A Guide to Understanding and Managing Cell Culture Contamination

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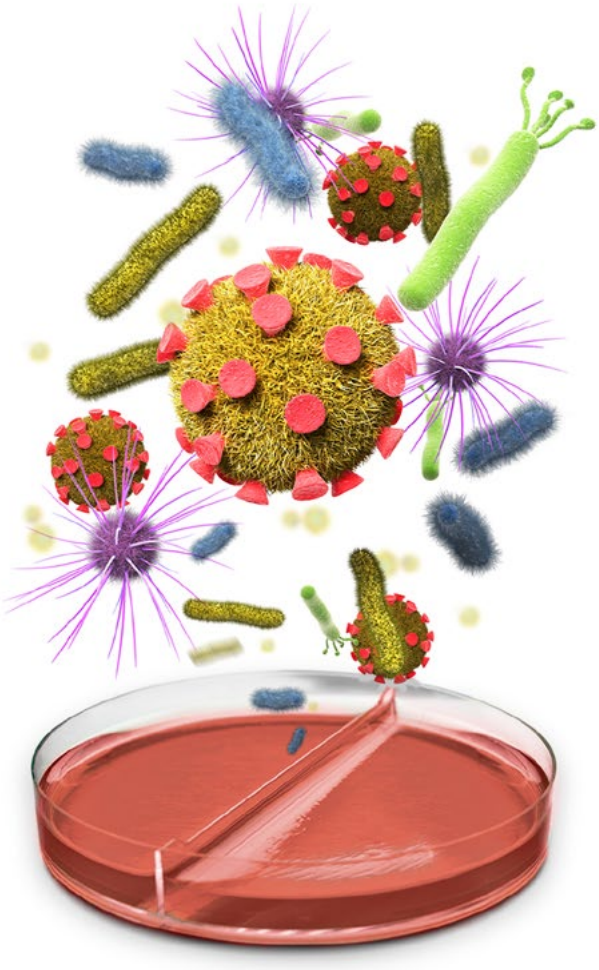

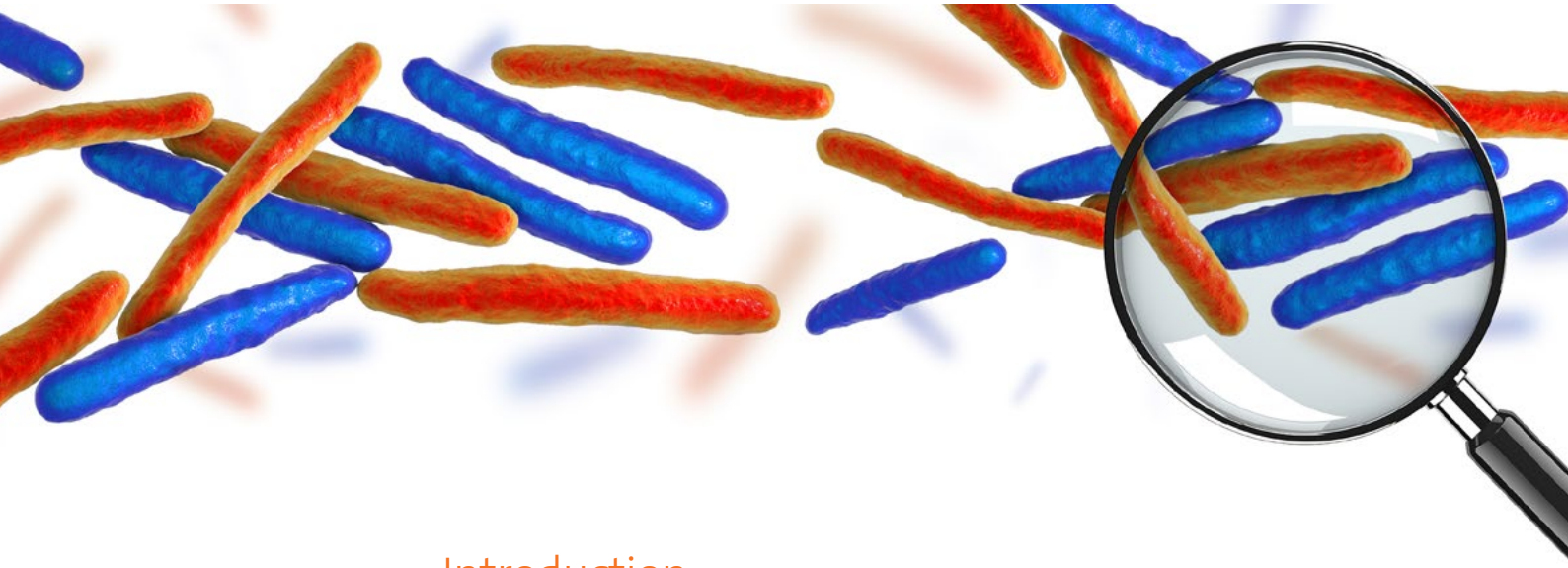

Introduction

No cell culture problem is as universal as that of culture loss due to contamination. All cell culture laboratories and cell culture workers have experienced it. Culture contaminants may be biological or chemical, seen or unseen, destructive or seemingly benign, but in all cases they adversely affect both the use of your cell cultures and the quality of your research. Contamination problems can be divided into three classes:

- *Minor annoyances* – when up to several plates or flasks are occasionally lost to contamination;
- *Serious problems* – when contamination frequency increases or entire experiments or cell cultures are lost;
- *Major catastrophes* – contaminants (usually other cell lines or mycoplasma) are discovered that call into doubt the validity of your past or current work.

The most obvious consequence of cell culture contamination is the loss of your time, money (for cells, culture vessels, media, and sera), and effort spent developing cultures and setting up experiments. However, the less obvious consequences are often more serious (Table 1). First, there are the adverse effects on cultures suffering from undetected chemical or biological contaminants. These hidden (cryptic) contaminants can achieve high densities altering the growth and characteristics of the cultures. Worse yet are the potentially inaccurate or erroneous results obtained by unknowingly working with these cryptically contaminated cultures.

Products, such as vaccines, drugs, or monoclonal antibodies, manufactured by these cultures will probably be useless. For some researchers, the most serious consequence of contamination is suffering the embarrassment and damage to their reputation that results when they notify collaborators or journals that their experimental results are faulty and must be retracted due to contaminants in their cultures.

Preventing all cell culture contamination has long been the dream of many researchers, but it is for most, an impractical, if not impossible, dream. **Contamination cannot be totally eliminated, but it can be managed to reduce both its frequency of occurrence and the seriousness of its consequences.** The goal of this guide is to review the nature of cell culture contamination and the problems it causes, and then to explore some of the key concepts and practical strategies for managing contamination to prevent the loss of valuable cultures and experiments.

Table 1. Some Consequences of Contamination

- Loss of time, money, and effort
- Adverse effects on the cultures
- Loss of valuable products
- Personal embarrassment
- Inaccurate or erroneous experimental results

Technical Assistance

The Corning Life Sciences Scientific Support Team is available to work with customers on their culturing problems. For contact information, visit:

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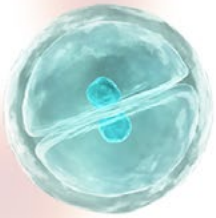
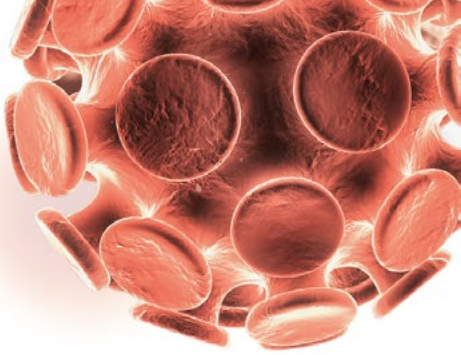




What Are the Major Cell Culture Contaminants?

A cell culture contaminant can be defined as some element in the culture system that is undesirable because of its possible adverse effects on either the system or its use. These elements can be divided into two main categories: **chemical contaminants** and **biological contaminants.**

Chemical Contamination

Chemical contamination is best described as the presence of any nonliving substance that results in undesirable effects on the culture system. To define further is difficult; even essential nutrients become toxic at high enough concentrations. Nor is toxicity the only concern since hormones and other growth factors found in serum can cause changes that, while not necessarily harmful to cultures, may be unwanted by researchers using the system.[reviewed in 1,2]

Media

The majority of chemical contaminants are found in cell culture media and come either from the reagents and water used to make them, or the additives, such as sera, used to supplement them. Reagents should always be of the highest quality and purity and must be properly stored to prevent deterioration. Ideally, they should be either certified for cell culture use by their manufacturer or evaluated by the researcher before use. Mistakes in media preparation protocols, reading reagent bottle labels, or weighing reagents are other common sources of chemical contamination.[3]

Table 2. Types and Sources of Potential Chemical Contaminants

- Metal ions, endotoxins, and other impurities in media, sera, and water
- Plasticizers in plastic tubing and storage bottles
- Free radicals generated in media by the photoactivation of tryptophan, riboflavin, or HEPES exposed to fluorescent light
- Impurities in gases used in CO_2 incubators
- Deposits on glassware, pipets, instruments, etc., left by disinfectants or detergents, antiscaling compounds in autoclave water, residues from aluminum foil or paper
- Residues from germicides or pesticides used to disinfect incubators, equipment, and labs

Sera

Sera used in media have long been a source of both biological and chemical contaminants. Due to cell culture-based screening programs currently used by good sera manufacturers, it is unusual to find a lot of fetal bovine serum that is toxic to a majority of cell cultures. However, it is common to find substantial variations in the growth promoting abilities of different lots of sera for particular cell culture systems, especially for cultures that have specialized or differentiated characteristics. Uncontrollable lot-to-lot variation in hormone and growth factor concentrations makes this problem inevitable; careful testing of sera before purchase, or switching to serum-free media can avoid these problems.

Remember also that serum proteins in the medium have the ability to bind substantial quantities of chemical contaminants, especially heavy metals, which may have entered the culture system from other sources, making them less available to the cells, and therefore, less toxic. As a result, switching from serum-containing medium to a serum-free system can unmask these toxic chemical contaminants, exposing the cells to their adverse effects.

Water

The water used for making media and washing glassware is a frequent source of chemical contamination and requires special care to ensure its quality. Traditionally, double or triple glass distillation was considered to be the best source of high quality water for cell culture media and solutions. Now most purification systems combine reverse osmosis, ion exchange, and ultrafiltration and are capable of removing trace metals, dissolved organic compounds, and endotoxins. However, these systems must be properly maintained and serviced to ensure continued water quality. Because of its aggressive solvent characteristics, highly purified water can leach potentially toxic metal ions from glassware or metal pipes, and plasticizers from plastic storage bags, vessels, or tubing. These contaminants can then end up in medium or deposited on storage vessels and pipets during washing and rinsing. Water used to generate steam in autoclaves may contain additives to reduce scale buildup in pipes; these potentially toxic additives can also end up on glassware.

Endotoxins

Endotoxins, the lipopolysaccharide-containing by-products of gram negative bacteria, are another source of chemical contaminants in cell culture systems. Endotoxins are commonly found in water, sera, and some culture additives (especially those manufactured using microbial fermentation) and can be readily quantified using the **L**imulus **A**mebocyte **L**ysate assay (LAL).

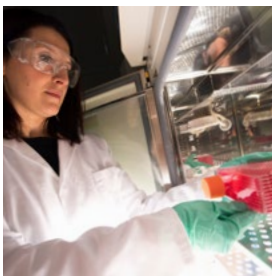
These highly biologically reactive molecules have major influences *in vivo* on humoral and cellular systems. Studies of endotoxins using *in vitro* systems have shown that they may affect the growth or performance of cultures and are a significant source of experimental variability.[reviewed in 6,39] Furthermore, since the use of cell culture-produced therapeutics, such as hybridomas and vaccines, are compromised by high endotoxin levels, efforts must be made to keep endotoxin levels in culture systems as low as possible.

