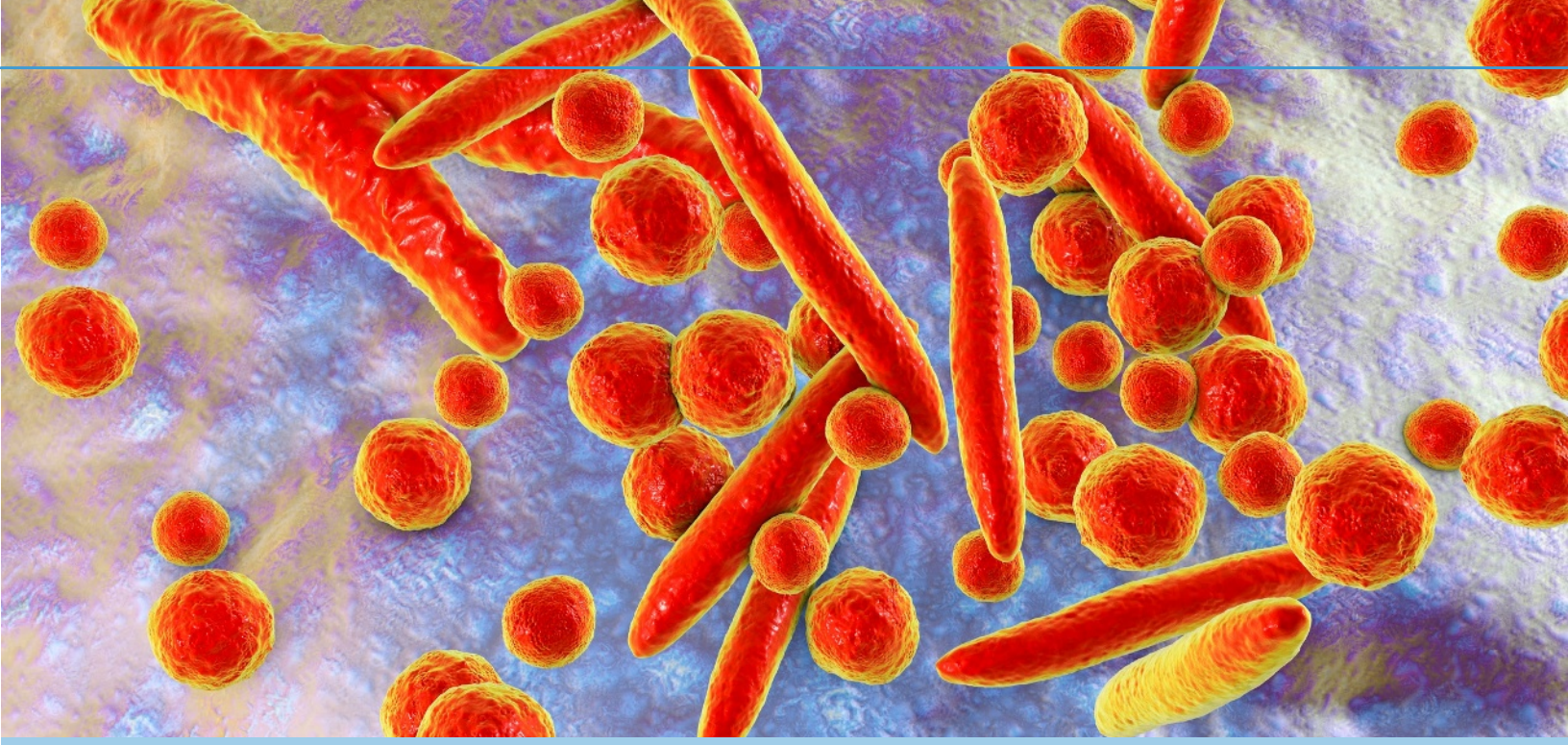

Image: Minimize talking in the lab when working with cell cultures, and avoid laughing as much as possible. One of the predominant species of mycoplasma comes from human mouths. Image from Dreamstime.

Keefe says that on one occasion she's witnessed primary cells brought back from a bacterial infection with antibiotics. But there was always a lingering doubt about how they had been changed. "It survived the treatment and tested negative for mycoplasma but the question if it had been altered because of the antibiotic treatment kept us from using it after the first experiment."

The next step involves sleuthing and sanitizing. "After we find a contamination, we have to find the reason and troubleshoot the problem based on the type of contaminant. This is followed by a deep clean of the work area, checking the health of other cells, etc.," explains Li.

In general, any infected cultures should be immediately removed from the space. Pour a 10% sodium hypochlorite (bleach) solution into the flask and let sit for a few hours to kill the offending microorganisms. All culture medium associated with or prepared at the same time as the contaminated cells should be tossed. The incubator and biosafety cabinets should also be thoroughly decontaminated with a diluted bleach solution. Note that this can be corrosive to metal, so rinse thoroughly and spray down with 70% ethanol or isopropanol.

Moving forward (but not without the basics)

Cell culture is moving at warp speed. With scientists switching from cell lines to primary cells and swapping two dimensions for three, attention to the details like basic aseptic technique has never been more important. By tweaking your cell culture set up, ensuring regular equipment maintenance and decontamination, and being more vigilant when in the hood, you can be sure your research continues on its trajectory, unencumbered by microbial hijackers.

About the Author

Lauren Tanabe has a Ph.D. in pharmacology and molecular signaling from Columbia University. She completed her postdoctoral work at the University of Michigan as a Dystonia Medical Research Foundation Fellow and at Wayne State University as an American Cancer Society Fellow.

Controlling Mycoplasma Contamination of Cell Cultures

This difficult but not impossible task requires a proactive approach.

It is well known that a significant percentage of cell cultures are contaminated with mycoplasma. The extent of this contamination problem has been surveyed many times over the last three decades and found to be a significant problem. It is thus safe to say that the chances of cultures in your lab being contaminated with mycoplasma now or in the future are moderate to high.

Don't despair, though. While preventing mycoplasma contamination is difficult, it is not impossible. We recently queried four experts to learn more about how you can protect your cell cultures from mycoplasma contamination and, if they do end up contaminated, what your best course of action is.

Our expert panel includes Maryellen de Mars, Ph.D., vice president, standards resource center at the ATCC (American Type Culture Collection); Dr. Jürgen Becker, product manager at Promocell; Amanda Capes Davis, Ph.D., founding manager of CellBank Australia and Chair of the International Cell Line Authentication Committee (ICLAC); and Mike Mortillaro, Ph.D., owner, Bulldog Bio.

What method(s) do you recommend for mycoplasma detection and why?

de Mars: Mycoplasma contamination is a serious concern for cell culturists because these bacteria can significantly affect the physiology and viability of cell lines. Therefore, the rapid and accurate identification of mycoplasma species is essential. Currently, three detection methods are used routinely: (1) the direct culture method, which uses both agar and broth; (2) the indirect method, which incorporates the Hoechst DNA stain; and (3) PCR-based testing, which typically uses universal primers that are specific to the 16S rRNA region.

To ensure that any possible contaminants are identified, both the direct and indirect methods should be performed simultaneously. Together, these methods can detect most known culturable and nonculturable mycoplasma species. Each test includes positive and negative controls, and complete testing requires four to five weeks.

PCR-based testing provides a quick and sensitive method for detecting mycoplasma contaminants in cell culture through the amplification of conserved target regions in the mycoplasma genome. Samples that are positive for mycoplasma contamination are

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easily recognized by a distinct PCR product of a defined size. This method is very accurate and rapid, with results typically available in less than one working day. At ATCC, we provide services and a kit for mycoplasma detection.

Becker: I would definitely choose a PCR-based detection method because it is the most sensitive and typically covers all mycoplasma strains relevant for cell culture contamination. Moreover, this method not only detects viable mycoplasmas but also dead mycoplasmas, giving you better information on the degree of contamination. The PCR-based assays are clearly more robust and reliable than enzyme-based assays, which strongly depend on the viability and cell metabolism (i.e., the cell culture conditions)—giving rise to false-negative results.

Mortillaro: We find that, when including the proper internal controls, a simple assay using PCR and visualizing on an agarose gel is very inexpensive and accurate without a big investment for a real-time PCR machine.