For additional information on endotoxin problems, search for keyword: "endotoxins" on our website at: **www.corning.com/lifesciences**

In the past, sera have been a major source of endotoxins in cell cultures. As improved endotoxin assays (LAL) led to an increased awareness of the potential cell culture problems associated with endotoxins, most manufacturers have significantly reduced levels in sera by handling the raw products under aseptic conditions. Poorly maintained water systems, especially systems using ion exchange resins, can harbor significant levels of endotoxin-producing bacteria and may need to be tested if endotoxin problems are suspected or discovered in the cultures.

Storage Vessels

Media stored in glass or plastic bottles that have previously contained solutions of heavy metals or organic compounds, such as electron microscopy stains, solvents, and pesticides, can be another source of contamination. The contaminants can be adsorbed onto the surface of the bottle or its cap (or absorbed into the bottle if it is plastic) during storage of the original solution. If during the washing process they are only partially removed, then once in contact with culture media they may slowly leach back into the solution. Residues from chemicals used to disinfect glassware, detergents used in washing, or some aluminum foils and wrapping papers for autoclaving or dry heat sterilization can also leave potentially toxic deposits on pipets, storage bottles, and instruments.



Fluorescent Lights

An important but often overlooked source of chemical contamination results from the exposure of media containing HEPES (N-[2-hydroxyl ethyl] piperazine-N'-[2-ethanesulfonic acid]) — an organic buffer commonly used to supplement bicarbonate-based buffers), riboflavin or tryptophan to normal fluorescent lighting. These media components can be photo-activated producing hydrogen peroxide and free radicals that are toxic to cells; the longer the exposure the greater the toxicity.[4] Short-term exposure of media to room or hood lighting when feeding cultures is usually not a significant problem; but leaving media on lab benches for extended periods, storing media in walk-in cold rooms with the lights on, or using refrigerators with glass doors where fluorescent light exposure is more extensive, will lead to a gradual deterioration in the quality of the media.

Incubators

The incubator, often considered a major source of biological contamination, can also be a source of chemical contamination. The gas mixtures (usually containing carbon dioxide to help regulate media pH) perfused through some incubators may contain toxic impurities, especially oils or other gases such as carbon monoxide that may have been previously used in the same storage cylinder or tank. This problem is very rare in medical grade gases, but more common in the less expensive industrial grade gas mixtures.[5]

Care must also be taken when installing new cylinders to make sure the correct gas cylinder is used. Other potential chemical contaminants are the toxic, volatile residues left behind after cleaning and disinfecting incubators. Disinfectant odors should not be detectable in a freshly cleaned incubator when it is placed back into use.

Keep in mind that chemical contaminants tend to be additive in cell culture; small amounts contributed from several different sources that are individually nontoxic, when combined together in medium, may end up overloading the detoxification capabilities of the cell culture resulting in toxicity-induced stress effects or even culture loss.

Biological Contamination

Biological contaminants can be subdivided into two groups based on the difficulty of detecting them in cultures:

1. Those which are usually easy to detect — bacteria, molds, and yeast;
2. Those which are more difficult to detect, and as a result potentially more serious culture problems, — viruses, protozoa, insects, mycoplasmas, and other cell lines.

For a comprehensive review, see references 7 and 8.

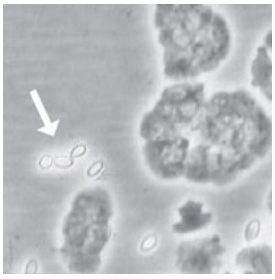


Figure 1. Photomicrograph of a low level yeast infection in a liver cell line (PLHC-1, ATCC® # CRL-2406TM). Budding yeast cells can been seen in several areas (arrows). At this low level of contamination, no medium turbidity would be seen; however, in the absence of antibiotics, the culture medium will probably become turbid within a day.

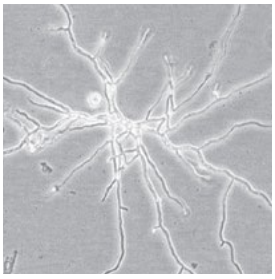


Figure 2. Photomicrograph of a small fungal colony growing in a cell culture. At this point, this colony would still be invisible to direct visual observation. If this culture was subcultured at this point, all of the cultures or experiments set up from it would soon be lost to fungal contamination.

Contaminants that go undetected are by far the most serious problem in cell culture. Ultimately, it is the length of time that a contaminant escapes detection that will determine the extent of damage it creates in your laboratory, research project, or to your reputation. (For a comprehensive review, see references 7 and 8.)

Bacteria, Molds, and Yeasts

Bacteria, molds, and yeasts are found virtually everywhere and are able to quickly colonize and flourish in the rich and relatively undefended environment provided by cell cultures. Because of their size and fast growth rates, these microbes are the most commonly encountered cell culture contaminants. In the absence of antibiotics, microbes can usually be readily detected in a culture within a few days of becoming contaminated, either by direct microscopic observation or by the effects they have on the culture (pH shifts, turbidity, and cell destruction; see Figures 1 and 2).

However, when antibiotics are routinely used in culture, resistant organisms may develop into slow growing, low level infections that are very difficult to detect by direct visual observation. Similar detection problems can occur with naturally slow growing organisms or very small or intracellular bacteria that are difficult to see during routine microscopic culture observation. These cryptic contaminants may persist indefinitely in cultures causing subtle but significant alterations in their behavior. By the time these cryptic contaminants are discovered, many experiments and cultures may have been compromised.

Viruses

Due to their extremely small size, viruses are the most difficult cell culture contaminants to detect in culture, requiring methods that are impractical for most research laboratories. Their small size also makes them very difficult to remove from media, sera, and other solutions of biological origin. Usually most viruses have stringent requirements for their original host species' cellular machinery (may also be tissue-specific) which greatly limits their ability to infect cell cultures from other species. Thus, although viruses may be more common in cell cultures than many researchers realize, they are usually **not considered** a serious problem unless they have cytopathic or other adverse effects on the cultures. (reviewed in 7,40) However, when Uphoff and colleagues[46] screened 577 human cell lines for the presence of murine leukemia viruses (MLV) they found nineteen (3.3%) were contaminated with MLV. Of these, 17 cell lines were shown to produce active retroviruses determined by using a product-enhanced reverse transcriptase PCR assay for reverse transcriptase activity. They believe passage of human tumor cells into immune-deficient mice to determine the tumorigenicity of the cells were most likely responsible for the MLV contaminations. It is also possible that the use of mouse feeder layers during the establishment of human cell lines or the practice of trying to clean up contaminated cell lines by passing them through mice could be responsible. This could also be a potential and serious problem for human stem cell lines grown on mouse feeder layers.

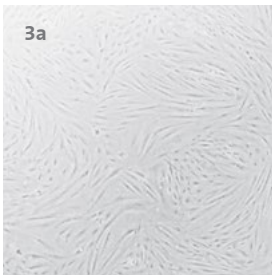
Since cytopathic viruses usually destroy the cultures they infect, they tend to be self-limiting. Thus, when cultures self-destruct for no apparent reason and no evidence of common biological contaminants can be found, cryptic viruses are often blamed (Figures 3a and 3b). Viruses are perfect culprits, unseen and undetectable; guilty without direct evidence. This is unfortunate, since the real cause of this culture destruction may be something else, possibly mycoplasma or a chemical contaminant, and as a result will go undetected to become a more serious problem.

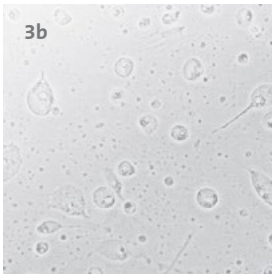
A major concern of using virally infected cell cultures is not their effects on the cultures but rather the potential health hazards they pose for laboratory personnel. Special safety precautions should always be used when working with tissues or cells from humans or other primates to avoid possible transmission of viral infection (HIV, hepatitis B, Epstein-Barr, simian herpes B virus, among others)

from the cell cultures to laboratory personnel.[9] Contact your safety office for additional assistance if in doubt as to appropriate procedures for working with potentially hazardous tissues, cultures, or viruses.[44,49]

Protozoa

Both parasitic and free-living, single-celled protozoa, such as amoebas, have occasionally been identified as cell culture contaminants. Usually of soil origin, amoebas can form spores and are readily isolated from the air, occasionally from tissues, as well as throat and nose swabs of laboratory personnel. They can cause cytopathic effects resembling viral damage and completely destroy a culture within ten days. Because of their slow growth and morphological similarities to cultured cells, amoebas are somewhat difficult to detect in culture, unless already suspected as contaminants.[7] Fortunately, reported cases of this class of contaminants are very rare, but it is important to be alert to the possibility of their occurrence.





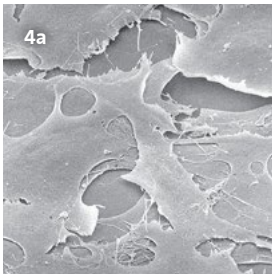
Figures 3a and 3b. Photomicrographs of a winter flounder (Pseudopleuronectes americanus) fibroblast-like cell culture. Figure 3a shows an apparently healthy early passage culture; Figure 3b shows the same culture approximately 24 hours later. Electron microscopy showed virus-like particles in these cells. Multiple attempts to establish cell lines from this species were unsuccessful and showed cytopathic effects that appeared to be caused by an unknown virus.

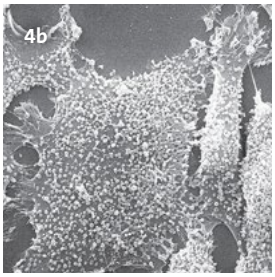
Invertebrates

Insects and arachnids commonly found in laboratory areas, especially flies, ants, cockroaches, and mites, can both be culture contaminants as well as important sources of microbial contamination. Warm rooms are common sites of infestation. By wandering in and out of culture vessels and sterile supplies as they search for food or shelter, they can randomly spread a variety of microbial contaminants. Occasionally they are detected by the trail of "footprints" (microbial colonies) they leave behind on agar plates, but usually they don't leave any visible signs of their visit other than random microbial contamination. Mites can be a serious problem in plant cell culture facilities, especially those doing large scale plant propagation. Although bacteria, molds, and yeast may sometimes appear to 'jump' from culture to culture, these multi-legged contaminants really can. While not nearly as common as other culture contaminants, it is important to be alert to the presence of these invertebrates in culture areas.

Mycoplasmas

Mycoplasmas were first detected in cell cultures by Robinson and coworkers in 1956. They were attempting to study the effects of PPLO (pleuropneumonia-like organisms — the original name for mycoplasma) on HeLa cells when they discovered that the control HeLa cultures were already contaminated by PPLO.[10] In addition, they discovered that the other cell lines currently in use in their laboratory were also infected with mycoplasma, a common characteristic of mycoplasma contamination. Based on mycoplasma testing done by the FDA, ATCC, and two major cell culture testing companies, at least 11% to 15% of the cell cultures in the United States are currently infected by mycoplasmas (Table 3). Since many of these cultures were from laboratories that test routinely for mycoplasma, the actual rates are probably higher in the many laboratories that do not test at all.[11-13] In Europe, mycoplasma contamination levels were found to be even higher: over 25% of 1949 cell cultures from the Netherlands and 37% of 327 cultures from former Czechoslovakia were positive.[14] The Czechoslovakia study had an interesting, but typical finding: 100% of the cultures from labs without mycoplasma testing programs were contaminated, but only 2% of the cultures from labs that tested regularly. Other countries may be worse: 65% of the cultures in Argentina and 80% in Japan were reported to be contaminated by mycoplasma in other studies.[11]





Figures 4a and 4b. These scanning electron micrographs show 3T6 cells (ATCC® # CCL-96TM) with (4b) and without (4a) mycoplasma infections. The level of contamination of these cells by the mycoplasma shown here is typical of contaminated cells. Examination of this contaminated culture by phase contrast microscopy did not show any evidence of contamination; nor did the medium show any turbidity.

Unfortunately, mycoplasmas are not relatively benign culture contaminants but have the ability to alter their host culture's cell function, growth, metabolism, morphology, attachment, membranes, virus propagation and yield, interferon induction and yield, cause chromosomal aberrations and damage, and cytopathic effects including plaque formation.[12] Thus, the validity of any research done using these unknowingly infected cultures is questionable at best. (See references 11, 12, and 15-18 for good overviews of this very serious mycoplasma contamination problem.)

What gives mycoplasmas this ability to readily infect so many cultures? Three basic characteristics: a) these simple, bacteria-like microbes are the smallest self-replicating organism known (0.3 to 0.8 µm in diameter), b) they lack a cell wall, and c) they are fastidious in their growth requirements. Their small size and lack of a cell wall allow mycoplasmas to grow to very high densities in cell culture (10^7 to 10^9 colony forming units/mL are common) often without any visible signs of contamination — no turbidity, pH changes, or even cytopathic effects (Figures 4a and 4b). Even careful microscopic observation of live cell cultures cannot detect their presence. These same two characteristics also make mycoplasmas, like viruses, very difficult to completely remove from sera by membrane filtration.[48] In addition, their fastidious growth requirements (unfortunately, easily provided for by cell cultures) make them very difficult to grow and detect using standard microbiological cultivation methods. Thus, these three simple characteristics, combined with their ability to alter virtually every cellular function and parameter, make mycoplasmas the most serious, widespread, and devastating culture contaminants.

Mycoplasmas have been described as the "crabgrass" of cell cultures, but this is too benign a description for what are the most significant and widespread cell culture contaminants in the world. Unfortunately, even with the advances in detection methods (discussed in detail later) mycoplasma infection rates (Table 3) have not changed noticeably since they were first detected in cell cultures. Aggressive management against mycoplasma contamination must be the central focus for any cell culture laboratory contamination or quality control program.[16]

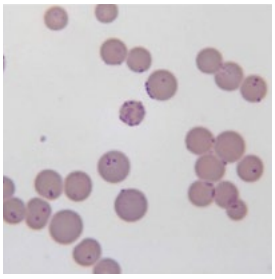


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Table 3. Mycoplasma Contamination of Cell Cultures

Number of Cultures Tested		Number Positive
Food and Drug Administration (FDA) (1970s to 1990s) (11)	20,000	Over 3,000 (15%)
Bionique Testing Laboratories (several years prior to 1993) (41)	11,000	1,218 (11.1%)
Microbiological Associates (1985–1993) (13)	2,863	370 (12.9%)
ATCC (1989 to 1994) (42)	5,362	752 (14%)

Table 4. Some HeLa Contaminated Cell Lines

Detroit 6 (CCL-3)	Detroit 98 (CCL-18)	WISH (CCL-25)*
Minnesota-EE (CCL-4)	NCTC 2544 (CCL-19)	Giardia Heart (CCL-27)
L132 (CCL 5)*	Conjunctiva (CCL-20.2)*	Wilm's Tumor (CCL-31)
Intestine 407 (CCL-6)*	AV3 (CCL-21)*	FL (CCL- 62)*
Chang Liver (CCL-13)	HEp-2 (CCL-23)*	
KB (CCL-17)*	J-111 (CCL-24)	

CCL Number is the ATCC catalog designation. All except CCL-20.2, CCL-31, and CCL-62 were shown to be HeLa by Gartler in 1968.[20] Those marked with an asterisk can be found in the Cell Biology Collection on the ATCC website (www.atcc.org) where they are marked as HeLa contaminants.

Cross-Contamination by other Cell Cultures

With the advent of improved karyotyping methods in the late 1950's, it soon became apparent that some cell lines were cross-contaminated by cells of other species.[7] In 1966, Gartler used isoenzyme analysis to show that 20 commonly used human cell lines were intraspecies contaminated by HeLa cells.[19,20] Contaminated is actually a misnomer since in fact 100% of the original cells had been replaced by the HeLa contaminant. Unfortunately, the scientific community was slow to respond to this very serious problem. Tests done at one research center on 246 cell lines over an 18-month period prior to 1976 showed that nearly 30% were incorrectly designated: 14% were the wrong species, and 25% of the human cell lines were HeLa cells.[21] A 1981 survey of cultures showed over 60 cell lines that were actually HeLa cells, 16 other human cell lines contaminated by non-HeLa human cell lines, and 12 cases of inter-species contamination (Table 4). Nor is the problem limited to contamination by HeLa cells. The advent of DNA analysis has shown that cells from a variety of sources have contaminated many other cell lines.[42] The Database of Cross-contaminated or Misidentified Cell Lines (as of December 2016) maintained by the International Cell Line Authentication Committee (ICLAC) (**iclac.org/databases/cross-contaminations**) shows 488 cell lines that are cross-contaminated or misidentified.[52] Every researcher using cell lines should check their cell lines on this site at least yearly to ensure their authenticity.

The seriousness of cross-contamination, while not as common as microbial contamination, cannot be overstated. The validity of experimental results from cultures having inter- or intra-species contamination is, at the very least, questionable. Furthermore, their use can lead to the embarrassment of having to retract published results. Whenever the invading cell is better adapted to the culture conditions and thus faster growing than the original cells, it will almost always completely replace them. Because of the outward physical similarities of different cell lines and the wide morphological variations that can be caused by the culture environment, it is impossible to rely only on microscopic observation to screen for cross-contamination of cultures. Simple accidents are one of the most common means by which other cell lines gain entry into cultures and will be discussed separately in the next section.

Remember, the seriousness of any culture contaminant is usually directly proportional to the difficulty of detecting it; those that go undetected the longest have the most serious consequences. Cultures containing nonlethal (but not harmless), cryptic chemical, or biological contaminants are sometimes used in research for months or even years before being uncovered; during this time the quality and validity of all research done with those cultures is compromised, as is the reputation of the researchers using them.

What Are the Sources of Biological Contaminants?

To reduce the frequency of biological contamination, it is important to know not only the nature and identity of the contaminants but also where they come from and how they gain entry into cultures. This section will detail some of the most common sources of biological contaminants.[3]

Table 5. How Do Biological Contaminants Enter Cultures?

- Contact with nonsterile supplies, media, or solutions
- Particulate or aerosol fallout during culture manipulation, transportation, or incubation
- Microorganisms swimming, crawling, or growing into culture vessels
- Accidents and mistakes

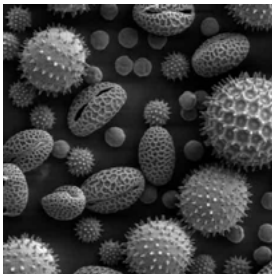
Nonsterile Supplies, Media, and Solutions

Unintentional use of nonsterile supplies, media, or solutions during routine cell culture procedures is a major source of biological contaminants. These products may be contaminated as a result of improper sterilization or storage, or may become contaminated during use.

Glassware, including storage bottles and pipets, is usually sterilized by autoclaving or dry heat sterilization. Serious contamination outbreaks are frequently traced to improper maintenance or operation of sterilization autoclaves and ovens. Packing too much into an autoclave or dry heat oven will cause uneven heating, resulting in pockets of nonsterile supplies. Using too short a sterilization cycle, especially for autoclaving volumes of liquids greater than 500 mL per vessel or solutions containing solids or viscous materials, such as agar or starches, is a common mistake. The size, mass, nature, and volume of the materials to be sterilized must always be considered and the cycle time appropriately adjusted to achieve sterility.[23,60] Then, once achieved, sterility must be maintained by properly storing the supplies and solutions in a dust- and insect-free area to prevent recontamination. Care must also be taken to avoid condensation on bottles of solutions stored in refrigerators and cold rooms. Of course, good aseptic technique is also required to maintain the sterility of properly sterilized supplies and solutions once they are in use.

Plastic disposable cell culture vessels, pipets, centrifuge tubes, etc., are usually sterilized by their manufacturer using a high intensity gamma or electron beam radiation source after they are sealed in their packaging. This is a very reliable process, and care must be taken when opening and resealing the packaging to avoid contaminating the products within.

Most media, sera, and other animal-derived biologicals are not heat sterilizable and require membrane filtration to remove biological contaminants. Radiation may also be used to accomplish this. Products filter sterilized in your laboratory should always be tested for sterility before use (discussed in detail later); commercially produced sterile products are tested by the manufacturer before being sold. While filtration through 0.2 µm membranes is very effective in removing most biological contaminants, it cannot guarantee the complete removal of viruses and mycoplasmas, especially in sera.[16,18,24] In an excellent review of the rates and sources of mycoplasma contamination,[25] Barile and coworkers reported that 104 out of 395 lots (26%) of commercial fetal bovine sera tested were contaminated by mycoplasma. They concluded in the early 1970s that animal sera were among the major sources of cell culture contamination by mycoplasma. Many sera manufacturers responded to this problem over the next decade by improving both filtration and testing procedures; they currently use serial filtration through at least three filter membranes rated at 0.1 µm or smaller to remove mycoplasmas. This approach has been very successful at reducing the problem of mycoplasma in sera and other animal-derived products.[16,54] While these products are no longer a major source of mycoplasma contamination, they must still be considered as potential sources to be evaluated whenever mycoplasmas are detected in cultures.



Airborne Particles and Aerosols

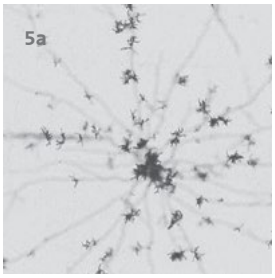
In most laboratories, the greatest sources of microbial contamination are airborne particles and aerosols generated during culture manipulations. The microbial laden particles are relatively large (generally 4 to 28 µm in diameter) and settle at a rate of approximately one foot per minute in still air. As a result, the air in a sealed, draft-free room or laboratory (no people, open windows or doors, air handling units, air conditioners, etc.) is virtually free of biological contaminants. However as soon as people enter the room, particles that have settled out will be easily resuspended. In addition certain equipment and activities can generate large amounts of microbial laden particulates and aerosols: pipetting devices, vacuum pumps and aspirators, centrifuges, blenders, sonicators, and heat sources such as radiators, ovens, refrigerators and freezers. Animal care facilities and the animals they house are especially serious particle and aerosol generators, and should always be kept as far from the culture area as possible.