Capes-Davis: It's easy to be overwhelmed by all the different choices. I suggest starting by looking at the kits or methods that relate to your area of expertise—for example, if you are a molecular biologist then PCR-based methods are your obvious choice. You need a method you can repeat easily, so it makes sense to go for your testing comfort zone if possible. For any kit or method, you need to know the number of species detected and the sensitivity of the method. Applying these two criteria will show which of the various methods or kits is the best choice for you.

The term "mycoplasma contamination" can be misleading because it's not caused by a single entity. There are many different species that can cause mycoplasma-type contamination, including related species such as *Acholeplasma laidlawii*.

What is the best way to eliminate mycoplasma contamination? When should you just discard the culture?

de Mars: Mycoplasma contamination in cell culture is often unavoidable. Since mycoplasma-infected cells are considered a source of contamination and can infect additional cultures in the lab, eliminating the contamination should occur as quickly as possible. ATCC recommends discarding all mycoplasma-contaminated cells and obtaining new starting materials whenever possible.

When cell cultures are too valuable or difficult to replace, there are chemical agents that can be used to eliminate mycoplasma infection. A variety of fluoroquinolones, tetracyclines, pleuromutilins, and macrolides have demonstrated strong antimycoplasma properties in cell culture. For example, BM-Cyclin and Plasmocin™ are often applied to mycoplasma-contaminated cells. BM-Cyclin, which is a combination of two antibiotics (tiamulin and minocycline), inhibits bacterial protein synthesis. Plasmocin, which is strongly active against mycoplasma species, contains two bactericidal components that affect protein synthesis and DNA replication, respectively.

Becker: If you cannot simply discard your cell culture and restart the experiment with a mycoplasma-free culture, we recommend our Mycoplasma-EX Kit, which contains a component that directly and selectively kills mycoplasmas very fast and efficiently (almost 100% elimination)—and does not simply inhibit mycoplasma growth as most antibiotics do.

Antibiotics are admittedly cheaper, but they have the disadvantage that treatments take a long time, mycoplasma resistance may occur, and the cultured mammalian cells themselves might be altered and impaired by antibiotics.

Mortillaro: The feedback we receive from our customers is that they typically dispose of infected cultures and decontaminate the areas in which these cultures were used. Multiple-antibiotic treatments can help, but are not always successful. In some cases, it is easy for the mycoplasma contamination to re-establish. Starting with seed cultures that are not contaminated is the best way to minimize exposure. These seed cultures can be tested early on for mycoplasma.

Capes-Davis: Discarding the culture is always your first and best outcome. Cell lines can be contaminated with more than one mycoplasma species. Also, treatment is toxic and may kill or alter the behavior of the cells. Mycoplasma can become resistant to antibiotics and often recurs after treatment, which may be due to an intracellular reservoir—many species invade their host cells, e.g., *Mycoplasma penetrans* (given that name for a good reason!). Only treat the culture if you have no other option.

If the cells are irreplaceable, you need a regime of antibiotics that works against mycoplasma. Penicillin and streptomycin do not work—penicillin acts against the rigid bacterial wall, which is not present in mycoplasma species. There are some excellent publications on mycoplasma eradication from Cord Uphoff and his colleagues at the DSMZ, so you can look at their methods and data to help you decide. Be careful to quarantine infected cultures from your other cell lines, and continue to test afterward to pick up any recurrences.

Can you suggest some best practices to avoid contamination as well as stop existing contamination from spreading?

de Mars: The best practices for avoiding contamination and preventing the spread of existing contamination include keeping a documented history of your cell line, following cell culture best practices, and routine testing. When possible, always use certified or registered cell lines that are confirmed to be negative for mycoplasma contamination. To both avoid and control the spread of mycoplasma during cell culture, we recommend the following laboratory practices:

- Only work with one cell line at a time. Aerosols and operator error are two of the most common means of transferring mycoplasma contamination.
- Always wear clean personal protective equipment (PPE) and adopt appropriate aseptic techniques.
- Use a vertical laminar flow hood that is isolated from other standard cell culture facilities. Disinfect inner surfaces of the hood between each use. Clean incubators that store cultures on a regular schedule.
- Avoid the indiscriminate use of antibiotics. This practice can permit repeated lapses in aseptic technique, will mask low-grade contaminations, and may lead to the emergence of resistant organisms.
- Promptly autoclave infected cultures, media, and reagents to avoid contamination of clean cell lines.