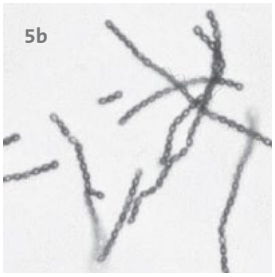
McGarrity used a cell culture that was intentionally infected with mycoplasma as a model to study how mycoplasmas are spread in a laminar flow hood during routine subculturing procedures.[26] (This reference is especially recommended for a better understanding of how mycoplasma can be spread in a lab.) Following trypsinization of the infected culture in a laminar flow hood, live mycoplasma were isolated from the technician, the outside of the flask, a hemocytometer, the pipettor, and the outside of the pipet discard pan. Live mycoplasma could even be successfully recovered from the surface of the laminar flow hood *four to six days later*. A clean culture, that was subcultured once a week in the same hood following the work with the contaminated cells, tested positive for mycoplasma after *only 6 weeks*. It is easy to understand from this study how the entry of a single mycoplasma infected culture into a laboratory can quickly lead to the infection of all the other cultures in the laboratory. This explains the frequent finding that if one culture in a laboratory is mycoplasma contaminated then usually most, if not all of the other cultures, will be as well. **Currently, the major source of mycoplasma contamination is infected cultures acquired from other research laboratories.**

Another major source of particulates and aerosols are laboratory personnel. Street clothes and dirty lab coats are dust magnets. Placing a dust-laden sleeve into a laminar flow hood generates a cloud of dust particles that can easily fall into and contaminate cultures during routine processing. Talking and sneezing can generate significant amounts of aerosols that have been shown to contain mycoplasma.[26] Mouth pipetting is both a source of mycoplasma contamination and a hazard to personnel and must not be permitted under any circumstances. Dry, flaky skin is another source of contamination-laden particles; this common condition is aggravated by the frequent hand washing required in the laboratory; even the lotions designed to moisten dry skin have occasionally been found to be contaminated. Routinely donning clean gloves before working in the hood is highly recommended both for personal safety and to prevent contamination. Some laboratory personnel shed yeast-containing particles for several days following bread making or beer brewing at home. Attempts by these individuals at cell culturing during this period often end in failure due to yeast contamination.

Incubators, especially those maintained at high humidity levels, can be a significant source of biological contamination in the laboratory. Dirty water reservoirs, and shelves or culture vessels soiled by spilled media, allow the growth of spore-generating fungi. The fans used in many incubators to circulate the air and prevent temperature stratification can then spread these spores and other particulates. Some incubators humidify incoming gases by bubbling them through the water reservoirs at the bottom of the incubator; the aerosols generated by this will quickly spread any contaminants in the water.

While laminar hoods and incubators are the major sites where biological contamination occurs, transporting cultures between these two sites also provides opportunities for contamination. Most cell culture laboratories try very hard to keep their incubators and laminar flow areas clean, but sometimes they overlook the potential sources of contamination found in less clean laboratory areas transversed going from one location to the other. Rooms containing open windows, air conditioners, microbiology and molecular biology work areas, and the other major particle generators discussed above, add to the potential hazards of moving cultures around the laboratory. This problem increases both with the distance traveled and when the culture vessels, such as dishes and microplates, are unsealed.





Figures 5a and 5b. Photomicrographs of contaminants growing on the outside surfaces of culture vessels. Eventually, these organisms may grow into the culture.

Microorganisms Swimming, Growing, and Crawling into Cultures

Unsealed culture plates and dishes, as well as flasks with loose caps to allow gas exchange, provide another common way for contaminants to enter cultures. It is very easy for the space between the top and bottom sidewalls of a dish, or a flask and its cap to become wet by capillary action with medium or condensation. This thin film of liquid then provides a liquid bridge or highway for microorganisms to either swim or grow into the culture vessel.

Even without any detectable film, fungi, as well as other microorganisms, can grow on the outside of culture vessels (Figures 5a and 5b); eventually their hyphae grow right up the side wall of the dish or past the cap into the neck of the flask. This is more often observed in long-term cultures (a month or more) maintained in the same unsealed culture vessel. Very small insects and other invertebrates can also make temporary visits into unsealed cultures, especially dishes and plates, leaving behind (unless they fall in and drown) only the contaminants carried on their feet.

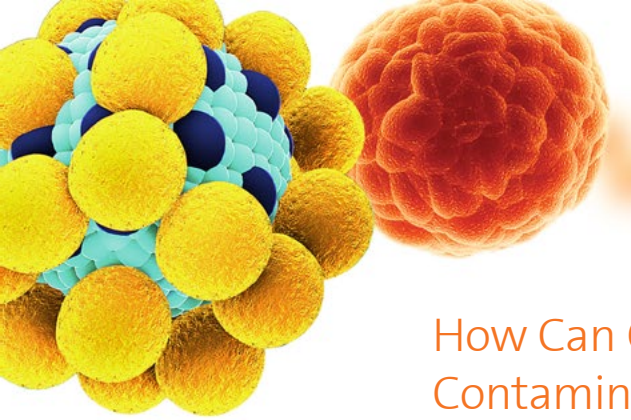
Accidents

Accidents are often overlooked as a significant source of cell culture problems. An accident is defined as "an undesirable or unfortunate happening, unintentionally caused and usually resulting in harm, injury, damage, or loss" (Webster's Encyclopedic Unabridged Dictionary, 1989). Cell culture-related accidents are one of the leading causes of cross-contamination by other cell cultures. The following actual cases demonstrate how relatively simple accidents can result in serious cross-contamination problems:

- A technician retrieved a vial labeled WI-38 from a liquid nitrogen freezer thinking it contained the widely used diploid human cell line. Once in culture, it was immediately discovered to be a plant cell line derived from a common strain of tobacco called Wisconsin 38, also designated WI-38.
- Two separate research laboratories, both attempting to develop cell lines from primary cultures, shared a walk-in incubator. One lab used the acronyms HL-1, HL-2, etc., to identify the primary cultures they derived from human lung. The other lab worked with cultures derived from human liver, but they too (unknowingly) used the identical coding system. It wasn't long before a culture mix up occurred between the two laboratories showing the importance of carefully naming cell lines.[(reviewed in 59)]

Fortunately, both of the above accidental cross-contamination cases, although serious, were caught before they caused catastrophic problems. But how many times have similar accidents occurred and not been caught? Based on continuing reports in the literature[7,8,19-22] many researchers have not been lucky enough to identify and correct their mistakes.

The information presented above is designed to provide you with an increased awareness and understanding of the nature of biological and chemical contamination, and its serious consequences. The rest of this guide will cover some basic ideas, techniques, and strategies for actively detecting and combating cell culture contamination in your own laboratory.



How Can Cell Culture Contamination be Controlled?

Cell cultures can be managed to reduce both the frequency and seriousness of culture-related problems, especially contamination. Lack of basic culture management procedures, especially in larger laboratories, frequently leads to long-term problems, making contamination more likely for everyone. One solution is to actively manage your cultures to reduce problems, and if necessary, set-up a program for use in your laboratory.[27,28] This program should be designed to meet the needs of your specific working conditions and be based on the nature of your past cell culture problems; it can be very simple and informal, or more structured if required.

The first step in managing cultures is to determine the extent and nature of the culture losses in your lab. Everyone in the laboratory should keep an accurate record for a month or more of all problems, no matter how minor or insignificant, that result in the loss of any cultures. These problems may not only be contamination-related but can also be from other causes such as incubator or equipment failures. Next, review the problems as a group to determine their nature, seriousness, and frequency. The group's findings may be surprising: what were thought to be individual and minor random occurrences of contamination often turn out to have a pattern and be more extensive than any individual realized. This problem sharing is often a painful process, but remember the goal is not to place blame but to appreciate the extent and nature of the problems confronting the laboratory. A critical part of this process is understanding the seriousness and actual costs of culture loss; placing a dollar value on these losses is often required before the full extent of the losses can be appreciated. It is very important for everyone in the laboratory to know and fully understand the answers to the following questions:

1. How much time, money, and effort have been invested in your cultures and experiments?
2. What are the consequences of their loss?
3. How expensive or difficult will it be to replace them?

Use the Cell Culture Log at the end of this guide to document and track your cell culture activities.

Once the nature and consequences of the problems in the laboratory are better understood, the need for a management system, if necessary, can be determined. Basic problem solving tools[2] can be used to help identify the source of problems; changes to minimize or prevent the problems from reoccurring can then be implemented.

The following suggestions, concepts, and strategies, combined with basic management techniques, can be used to reduce and control contamination (Table 6). These may require modification to fit your own needs and situation.

Table 6. Steps For Reducing Contamination Problems

- Use good aseptic techniques
- Reduce accidents
- Keep the laboratory clean
- Routinely monitor for contamination
- Use frozen cell repository strategically
- Use antibiotics sparingly if at all

Use Good Aseptic Techniques

Aseptic technique is designed to provide a barrier between microorganisms in the environment and your cultures and sterile supplies, yet permit you to work with them. There are many successful techniques for achieving and maintaining aseptic cell cultures; ultimately, your technique is "good" if it routinely protects both you and your cultures from contamination. Teaching aseptic technique is beyond the scope of this guide; the goal here is to review some of its basic tenets and present some suggestions for improving it. The reader is referred to Freshney[3] for a basic introduction to this very important area.

The first step in developing sound, rational aseptic techniques is a solid understanding of both the nature and potential sources of biological contamination. This is reviewed in the beginning of this guide and covered in many of the references.

The second step, based on the nature of your work, is to determine the level of risk or danger to yourself and other laboratory personnel and then design your culture techniques accordingly. This is especially true when working with cultures that are virally contaminated or derived from human and other primate sources. Ensure that all laboratory personnel have been trained in the safe handling and disposal of any potentially hazardous cultures and materials; refer to your facility's safety office for any necessary assistance or guidance.[9]

Next, based on the potential costs and consequences if the cultures are lost, determine how rigorous your technique must be and what degree of redundancy if any, is required. Very valuable or irreplaceable cultures can be carried by two or more workers using media from different sources and separate incubators to reduce the chance of their simultaneous loss.[27,28] Workers should wear clean gloves while working with cell cultures and change them periodically to prevent cross contamination. Evaluate if they need to be gowned or masked to reduce the potential for contamination. The nature of your working environment and any problems it may present must also be considered in choosing appropriate aseptic techniques. Certified laminar flow hoods and safety cabinets are recommended for use whenever possible. Some of the aseptic techniques taught in introductory microbiology classes for use on the open bench, such as flaming, while popular, are not appropriate or necessary in laminar flow hoods.[16]

The following suggestions are recommended to reduce the probability of contamination:

- Wearing gloves will minimize the chance of contaminating your cell cultures with microorganisms.
- Make it more difficult for microorganisms to gain entry by using sealed culture vessels whenever possible, especially for long-term cultures. The multiwell plates can be sealed with labeling tape or placed in sealable bags, 35 and 60 mm dishes can be placed inside 150 or 245 mm dishes. Use vented cap flasks (Figure 6) whenever possible. These have hydrophobic filter membranes that allow sterile gas exchange but prevent the passage of microorganisms or liquids.
- Avoid pouring media from cell culture flasks or sterile bottles by using 50 or 100 mL pipets or aseptic tubing sets to transfer larger volumes. Using disposable aspirator pipets and a vacuum pump is an economical way to quickly and safely remove medium from cultures. A drop of medium remaining on the vessel's threads after pouring can form a liquid bridge when the cap is replaced providing a means of entry for bacteria, yeasts, and molds. If pouring cannot be avoided, carefully remove any traces of media from the neck of the vessel with a sterile gauze or alcohol pad.
- Always carry unsealed cultures in trays or boxes to minimize contact with airborne contaminants. Square 245 mm dishes are excellent carriers for 96- and 384-well microplates, as well as for 35 mm and 60 mm dishes.
- Do not use the hood as a storage area. Storing unnecessary boxes, bottles, cans, etc. in the hood, besides adding to the bioburden, disrupts the airflow patterns.
- Never mouth pipette. Besides the risk of injury to laboratory personnel, mouth pipetting has been implicated as the likely source of human mycoplasma species (M. orale and M. salivarium) often found in cell cultures.[15]
- Use clean lab coats or other protective clothing to protect against shedding contaminants from skin or clothes. Their use should be restricted to the cell culture area to avoid exposure to dirt and dust from other areas.
- Work with only one cell line at a time in the hood, and always use separate bottles of media, solutions, etc., for each cell line to avoid possible cross-contamination. Use disinfectant to wipe down the hood's work surfaces between cell lines.
- Frequently clean water baths used for warming media or solutions. Better yet, avoid water baths entirely by using an incubator room or a bath filled with heated metal or glass beads for warming media and solutions. Wetting the outside of a bottle or tube with contaminated water before bringing it into a hood is never a good idea.[17]
- Use antibiotic-free media for all routine culture work; this is a very important concept and will be discussed in detail below.
- To reduce the possibility of contamination always use filtered pipet tips when using single- and multi-channel pipettors for cell culture.