Becker: We recommend good laboratory practice, i.e., working under conditions where infection of cell culture and spreading of contamination are avoided. Cell culture labs should have respective standard operating procedures (SOPs) in place and train all personnel already working in the lab or joining the group. Use reliable, validated disinfectants regularly to disinfect workbenches, laminar flows, water baths, etc. For example, 70% ethanol is not a safe, reliable disinfectant. Our PromoCidal or Spore-EX are highly efficient, thoroughly validated and non-irritant, non-corrosive surface disinfectants. And don't forget that water baths and the water in CO2 incubators can harbor mycoplasmas. Use reliable water stabilizers/cleaners such as Aquaguard to further reduce contamination risks.

Test existing cell cultures (e.g., from former lab members) as well as cell cultures coming into the lab from outside (cell banks, other research groups) for mycoplasma contamination before starting to work with them. Test cell cultures regularly for mycoplasma contamination; mycoplasma-free cultures can be contaminated at any time, for instance, by the lab staff itself or contaminated media components. If mycoplasma contamination is detected, discard the cell culture, or try to eliminate contamination (if you can not discard the contaminated cells). Try to identify and eliminate the source of mycoplasma contamination.

Mortillaro: Avoiding seed cultures that are already contaminated with mycoplasma is the first and most important step. You can also disinfect using various reagents. We recommend MycoClean™. We also offer an elegant approach to physically isolate (essentially quarantine) your cell culture plates during growth/incubation and transport from other cell dishes and the general cell culture environment that may be contaminated—FastGene Cell Culture Protective Trays.

Capes-Davis: The best way to avoid contamination is to separate cultures from one another. In the early days, mycoplasma contamination was linked to particular reagents, e.g., trypsin or serum. Today, the culprits are likely to be the other cell lines that you or your colleagues are growing in the same room. Keep separate supplies of media and other reagents for each cell line, and never handle them in the biosafety cabinet at the same time. Wipe down the work surface and run the cabinet for a little while between cell lines.

You should be aware that mycoplasmas survive in dried form very nicely—back in 2001, a lab looked at the viability of dried mycoplasma, and they showed that many common species in cell culture remained viable after 168 days. So it is important to wipe down your work surface with 70% ethanol or a similar disinfectant and continue your testing so you can deal with the positive cell lines as they arise.

How often should testing be done? Are there specific times/occasions it should be done?

de Mars: There is no limit to the number of times one should perform mycoplasma testing, but it is preferable to screen cultures on a regular schedule. Routine testing helps ensure that the experiments performed in your lab are verifiable and reproducible. According to best practices, there are specific occasions when testing should occur. Mycoplasma testing should be performed upon the receipt of an initial vial of cells, particularly if it is from an unreliable source; this should occur before a master cell bank is generated. Mycoplasma testing should then be repeated when a master cell bank is expanded to a working cell bank. If cells are isolated from primary tissue, it may be necessary to use antibiotics in the primary culture. Antibiotics should be removed as

Image: Mycoplasma contamination is a serious concern for cell culturists because these bacteria can significantly affect the physiology and viability of cell lines. Image from Dreamstime.

soon as possible, and the culture should be tested for mycoplasma after at least two passages in the absence of antibiotics.

Becker: Test cell cultures before starting your experiments. We recommend testing of cell cultures on a monthly basis as well as before cryopreservation. Test each incoming cell culture immediately.

Mortillaro: Guidelines should be dictated by an individual lab's requirements and related to past contamination history as well as the number of new seed stocks and personnel being added. Usually we see our customers test all cultures anywhere from once a week to once a year. The worst habit is to test stocks after they exhibit signs of contamination, because at that point experimental results can be questioned and cross-contamination issues become more complicated.

Capes-Davis: Test regularly rather than stressing about exactly how often it should be done. If you test for mycoplasma regularly, you will remove the highest-risk samples and then you are just ensuring that no new contaminants arrive to disturb the status quo. Always test cell lines when they arrive in the lab, and make sure you test before you publish so you can report what you have done and confirm that cells are mycoplasma-free.