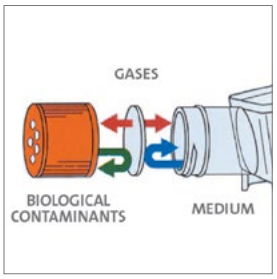


Figure 6. Vented cap flasks greatly reduce the opportunities for contamination in culture systems requiring gas exchange.

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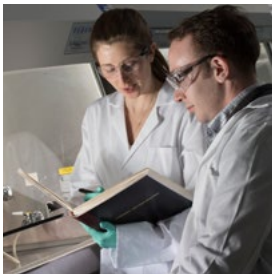
- Whenever possible, package sterile solutions, such as trypsin, L-glutamine, and antibiotics, in small volumes (i.e., stored in 15 mL tubes) to reduce the number of times each tube must be entered and thus reduces the probability of contamination.
- Biosafety cabinets and hoods should be turned on at least 15 minutes prior to use each day. Alternatively, keep hoods running 24 hours a day during the work week. Work surfaces should be wiped down with 70% ethanol, or other suitable disinfectants,[44] before and after each use and between cell lines.
- Do not use open flames, especially Bunsen burners, in laminar flow hoods. It is unnecessary, can damage the HEPA filter, and be counterproductive. The Centers for Disease Control and Prevention (CDC) states: "Open flames are not required in the near microbe-free environment of a biological safety cabinet. An open flame in a BSC, however, creates turbulence that disrupts the pattern of HEPA-filtered air being supplied to the work surface".[61] It is also a major safety issue. Serious hood explosions, fires, and injuries have resulted from gas leaking from Bunsen burners or an open flame igniting alcohol used as a disinfectant.
- Doors in the culture area should be kept closed while hood is in use. Opening a door can create a back draft and disrupts laminar flow in hoods. Consider replacing traditional doors with sliding doors to eliminate this problem, especially in heavy traffic areas.
- Minimize foot traffic behind and close to the BSC when performing work. There is increased likelihood of airflow disruption if the traffic is high, plus one can be easily distracted from their work if participating in discussions with colleagues.
- Do not use germicidal ultraviolet (UV) lamps to disinfect hoods: The NIH, CDC, NSF/ANSI, and the American Biological Safety Association all agree that ultraviolet lamps are not recommended, nor are they necessary. NSF Standard 49, the industry testing standard for all biohazard cabinetry, does not provide any performance criteria for UV lighting and specifically states "UV lighting is not recommended in Class II (laminar flow) biohazard cabinetry". Numerous factors affect the activity of the germicidal effect of UV light, which require regular cleaning, maintenance and monitoring to ensure germicidal activity. In addition, there are safety hazards associated with UV light exposure, which include cornea burns and skin cancer. (Ref. 43, 45, 58; see also Biosafety Technical Bulletin: Ultraviolet Lights in Biological Safety Cabinets **https://ncifrederick.cancer.gov/ehs/ibc/Media/Documents/UVLights.pdf**).

Reduce Opportunities for Accidents



Accidents usually involve people, and reducing them must take into consideration both human nature and stress. Accidents are far more likely on: a) Friday afternoons, b) the day before a vacation begins, c) with new employees, or d) when people are stressed, overworked, or rushed. The following suggestions can help reduce the confusion and misunderstanding that cause many accidents to happen in the laboratory.

- Be very careful when labeling solutions, cultures, etc. Always clearly indicate if solutions or other supplies have been sterilized. Reduce misunderstandings in crowded or busy labs by using a color coding system: assign each worker their own color for labeling tape and marking pen inks.
- Be very careful with the use and choice of acronyms. Everyone in the laboratory should understand and agree to their meaning.
- Whenever possible use standardized record-keeping forms; this simplifies their use and makes it more likely that good records will be kept.
- Use written protocols and formulation sheets when preparing media and solutions, listing the reagents used, lot numbers, weights, volumes, pH, and any special treatments that were done. These will both reduce the potential for errors as well as provide a valuable aid in tracking down the cause of problems.

Clean up the Work Area and Surrounding Environment

Reducing the amount of airborne particulates and aerosols in the laboratory, especially around the incubator and the laminar flow hood, will reduce the amount of contamination. Routinely wipe floors and work surfaces to keep down dust. Incubators, especially those that maintain high humidity levels, require periodic cleaning and disinfecting. Often overlooked but important sources of contaminants are the water baths used to thaw sera and warm media. Dirty water baths not only coat bottles with a layer of heavily contaminated water right before they are placed under the hood, but the water dripping from bottles generates heavily contaminated aerosols which can end up on lab coats and hands. Water baths should be emptied and cleaned on a regular basis, well before odor

or visible turbidity develops. Pipet disposal trays and buckets, and other waste containers provide a source of potentially heavily contaminated materials in close proximity to the laminar flow hood and are a potential mycoplasma source.[26] Waste containers should be emptied daily and the wastes disposed of safely. Autoclaving of any wastes that have been in contact with cells is recommended.

The cooling coils on refrigerators and freezers are a major source of microbial laden airborne particulates that are often overlooked in otherwise very clean laboratories. These should be vacuumed at least yearly; besides removing a significant source of contamination; regular vacuuming will extend the life of the cooling units and allow them to run more efficiently.

Wet ice can be a source of contamination in a laminar flow hood. Alternate cooling methods should be used if possible.

Some laboratories may also need to consider a pest management program to reduce the presence of mice, ants, cockroaches, and other multi-legged creatures that can be sources of contamination. Potted plants, although attractive, can provide a home for these creatures and should not be kept in the culture vicinity. Care must be taken when using pesticides as part of a pest management program to prevent accidentally chemically contaminating the cultures in the laboratory.

Sterility Testing

The best strategy for reducing contamination is to be proactive by routinely monitoring supplies, media and solutions, work areas and most importantly, cell cultures for contaminants before they are used in critical applications and experiments. The key to developing a realistic contamination monitoring program is to keep it as simple as possible so that people use it, yet ensure that it can get the job done. Unfortunately there are no easy solutions: no single microbiological medium can detect all types of biological contaminants, and practical testing methods often miss low levels of contaminants. The process of detection is made even more difficult by the presence of antibiotics. The techniques and concepts presented below offer some practical approaches for monitoring contamination that can be readily adapted to meet the needs of most cell culture laboratories.

All autoclaves and dry heat ovens used to sterilize glassware, solutions and other supplies must be regularly maintained, and personnel properly trained in their loading and operation. Thermometers and chart recorders should be tested and calibrated periodically to ensure their accuracy. Inexpensive (when compared to the cost of a single autoclave failure) autoclave thermometers, spore test strips and capsules, or other testing devices can be placed inside autoclaves or into bottles of solutions or other packaged supplies during every run, or as necessary, to ensure proper loading and operation.

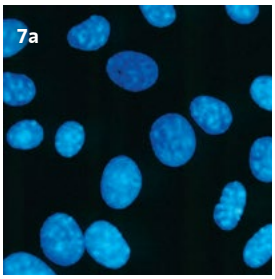
Samples of all in-house filter-sterilized solutions should be tested for sterility each time they are prepared and the solutions not used until testing is complete. Standard microbiological testing methods for bacteria, yeasts, and fungi usually require placing samples for testing into several different broths (trypticase soy, thioglycolate, and Sabouraud broths, for example) and semisolid media (brain-heart infusion, blood agar), and then incubating them at both 30°C and 37°C for at least two weeks.[29]

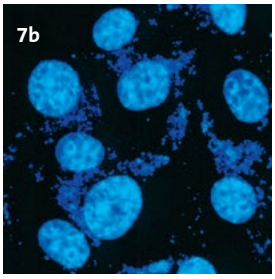
Cell culture media, especially unopened bottles of media that are outdated or no longer used in the lab (as long as they do not contain any antibiotics) can provide a very rich, readily available and useful substitute for standard microbiological media. A small amount of serum (3% to 5% — again outdated or unwanted sera can be used) should be added to promote growth. The medium can be dispensed in 10 mL amounts into sterile 16 x 125 mm glass or plastic screw cap culture tubes or clear 15 mL plastic centrifuge tubes and be stored at 4°C until needed. The sterility of either filtered solutions or cultures and products suspected of being contaminated can be routinely and easily checked by placing a small sample into each of two tubes and incubating one at 30°C and the other at 37°C for at least two weeks.

This sterility test media substitute is also very useful for evaluating the amount or source of particulate contamination in an area, near a piece of equipment, or by a technique. Hoods, and especially incubators, are frequently blamed by laboratory personnel as the source of their contamination problems as in: “my cultures keep getting contaminated because something is wrong with the hood” (or incubator). Until these areas are screened and eliminated as the source of the problem, the real problem, often simply poor aseptic technique, cannot be dealt with effectively. These suspected problem areas can be screened by dispensing the test medium into 96-well culture microplates or 100 mm culture dishes (use agar-gelled media for the dishes). The vessels are then opened (with unopened vessels as controls) for 30 to 60 minutes at several locations within the test site prior to being sealed

and incubated. Cultures can be initially checked for contamination after two to three days, although slow growing contaminants may take two weeks or longer to appear. The rate of contamination (number of colonies or contaminated wells/vessel or unit area/unit time) can then be calculated and analyzed. Besides giving an accurate level of the bioburden in that area, microscopic observation of the contaminants in the liquid test media also allows their morphological comparison with the microorganisms found causing problems in the cell cultures. Past experience with this approach has shown it is a very useful tool when teaching aseptic technique as it clearly demonstrates that the air in a room or even inside a humidified incubator, is usually not a major source of contamination in a well-maintained laboratory. It is also a useful tool in tracking down mysterious contamination outbreaks.

Detecting Mycoplasma in Cultures





Figures 7a and 7b. Photomicrographs (1000X) of VERO cells stained with Hoechst 33258 dye. DNA-containing nuclei and mycoplasma stain brightly under ultraviolet light allowing the clean culture (7a) to be easily distinguished from the infected culture (7b). Photomicrographs courtesy of Bionique Testing Laboratories, Inc.

No monitoring program is complete unless it can effectively detect contaminated cultures, especially those infected by mycoplasma. Unfortunately mycoplasma detection is not simple, and because of this and a lack of awareness, many cell culture users simply don't bother to test — as many as 50% (Table 7.) As a result, it is estimated that at least 15% of all cell cultures in the United States are contaminated with mycoplasma. Because of these outrageously high levels of contamination and the proven ease with which mycoplasmas can be spread from contaminated cultures,[26] it is very important to quarantine all cultures coming into the laboratory until they have been tested for mycoplasma. This is especially true of gifts of cell lines from other labs; often these free "gifts" end up infecting your cultures and causing problems.