Tips for a Contamination-Free CO_2 Incubator

Avoiding contamination includes things as simple as pulling up your sleeves and using long sterile gloves.

The CO_2 incubator provides a simulated human body–like environment for cell cultures and tissues to live and thrive. Unlike the body, however, the incubator is not equipped with a pathogen-fighting immune system and must be kept sterile by the user. Pathogens typically enter the incubator in one of a few ways: through contamination within cell culture dishes due to poor aseptic technique, in the airflow when the incubator is opened, from the user's contaminated skin or clothing, or in the water pan. This article highlights tips for avoiding incubator contamination.

Keep chamber doors closed as much as possible— Most modern incubators have an inner chamber door and the finest incubators will have separate doors for each shelf. Since many pathogens are airborne, keeping ambient air from entering the chamber is critical. The easiest way to prevent airborne pathogens from entering is to only open doors when absolutely necessary and for as little time as possible. Positive air pressure within the chamber is also a very effective way to keep outside air from entering the growth chamber, but not all incubators are equipped with this feature.

Watch the sleeves of your lab coat—Your skin and clothing are a likely source of contamination. Exposed skin and the long sleeves of a lab coat or sweatshirt should never come in contact with the incubator chamber or cell culture vessels. It is recommended to pull up your sleeves and use long sterile gloves before handling cell culture materials.

Change and clean the water pan regularly—It is recommended that the water in the pan be changed weekly. Replacement water should be distilled or mineral-free, no purer than 1 mega ohm. Copper disks or disinfectants (without chlorine) added to the water can also help eliminate potential contamination. Never let the water pan run dry or be overfilled. A maintained water pan is key to preventing contamination.

Decontaminate secondary cell culture containers—When working with many cell culture dishes and flasks at a time, it is common to use a secondary vessel such as a flat, short-walled container to carry them. These secondary containers should be thoroughly cleaned with disinfectants that will kill both bacteria and fungi.

Do not overfill the incubator—An incubator filled to the brim with dishes or flasks can disrupt the airflow and cause improper air filtration and disrupt temperature uniformity. A temperature difference within the incubator can cause condensation to occur, which can act as a haven for pathogens. The

incubator's manual should have guidelines for ideal working conditions.

Keep incubator away from contamination sources—Placement of the incubator within the lab space is crucial. If kept under a laminar flow hood, for example, contamination from the underside of the hood, a researcher's shoes, or the floor could easily find its way into the incubator. It is best to install the incubator on a flat surface away from hoods and water baths. Also, the higher the incubator is off the ground, the better for preventing contamination from particles that can be brushed up from walking on the nearby floor.

Know thy incubator—Your incubator may or may not have built-in contamination prevention features. It is important to understand how best to use your particular incubator to prevent contamination. Consult the incubator manual to find if your incubator incorporates the below contamination prevention features.

Positive chamber pressure—It is best if there is a slight positive pressure within the cell culture chamber within the incubator. This prevents contaminated laboratory air from entering the chamber.

Air filtration—Air within the growth chamber should continuously flow through HEPA filters. It is ideal for the HEPA filter to be located outside of the growth chamber. If located internally, there is a risk of contamination if the motor that blows the air stops. In this case, contaminations within the HEPA filter can fall onto cells. HEPA filters should also be replaced every 6 to 12 months depending on usage.

Removable gasket—The incubator gasket creates the seal around the inner door and is an area where humidity can build, resulting in the growth of contamination if left unchecked. Ideally, the gasket will be removable and easy to clean. If using a v-gasket, beware of the direction of the flap. An outwardly pointed flap will collect particles from entering the growth chamber while an inwardly pointed flap will collect humidity and can act as a breeding ground for contamination.

Image: The easiest way to prevent airborne pathogens from entering an incubator is to only open doors when absolutely necessary and for as little time as possible. Image from Dreamstime.

Rounded corners—Smooth, rounded corners within the incubator can eliminate hiding places for potential contaminants. If your incubator has sharp corners, make sure that the corners are thoroughly cleaned to prevent contamination.

Sterilization protocols—Many incubators have automated high-temperature sterilization cycles for fast and easy complete sterilization within the growth chamber. If there is not an automated sterilization cycle, an external source of dry high-heat or hydrogen peroxide gas can be used. When manually cleaning the chamber, it is recommended that labs create SOPs that include cleaning with a 70% solution of isopropyl alcohol or other disinfectant. UV light can also be used to sterilize but will only be effective in areas where the light shines at sufficient intensity and penetration, missing areas such as the bottoms of the shelves.

Copper interior—Copper has antimicrobial properties that can protect against contaminants introduced by a user or perhaps by the bottoms of culture plates. Chamber shelves can be coated with copper to further reduce the chances of contamination.

Avoiding Tissue Culture Contamination with Other Cell Types

Contamination needs to be kept under control, as it compromises the overall reproducibility of experimental results.

Results of an online survey conducted by the Global Biological Standards Institute (GBSI), confirmed what many cell scientists already know.

More than half of respondents—52%—"never" perform authentication or other species-related quality control tests on the cell lines used in their experiments. According to survey results, 74% never conduct STR (short-tandem repeat) DNA profiling, the accepted standard for authentication.

Although respondents were more likely to perform some sterility-related microbial contamination quality controls, particularly visual inspections, such measures will not typically detect mycoplasma bacteria-–infected cells.

Cell and tissue culture contamination with microbial contamination and other cell types not only affects the culture in question, but also compromises overall reproducibility of experimental results.

With the continuing increase in the use of cell culture for biological research, vaccine production, and production of therapeutic proteins for personalized medicine and emerging regenerative medicine applications, culture contamination remains a significant concern. Based on submissions to major cell repositories in the last decade, it is estimated that between 18% and 36% of cell lines may be contaminated or misidentified.

In 2008, for example, 40 human thyroid cancer cell lines were analyzed by STR profiling. Only 23 unique profiles were obtained, and many of the cross-contaminating cell lines were not thyroid in origin. These cell lines had been previously used for two decades in thyroid cancer research.

Due to the scope of this problem, the NIH and many journals now recommend or require cell-line authentication prior to grant approval or acceptance for publication, making it critical for scientists using cell cultures for research.

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Avoiding contamination with other cell types

General considerations

- Maintain good cell culture practices in general: to help ensure maintenance of a single cell type handle only a single cell line in a bio-safety hood at any one time.
- Clean the workspace thoroughly between using iusest for specific cell lines.
- Dedicate equipment to a single cell line including bottles and aliquots of cell culture medium and other reagents.
- Clearly label bottles; aliquots should also be clearly labeled and never be shared between cell lines.
- Empty waste receptacles regularly and use a disinfectant effective against both microbes and cell cultures.

Taking specific precautions

- Obtain cell lines from reputable cell banks.
- Develop and maintain a controlled cell banking regime of master and working cell banks. Adherence to these routines helps avoid contamination, cross-contamination, and genetic and phenotypic instability that can increase with each successive subculture.
- Perform authentication tests including STR profiling, isoenzyme analysis, and contamination tests.
- Avoid cell line transfers to colleagues. If transfers do occur, cells should be accompanied with by comprehensive documentation verifying the integrity of the material.

Obtaining cell lines from reputable cell banks, periodically checking the characteristics of the cell lines, and practicing good aseptic technique will help avoid cross-contamination. DNA fingerprinting, karyotype analysis, and isotype analysis can confirm the presence or absence of cross-contamination in your cell cultures.

Continuous monitoring of cultured cells

Regular checks of cell-line morphology and growth characteristics by phase contrast microscopy and comparison with reference images can indicate early problems. Such clues include identification of unexpected morphology or growth characteristics in a cell line soon after recovery from frozen storage that could mean recovery of the wrong vial from storage. Printed labels including the cell- line name in its correct format must have adhesive suitable for low temperature storage, as inappropriately adhered labels will detach, leaving an unlabeled vial in the inventory.

The American Type Culture Collection (ATCC) has developed a Standard for human cell-line authentication that recommends STR profiling. STR analysis can be performed in most laboratories that can execute molecular techniques providing an easy, low-cost, and reliable method for the authentication of human cell lines.