There are two basic testing methods for mycoplasma: direct culture in media, or indirect tests that measure specific characteristics of mycoplasma.[16] Direct culture is the most effective and sensitive method for detecting mycoplasma, but it is also the most difficult and time-consuming. It requires several carefully tested liquid and semisolid media and controlled environmental conditions (See reference 30 for detailed protocols), and must be run with live mycoplasma controls. Additionally, although direct culture is the most sensitive method, it is the slowest (requiring up to 28 days) and it may not reliably detect some fastidious strains of mycoplasma, making it less than 100% effective. Budget permitting, direct culture testing is best contracted to an outside testing facility for two reasons: first, given the ease with which mycoplasma can spread in the laboratory, bringing live mycoplasma into a cell culture facility for the required controls is not recommended; second, to do it well, direct testing requires a serious effort and commitment of resources better spent in doing cell culture. These tests are commercially available at a reasonable cost from several cell culture testing companies. (Visit **www.atcc.org** or **www.bionique.com** for additional information on mycoplasma testing services.)

There are a wide variety of indirect test methods available for mycoplasma detection, including PCR-based kits, DNA fluorochrome staining, autoradiography, ELISA, immunofluorescence, and specific biochemical assays. These tests are faster than direct culture, all are commercially available in kit form, and they can detect the fastidious, difficult to cultivate strains that are occasionally missed by direct culture. However, they have traditionally lacked the sensitivity of direct culture, requiring much higher levels of contamination for detection. As a result, they had more frequent false negatives than direct culture methods, potentially leaving researchers who rely solely on a single indirect test with a false sense of security.[reviewed in 11,12,18,35,51]

A widely used and highly recommended indirect test is DNA fluorochrome staining.[31,47] This easy and relatively fast procedure stains DNA using a fluorescent dye. When stained and fixed cells are examined under a UV microscope equipped with the proper filter package, DNA fluoresces brightly (Figures 7a and 7b). Not only will this test detect mycoplasma but as an added benefit it will also detect any other microbial contaminants. This staining method can be combined with an indicator cell line to increase its sensitivity. Interpreting results is not always easy, especially with hybridoma cultures; suitable positive and negative control slides should always be used to help interpret staining results. These positive and negative mycoplasma control slides are commercially available; since they have already been fixed, they are safe to use in the laboratory.

The Mycoplasma Detection using DNA Staining Protocol (CLS-AN-025) is available at **www.corning.com/lifesciences**

Recently, the emergence of improved PCR methods has expanded the options for quality control testing for both cell lines and some cell-based therapeutics. These improved methods are more sensitive than DNA fluorochrome staining and other indirect methods. And in some instances, they offer comparable detection efficiency with relation to culture methods. However, since they are unable to distinguish between DNA from viable or nonviable organisms, they can generate false positive results. Misleading results can also be caused by PCR amplicon contamination arising from environmental and carryover of amplicons from earlier PCR runs or interference from components in the medium or cells. Consequently, it is essential that laboratories using PCR address the issue of containment within the facility layout, workflow process, and development of stringent techniques.[17]

The best overall testing approach is a combination of both methods: direct culture can provide very high sensitivity while DNA fluorochrome staining can detect any fastidious mycoplasma that the direct culture misses. Both the FDA and USDA requires this approach for cell culture derived products, such as monoclonal antibodies, vaccines and drugs, and the cells required to produce them. If resources do not permit the combined approach, then the DNA fluorochrome staining procedure using an indicator cell line, combined with one other indirect test method should provide a satisfactory level of security for most research applications.

Because of the very serious nature of mycoplasma contamination and its widespread distribution, it is important to review the major sources of mycoplasma contamination and the basic steps for preventing it from happening in your laboratory:

1. Currently, the number one source of mycoplasma contamination is other infected cell lines; it is essential to quarantine all cultures brought into the laboratory until they have been screened for mycoplasma contamination, and to use only tested cultures in research.
2. The second common source is the cell culturist; good training especially in aseptic technique combined with the strategic use of a tested cell repository and limited use of antibiotics will greatly reduce the opportunities for contamination via this route.
3. The last important source of mycoplasma are sera and other biologicals that are sterilized by filtration; fortunately, due to better filtration practices and testing methods problems from this area have been greatly reduced.[54] Buy only from sources that have a good reputation and that use reliable acceptable filtration (0.1 µm or smaller) and testing procedures.

Detecting Other Biological Contaminants in Cultures

The traditional microbiological media described earlier for testing the sterility of solutions can be adapted for testing cultures for bacteria, yeasts, and fungi[29] However, the direct culture tests and the indirect DNA fluorochrome test for mycoplasma, although not designed for this purpose, will also detect most bacteria, yeasts, and fungi, including intracellular forms, reducing the need for the traditional tests. Special culture procedures are also available for detecting suspected protozoan contaminants in culture.(referenced in 32)

There are several other important quality control tests that should be used to both identify and characterize the cell cultures used in your research. Especially serious is the widespread problem of cross-contamination by other cell lines described earlier, but cells are also continually evolving in culture: important characteristics can be lost, mutations can occur, or chromosomes can undergo rearrangements or changes in number. Monitoring these changes is important because altered cell cultures can have a significant impact on the reproducibility of your research.(reviewed in 33) The following characterization methods are recommended for monitoring cell line identity; refer to the cited references for details. Most laboratories should incorporate at least one of these methods as part of their monitoring program:(reviewed in 52)

- Chromosomal analysis/karyotyping, a relatively simple method that involves preparing a metaphase spread with chromosome banding and painting to determine the modal chromosome number and presence of any unique marker chromosomes.[34]
- Isoenzyme analysis using electrophoresis to generate a protein 'fingerprint' that can be used to determine species or for future comparisons.[33] This was the method originally used by Gartler in the 1960s to show cross-contamination of human cell lines by HeLa cells.[19,20]
- Immunological or biochemical techniques to detect markers that are unique to the tissue, cell line, or the species from which it is derived.[33]
- DNA fingerprinting, measures variation in length within mini-satellite DNA containing variable numbers of tandem repeat sequences to detect both intra- and inter-species contamination.[35]
- Polymerase chain reaction (PCR) fragment analyzes amplification of specific genes or gene families.
- DNA barcode regions are evaluated by sequencing DNA fragments from the mitochondrial gene cytochrome c oxidase subunit I.
- Short tandem repeat (STR) profiling detects variation in length within microsatellite DNA containing variable numbers of short tandem repeat sequences. This method has become the current international reference standard and is highly recommended as an easy and economical approach to confirm cell line identity. These tests are also commercially available at a very reasonable cost.

The results from these identity tests can serve as an important baseline against which any future changes can be compared.

Recommendations for a Cell Culture Testing Program

The cell culture testing program you choose should be the best you can afford, as it is the cornerstone of your research and money well spent. An inadequate program (or worse, no program at all) provides a false sense of security and can eventually lead to failure compromising the validity of your research. The following steps are recommended for setting up a sound, yet practical culture monitoring program:

1. Test all current in-house cell lines using some of the methods described above to ensure they are free from mycoplasma and other microbial contaminants, and to check their identity. Then incorporate these tested cultures into your cell repository and rely only on them for all future experiments.
2. Quarantine and then test all incoming cell lines and any cultures currently stored in your cell repository that were not tested when they were frozen.
3. Test all cell lines that are in continuous use at least every three to four months and any time they behave suspiciously. Better yet, save time, money, and effort by periodically discarding these cultures and replacing them with cultures from your tested cell repository. (This strategy will be discussed in detail later in the section on using a cell repository.)
4. New lots of sera should be evaluated for any critical applications before widespread use. The simplest test method is to use the new serum in an indicator cell culture for several weeks and then test the culture for mycoplasma contamination using DNA staining or other suitable test.

Detecting Chemical Contaminants

Determining that a chemical contaminant is the cause of a cell culture problem is usually much more difficult than with biological contaminants because it is so hard to detect. Often the first signs that something is wrong are widespread alterations in the growth, behavior or morphology of the cultures in the laboratory; however, it may take weeks before these changes are noticed. Once noticed, the cause is frequently misconstrued to be of biological origin; only after extensive and unsuccessful testing for the usual microbial suspects does attention focus on the possibility it might be a chemical contaminant.

For additional information on solving chemical contamination problems, refer to the Corning Guide for Identifying and Correcting Common Cell Growth Problems (CLS-AN-043) at:
www.corning.com/lifesciences

Begin the problem-solving process by identifying all changes that have occurred in the lab in the weeks prior to the problem being noticed, especially in equipment, solutions, media, and supplies, that may be related to the problem.[2] Good record keeping is essential for this process to be successful. Bring together laboratory personnel to brainstorm for all of the possible causes and then select the best possibilities for evaluation. Simple comparison experiments can then be done to eliminate each possibility as the source of the problem; media, solutions, sera, and other products to use as controls in the testing can be obtained from other labs or sources. The best way to avoid chemical contamination is to test all new lots of reagents, media, and especially sera, and test the water purity at least yearly using the most sensitive culture assay available.

Strategic Use of a Frozen Cell Repository

A cryogenic cell repository is commonly used in laboratories to reduce the need to carry large numbers of cultures and to provide replacements for cultures lost to contamination or accidents. Freezing cultures also stops biological time for them, preventing them from acquiring the altered characteristics that can normally occur in actively growing cells as a result of environmental or age related changes. However, a cell repository is only a reliable resource if the cultures it contains have been properly tested, labeled, and stored.[(reviewed in 36,44)]

Equally important, a cell repository can also be used strategically to convert continuously carried cultures into a series of short-term cultures, thereby greatly reducing both the amount of quality control testing required and potential problems from cryptic contaminants.[(reviewed in 53)] When cultures are continuously carried for long periods in the laboratory they should be tested for contaminants at least every three to four months (more often for critical applications). If they are not tested regularly, then when a cryptic contaminant, such as a mycoplasma or another cell line, is finally uncovered, it is impossible to determine how long it has been in the culture and how much research has been invalidated by its presence. In addition, if the contaminant is mycoplasma, it is likely to have spread by then to other cultures. However, regular testing, although very important to ensure the integrity of your cultures, can require considerable effort, especially in laboratories using multiple cell lines. Rather than test cultures several times a year, it is easier to simply discard them every three months replacing them from the repository with cultures from the same lot or batch that have been previously tested to ensure their integrity.

Tested stocks should be set up in the cell repository for each culture that is routinely used in your laboratory. The cultures should be grown for at least two weeks in antibiotic-free media, and then thoroughly tested to check their viability, ensure they are free of contamination, and confirm their identity and presence of any important characteristics. Testing should be done both immediately before and after freezing; however, if you don't mind assuming some added risk, testing can be left until after freezing. The freezer stock should always be prepared from pooled cultures and contain enough vials, assuming a consumption rate of five vials per year (or higher based on your experience), to last the planned lifetime of any research projects involving them. A better alternative may be to first develop a seed or master stock (10 to 20 vials is usually sufficient, depending on your envisioned needs), and then from that develop a working stock (approximately 20 vials). When the original working stock is depleted, it is replaced by using a vial from the seed stock to develop a new working stock. Assuming a consumption rate of five vials per year, each working stock will be good for 4 years, with the seed stock lasting for 40 to 80 years. Hopefully, this will be long enough to finish a research project. This approach reduces the amount of routine testing to practical levels since only newly introduced cultures will require testing. Equally important, discarding cultures after growing them for three months also destroys any undiscovered biological contaminants that may have gained access to the cultures, limiting both their damage to the integrity of the research and their spread to other cultures.[53]

Strategic Use of Antibiotics

When used intelligently, antibiotics are a useful tool in cell culture, but they can be very dangerous when overused or used incorrectly. Experienced cell culture users have recommended for many years that antibiotics never be used routinely in culture media.[3,7,12,17,18,26,27,49] In a major study, Barile found that 72% of cultures grown continuously in antibiotics were contaminated by mycoplasma, but only 7% grown without antibiotics were contaminated, a 10-fold difference.[37] Similar results are common: workers who routinely and continuously use antibiotics in their media tend to have higher contamination problems, including mycoplasma, than workers who do not. Over-reliance on antibiotics leads to poor aseptic technique. It also leads to increased antibiotic resistance among common culture contaminants. In an ongoing study[41] of the antibiotic sensitivity of culture-derived mycoplasmas, 80% were resistant to gentamycin, 98% to erythromycin, and 73% to kanamycin, all commonly used antibiotics widely claimed to be effective against mycoplasmas. Mycoplasmas also showed resistance to the antibiotics recommended and sold specifically for cleaning up mycoplasma infected cultures: 15% were resistant to ciprofloxacin, 28% to lincomycin, and 21% to tylosin.