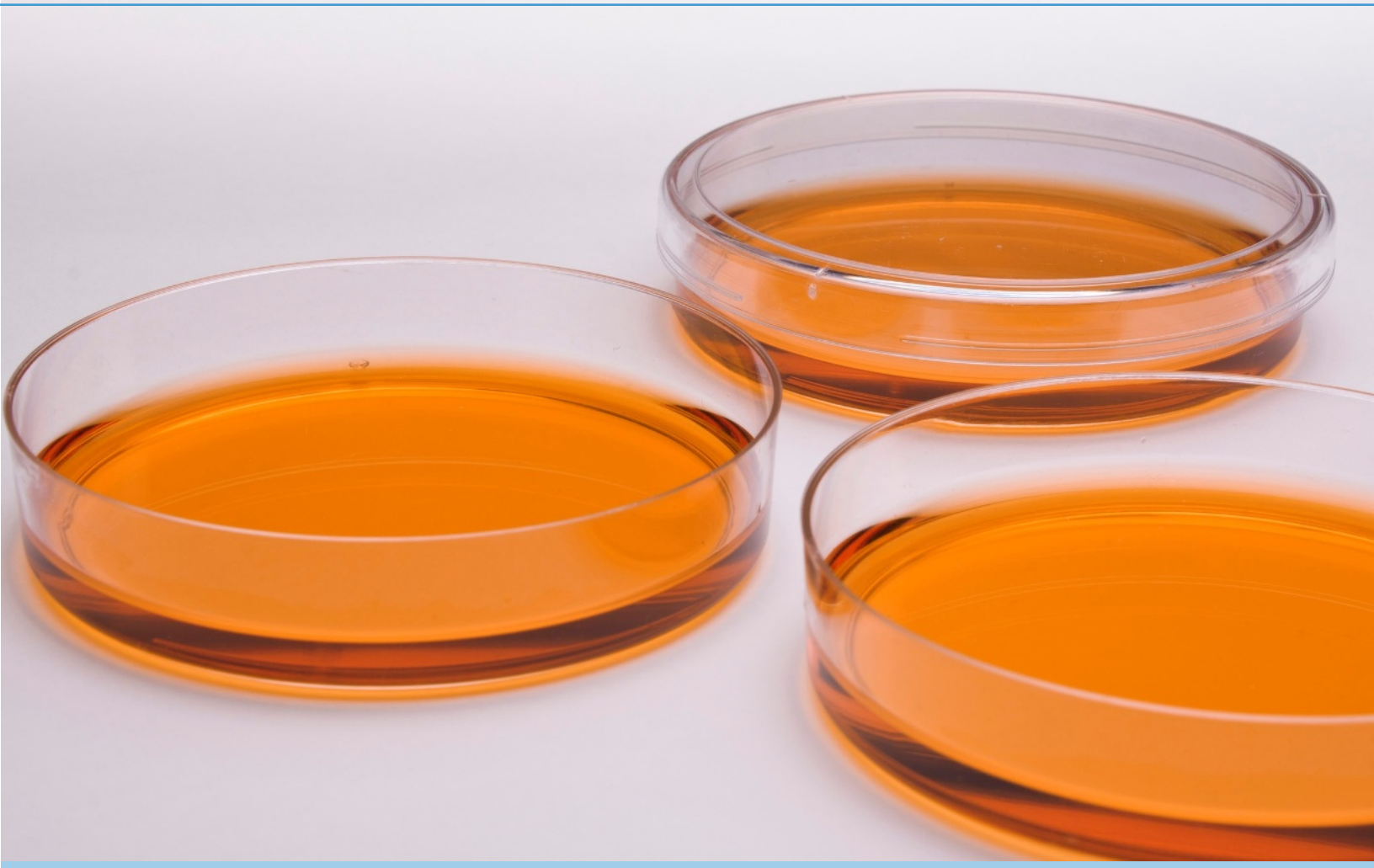

Image: It has been estimated that between 18% and 36% of cell lines may be contaminated or misidentified. Image from Dreamstime.

A combination of regular identity testing and vigilance of good cell culture practices is required to provide valid cell cultures and to ensure that research is acceptable for peer review. Failure to adhere to these guidelines will lead to an undesirable legacy and invalid research.