Why does the routine use of antibiotics lead to higher rates of mycoplasma contamination?(reviewed in 50) Everyone generates and sheds a relatively constant flow of particles consisting of fibers, aerosols, and droplets, as they work in the laboratory. These particles can have a mixture of bacteria, yeast, fungi, and even mycoplasmas bound to them. If one of these contamination-laden particles enters an antibiotic-free culture, the chances are that at least one of the contaminants will produce a highly visible infection within 24 to 48 hours. As a result the contaminant is quickly detected and the culture discarded. It is very unlikely that particles shed by laboratory personnel would ever consist of just difficult to detect contaminants, such as mycoplasmas, that could enter cultures and not cause visible signs of contamination. However, if the culture contains antibiotics, there is a chance that the antibiotics will prevent the growth of the usually more easily detected contaminants but allow mycoplasma or other cryptic contaminants to grow undetected. As a result, instead of being discarded, the cryptically infected culture remains in use, is utilized in experiments, and becomes a potential source of serious contamination for the other cultures in the laboratory.

Antibiotics should never be used as a substitute for good aseptic technique; however they can be used strategically to reduce the loss of critical experiments and cultures. The key is to use them only for short-term applications: for the first week or two of primary cultures, during the initial production stages of hybridomas, for experiments in general where the cultures will be terminated in the end. Whatever their use, the antibiotics ultimately chosen should be proven effective, noncytotoxic, and stable.[37,50]

Curing Contaminated Cultures

Autoclaving is the preferred method for dealing with contaminated cultures — it always works and is guaranteed to keep the infection from spreading to other cultures. However, occasionally contamination will be found in a valuable culture that cannot be replaced and attempts will be made to save it. This is a task that should not be undertaken lightly as it usually entails considerable effort and frequently turns out to be unsuccessful.[17,57] In addition, cultures can lose important characteristics as a result of the clean-up procedure. If the contaminant is a fungus or yeast, success is unlikely since antifungal agents, such as amphotericin B (Fungizone) and Nystatin, will not kill these organisms, but only prevent their growth. Many bacterial culture contaminants come from human or animal sources and are likely to have developed resistance to most commonly used cell culture antibiotics.

However, most clean up attempts are usually made against mycoplasma infected cultures. Treating with antibiotics is the most widely used approach, but as discussed earlier, cell culture mycoplasma strains are often resistant to some of the antibiotics specifically recommended for cleaning up mycoplasma infected cultures. Furthermore, the more attempts made at cleaning up contaminated cultures with these antibiotics the more likely resistant mycoplasma strains will develop. Other approaches, usually combining the use of antibiotics with specific antisera or other chemical treatments, can be used as well.[reviewed in 11,16,17,37,57] However, none of these methods are 100% successful and clean up should only be tried as a last resort. **Caution:** often these treatments reduce the level of contamination below that which can be detected by indirect methods such as DNA staining or PCR. As a result, clean-up attempts often appear successful for the first month or more following treatment because the low level of surviving mycoplasmas can escape detection. But eventually the few remaining undetected mycoplasmas recover leading to more serious problems. Budget permitting, there are commercially available mycoplasma clean-up services for contaminated cultures, they are relatively expensive but usually successful.[17, 57]

A Final Warning

It has been over 50 years since Gartler was brave enough to present his findings on HeLa cell contamination at the Second Decennial Review Conference on Cell Tissue and Organ Culture in Bedford, Pennsylvania.[19] His work showed that virtually all of the heteroploid human cell lines then in use were actually HeLa cell contaminants. Unfortunately, this work was dismissed by a large portion of the scientific community and these HeLa contaminants continue to this day to be passed around and used by researchers around the globe. In the United States alone, losses due to cell culture contamination, especially by the widely traveled HeLa cell line, other cell lines and mycoplasma cost cell culture users hundreds of millions of dollars annually; this is money that could otherwise be used for additional research.[55]

Unfortunately these serious problems do not appear to be getting better. As shown by the survey results in Table 7, mycoplasma contamination is still a problem for most cell culture workers. At least 23% of respondents have experienced mycoplasma contamination of their cultures, but an additional 44% suspected mycoplasma contamination but were not sure. The reason for their uncertainty is clarified by the response to question #4 from Table 7: 50% of all respondents do not test for mycoplasma; as a result they are unaware of the status of their cultures. The answer to question #5 from Table 7 points out one important reason for widespread contamination problems — the overuse of antibiotics. With 65% of respondents using antibiotics on a regular basis, the continued frequent occurrence of cryptic contaminants, especially mycoplasmas, is likely.

More recent surveys show the problems of cross cell contamination are also being ignored by many researchers. Buehring, et al.[56] reported in 2004 that just one-third of 483 researchers from 48 countries tested their cell lines to authenticate their identity. A 2015 online survey[55] showed the majority (52%) of the 446 researchers responding never perform authentication or other species-related quality control tests on the cell lines used in their research programs. Some journals have finally begun requiring evidence of cell line authentication with all submitted manuscripts using continuous cell lines. Hopefully, this will help push researchers to better deal with these problems of cell line cross-contamination and misidentification. There are no valid excuses for wasting valuable time, money, and resources by using contaminated cells in research. Test all of the cultured cells used in your research program, both for mycoplasma and authenticate that their identity is correct.[52] Check the literature, especially the lists of contaminated cell lines that can be found on national cell repositories such as ATCC, DSMZ, ECAAC, and JCRB, or at the Database of Cross-contaminated or Misidentified Cell Lines maintained by the International Cell Line Authentication Committee (ICLAC) (**iclac.org/databases/cross-contaminations**).

Table 7. Contamination Survey Results*

A. Do you consider microbial contamination (bacteria, yeast, fungi, mycoplasma) of your cultures to currently be a problem?

- **50%** Yes, minor
- **8%** Yes, serious
- **33%** No
- **9%** Not sure

B. How often is it a problem?

- **67%** 1-5 times/year
- **20%** 6-10 times/year
- **12%** More than 10 times/year

C. Have you ever encountered mycoplasma contamination in any of your cultures?

- **9%** Yes, once
- **14%** Yes, several times
- **33%** Never
- **44%** Maybe, not sure

D. Do you currently test your cultures for mycoplasma?

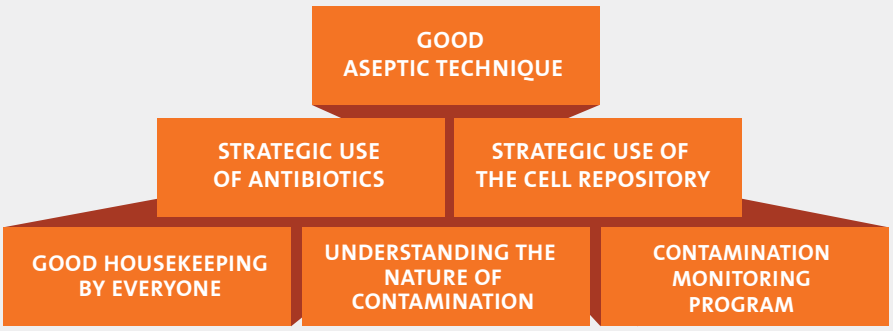
- **50%** No
- **32%** Yes, occasionally
- **18%** Yes, an average of 4 times/year

E. Do you use antibiotics in your culture medium?

- **65%** Yes, usually
- **7%** Yes, short-term only
- **17%** Occasionally
- **11%** Never

*Combined summary of three surveys (130 respondents) conducted at Corning seminars in Baltimore, Boston, and St. Louis in 1990.

Table 8. Key Building Blocks For Successfully Managing Cell Culture Contamination



Cell culture contamination will never be totally eliminated, but through good training,[44,49] a better understanding of the nature of contamination, and the implementation of some basic concepts, it can be better controlled and its damage greatly reduced.

The information in this guide has been compiled to provide you with the foundation (Table 8) upon which you can build a contamination management program designed to fit your own needs. For additional assistance in these areas, visit **www.corning.com/lifesciences**, or contact Corning Scientific Support at 1.800.492.1110; outside the United States, call +1.978.442.2200.