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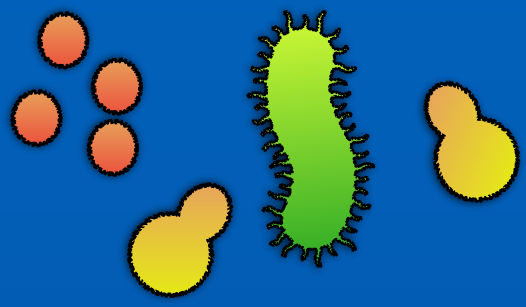
KNOW THE ENEMY

Contamination can cost lots of time and money. Knowing what to look for can downgrade a potential crisis to a minor annoyance



Bacteria	Most common type of infection Causes turbidity, changes pH Under microscope look like tiny, moving, granules
Yeast	Causes turbidity, no change in pH until later stages Under microscope appear as tiny, bright spheroids
Mycoplasma	Oftentimes invisible Can persist in culture without causing cell death, but may change cell behavior and influence experiments Requires testing with mycoplasma detection kits
Mold	Causes turbidity, no change in pH until later stages Under microscope appear as thin filaments
Virus	Invisible Can put scientists at risk Requires antibody staining, ELISA assays, or PCR with viral primers
Endotoxin	Found in water, sera, and certain reagents, may affect growth of cultures and influence experiments Look for "endotoxin free" reagents
Cross Contamination	Morphology may offer clue that cells are cross-contaminated Requires STR analysis Obtain cells from reputable cell banks and vendors Avoid accepting cells from other labs without authenticating

PREVENT & PROTECT

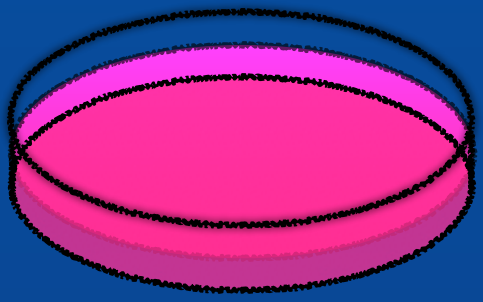
Practice aseptic technique

Check cells under microscope everyday

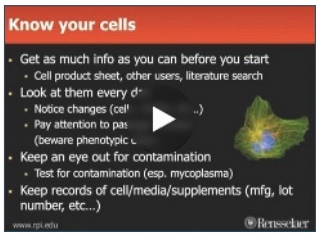
Routinely test for mycoplasma and decontaminate all equipment

Let hood warm up properly and don't overcrowd with supplies

Zip it! Talking while working can contaminate your cultures

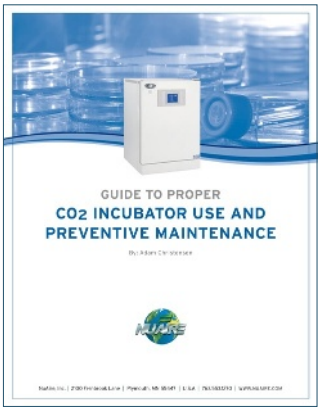


Resources



[Webinar] How to Set Up & Run a Successful Cell Culture Lab

This webinar touches on three factors that contribute to a successful lab as well as offer proven tips on how to ensure that your research yields reliable results.



[White Paper] CO_2 Incubator: Proper Use and Preventive Maintenance

This guide helps lab managers understand risks and best practices as they develop training for CO_2 incubator users and establish a preventive maintenance program.

[White Paper] Ultralow Freezer: Proper Use and Preventive Maintenance

Minus 80 freezers generally have a service life of 12 to 15 years. The service life of the freezer may be increased by following the maintenance tips in this document for setup, operation, and preventive maintenance.

[ebook] 10 Steps to Cleaning a Spill in a Biosafety Cabinet

It happens at some point to even the most seasoned laboratory technician that a spill occurs within the Biosafety Cabinet (BSC). Taking precautionary measures before and during your work with hazardous materials could help keep you and others safe. Remember, if a spill occurs, don't panic. Here are some simple steps to keep you and your laboratory safe.

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