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References

1. Waymouth, C. Construction of Tissue Culture Media. Chapter 2 in Growth, Nutrition and Metabolism of Cells in Culture; G. H. Rothblat and V. J. Cristofalo, eds. (Academic Press, New York, 1972) 11-47.
2. Ryan, J. A. General Guide for Identifying and Correcting Common Cell Culture Growth and Attachment Problems. Corning Technical Bulletin at www.corning.com/lifesciences.
3. Freshney, R. I. Culture of Animal Cells: A Manual of Basic Technique. (Alan R. Liss, New York, 1981) Chapters 5, 10, and 17.
4. Wang, R. J. Effect of Room Fluorescent Light on the Deterioration of Tissue Culture Medium. In Vitro 12:19-22 (1976).
5. McLimans, W. F. The Gaseous Environment of the Mammalian Cell in Culture. Chapter 5 in Growth, Nutrition and Metabolism of Cells in Culture; G. H. Rothblat and V. J. Cristofalo, eds. (Academic Press, New York, 1972) 137-170.
6. Case-Gould, M. J. Endotoxin in Vertebrate Cell Culture: Its Measurement and Significance. In Uses and Standardization of Vertebrate Cell Lines, (Tissue Culture Association, Gaithersburg, MD, 1984) 125-136.
7. Fogh, J., Holmgren, N. B. and Ludovici, P. P. A Review of Cell Culture Contaminations. In Vitro 7(1):26-41 (1971).
8. Contaminants in Tissue Culture. J. Fogh, ed. (Academic Press, New York, 1973).
9. Barkley, W. E. Safety Considerations in the Cell Culture Laboratory. In Methods in Enzymology: Cell Culture, Vol. 58. W. B. Jacoby and I. H. Pastan, eds. (Academic Press, New York, 1979) 36-44.
10. Robinson, L. B., Wichelhausen, R. H. and Roizman, B. Contamination of Human Cell Cultures by Pleuropneumonia like Organisms. Science 124:1147-1148 (1956).
11. Rottem, S. and Barile, M. F. Beware of Mycoplasmas. Trends in Biotechnology 11:143-150 (1993).
12. Lincoln, K. L., and Gabridge, M. G. Cell Culture Contamination: Sources, Consequences, Prevention and Elimination. In Methods in Cell Biology, Vol. 57 (Academic Press, New York, 1988) 49-65.
13. Luzak, J., Pawar, S. A., Knower, S. A., Cox, M. S., Dubose Jr., J. and Harbell, J. W. Trends in the Incidence and Distribution of Mycoplasma Contamination Detected in Cell Lines and Their Products (Presented at the World Congress on Cell and Tissue Culture, June, 1993).
14. McGarrity, G. J. Working Group on Cell Culture Mycoplasmas. Annual report to the International Research Program in Comparative Mycoplasmology (International Organization of Mycoplasmology, 1988).
15. Barile, M. Mycoplasma Contamination of Cell Cultures: a Status Report. In Cell Culture and Its Applications. (Academic Press, New York, 1977) 291-334.
16. Lincoln, C. K., and Lundin, D. J. Mycoplasma Detection and Control. United States Federation for Culture Collection Newsletter 20 (4):1-3 (1990).
17. Ryan, J. A. and Mariano, J. A. Frequently Asked Mycoplasma Questions. Bionique Testing Laboratories Mycoplasma Resource Center, Saranac Lake, NY www.bionique.com/mycoplasma-resources.html (2010).
18. McGarrity, G. J., Sarama, J. and Vanaman, V. Cell Culture Techniques. ASM News 51:170-183 (1985).
19. Gartler, S. M. Genetic Markers as Tracers in Cell Culture. National Cancer Institute Monograph 26:167-195 (1967).
20. Gartler, S. M. Apparent HeLa Cell Contamination of Human Heteroploid Cell Lines. Nature 217:750-751 (1968).
21. Stulburg, C. S., Peterson, Jr., W. D., and Simpson, W. F. Identification of Cells in Culture. Amer. J. Hematology 1:237-242 (1976).
22. Nelson-Rees, W. A., Daniels, D. W. and Flandermeyer, R. R. Cross-Contamination of Cells in Culture. Science 212:446-452 (1981).
23. Bader, F. G. Sterilization: Prevention of Contamination. In Manual of Industrial Microbiology and Biotechnology, A. L. Demain and N. A. Solomon, eds. (American Society of Microbiology, Washington DC, 1986). 345-362.
24. Roche, K. L. and Levy, R. V. Methods used to Validate Microporous Membranes for the Removal of Mycoplasma. BioPharm, 22-33, (April 1992).
25. Barile, M. F., Hopps, H. E., Grabowski, M. W., Riggs, D. B. and DelGiudice, R. A. The Identification and Sources of Mycoplasmas Isolated from Contaminated Cell Cultures. Ann. NY Acad. Sci. 225:251-264 (1973).
26. McGarrity, G. J. Spread and Control of Mycoplasmal Infection of Cell Cultures. In Vitro 12:643-647 (1976).
27. Wolf, K. and Quimby, M. C. Towards a Practical Fail-Safe System of Managing Poikilothermic Vertebrate Cell Lines in Culture. In Vitro 8(4):316-321 (1973).
28. Wolf, K. Laboratory Management of Cell Cultures. In Methods in Enzymology: Cell Culture, Vol. 58, W. B. Jacoby and I. H. Pasten, eds. (Academic Press, New York, 1979) 116-119.
29. McGarrity, G J. Detection of Contamination. In Methods in Enzymology: Cell Culture, Vol. 58, W. B. Jacoby and I. H. Pasten, eds. (Academic Press, New York, 1979) 18-29.
30. Barile, M. and McGarrity, G. J. Isolation of Mycoplasmas from Cell Cultures by Agar and Broth Techniques. In Methods in Mycoplasmology, Vol. 2, J. G. Tully and S. Razin, eds. (Academic Press, New York, 1983) 159-165.
31. McGarrity G. J., Steiner, T. and Vanaman, V. Detection of Mycoplasmal Infection of Cell Cultures by DNA Fluorochrome Staining. In Methods in Mycoplasmology, Vol. 2, J. G. Tully and S. Razin, eds. (Academic Press, New York, 1983) 183-190.
32. Dilworth, S, Hay, R. J., and Daggett, P. M. Procedures in Use at the ATCC for Detection of Protozoan Contaminants in Cultured Cells. Procedure 16655, TCA Manual 5 (3):1107-1110 (1979).
33. Peterson, Jr, W. D., Simpson, W. F. and Hukku, B. Cell Culture Characterization: Monitoring for Cell Identification. In Methods in Enzymology: Cell Culture, Vol. 58, W. B. Jacoby and I. H. Pasten, eds. (Academic Press, New York, 1979) 164-177.
34. Worton, R. G. and Duff C. Karyotyping. In Methods in Enzymology: Cell Culture, Vol. 58, W. B. Jacoby and I. H. Pasten, eds. (Academic Press, New York, 1979) 322-345.
35. Reid, Y. A., and Lou, X. Armstrong, S.E., Mariano, J.A., Martino, C.A., and Ryan, J.A. 2012. Identifying Mycoplasma Contamination: Concepts and Tools, In Microbial Identification: The Keys to a Successful Program. Edited by Mary Griffin and Dona Reber, PDA and Davis Healthcare International Publishing. Chapter 8:185-220. In Vitro, 29A:120A (1993).
36. Ryan, J. A. General Guide for Cryogenically Storing Animal Cell Cultures. Corning Technical Bulletin at www.corning.com/lifesciences.
37. Barile, M. F. Mycoplasmal Contamination of Cell Cultures: Mycoplasma-Virus-Cell Culture Interactions. In Contamination in Tissue Culture, J. Fogh, ed. (Academic Press, Inc., New York, 1973) 131-171.
38. Perlman, D. Use of Antibiotics in Cell Culture Media. In Methods in Enzymology: Cell Culture, Vol. 58, W. B. Jacoby and I. H. Pasten, eds. (Academic Press, New York, 1979) 110-116.
39. Ryan, J. A. Endotoxins and Cell Culture. Corning Technical Bulletin at www.corning.com/lifesciences.
40. Weiss, R. A. Why Cell Biologists Should Be Aware of Genetically Transmitted Viruses. In National Cancer Institute Monograph 48:183-190 (1978).
41. Lundin, D. J. and Lincoln, C. K. Mycoplasmal Contamination of Cell Cultures within the Clinical Diagnostic Laboratory. Amer. Clin. Lab. (April 1994).
42. Hay, R. J., Reid, Y. A., Miranda, M. G. Chen, T. C. Current Techniques for Cell Line Authentication. Biotechnology International 143-147.
43. NSF49-2004a NSF International Standards. Class II (Laminar Flow) Biohazard Cabinetry. Ann Arbor, MI.
44. Geraghty, R. J.,Capes-Davis, A., Davis, J. M., Downward, J., Freshney, R. I., Knezevic, I., Lovell-Badge, R., Masters, J. R. W., Meredith, J., Stacey, G.N., Thraves, P., and Vias, M. Guidelines for the Use of Cell Lines in Biomedical Research. British Journal of Cancer (2014) 111:1021-1046. (Available online)
45. Meechan, P. J. and Wilson, C. Use of Ultraviolet Lights in Biological Safety Cabinets: A Contrarian View. Applied Biosafety, 11(4):222-227.
46. Uphoff, C. C., Lange, S., Denkmann, S. A., Garritsen. H, S, P. and Drexler, H. G. Prevalence and Characterization of Murine Leukemia Virus Contamination in Human Cell Lines. PLoS ONE 10(4):e0125622. doi:10.1371/journal.pone.0125622 (2015).
47. Ryan, J. A. Mycoplasma Detection using DNA Staining Protocol at www.corning.com/lifesciences.
48. Akers, J. A., Meltzer, T. H. and Jornitz, M. W. The Reoccurrence of Mycoplasma Contamination: Prevention Strategies. BioProcess International Filtration Supplement. Available at www.bioprocessintl.com.
49. Coecke, S., et al. A Report of the Second ECVAM Task Force on Good Cell Culture Practice. ATLA 33, 261-287 (2005).
50. Ryan, J. A. and Mariano, J. A. Why Does Over Use of Antibiotics Result in Higher Mycoplasma Contamination Rates? Bionique Testing Laboratories, Mycoplasma Resource Center, Saranac Lake, NY. www.bionique.com/mycoplasma-resources/faq/mycoplasma-contamination-antibiotics-overuse.html (2010).
51. Armstrong, S. E., Mariano, J. A., Martino, C. A., and Ryan, J. A. 2012. Identifying Mycoplasma Contamination: Concepts and Tools, In Microbial Identification: The Keys to a Successful Program. Edited by Mary Griffin and Dona Reber, PDA and Davis Healthcare International Publishing. Chapter 8:185-220.
52. Capes-Davis, A.. Theodosopoulos, G., Atkin, I., Drexler, H. G., Kohara, A., MacLeod, R. A. F., Masters, J. R., Nakamura, Y., Reid, Y. A., Reddel, R. R. and Freshney, R. I. Check Your Cultures! A list of Cross-contaminated or Misidentified Cell Lines. Int. J. Cancer. 127:1-8 (2010).
53. Ryan, J. A. and Mariano, J. A. Creating a Certified Working Cell Bank for Important Cell Lines in Your Research Program. Bionique Testing Laboratories, Mycoplasma Resource Center, Saranac Lake, NY. www.bionique.com/mycoplasma-resources/technical-articles/certified-working-cell-bank.html (2010).
54. Armstrong, S. E., Mariano, J. A. and Lundin, D. J The Scope of Mycoplasma Contamination Within The Biopharmaceutical Industry. Biologicals 38 (2010) 211-213.
55. Freedman, L. P., Gibson,M. C., Wisman, R., Ethier, S. P., Soule, H. R., Reid, V. A. and Neve, R. M. The Culture of Cell Culture Practices and Authentication—Results from a 2015 Survey. BioTechniques 59:189-192 (October 2015).
56. Buehring, G. C., Eby, E. A. and Eby, M. J. Cell Line Cross-contamination: How Aware Are Mammalian Cell Culturists of the Problem and How To Monitor It? In Vitro Cell. Dev. Biol. Anim. 40:211-215 (2004).
57. Drexler, H. G. and Uphoff, C. C. Mycoplasma Contamination of Cell Cultures: Incidence, Sources, Effects, Detection, Elimination, Prevention. Cytotechnology 39:75-90 (2002).
58. Burgener, J. Position Paper on the Use of Ultraviolet Lights in Biological Safety Cabinets. Applied Biosafety, 11(4):228-230 (2006).
59. Yu, M et al. A Resource For Cell Line Authentication, Annotation and Quality Control. Nature, 520:307-311. doi:10.1038/nature 14397 (April 16, 2015).
60. Healthcare Infection Control Practices Advisory Committee (HICPAC) Guideline for Disinfection and Sterilization in Healthcare Facilities (www.cdc.gov/hicpac/Disinfection_Sterilization/toc.html), Centers for Disease Control, Atlanta GA (2008).
61. The Center for Disease Control and Prevention (CDC) in the Biosafety in Microbiological and Biomedical Laboratories. 5th edition HHS Publication No. (CDC) 21-1112, Revised December 2009.

Cell Culture Log

Cell Line _____ **Date acquired** _____

Full Name _____ **Who was the cell line acquired from?** _____

Abbreviated Form _____

Morphology of healthy cells (description or picture)

Morphology of unhealthy or stressed cells (description or picture)

This can be done by reducing the serum concentration by 75% for 24-48 hours and describe any observable changes in morphology.

Is the cell line certified contaminant free? (e.g., Mycoplasma) __ YES __ NO

Doubling time _____

__ Adherent or __ Suspension

Growth surface conditions (TC-treated or unique surface required, specify)

_____

Unique characteristics (Primary, Engineered..)

_____

Medium used for culture _____

FBS? __ YES __ NO if yes, what % FBS? _____

Glutamine _____

NOTES

Antibiotics __ YES __ NO if yes, what antibiotic(s)? _____

Other Additives

_____

Mycolplasma testing dates _____

Mycoplasma results _____

Has a contamination check been done prior to cryopreserving Parantal stocks? __ YES __ NO

Cryopreservation solution _____

Number of cryopreserved Parent stocks _____

Number of cryopreserved working stocks _____

Acceptable passage range _____

Ordering Information

Corning® Cell Culture Flasks

Cat. No.	Surface Area (cm²)	Flask Style	Neck Style	Cap Style	Qty/Pk	Qty/Cs
430639	25	Rectangular	Canted	Vent	20	200
430641U	75	Rectangular	Canted	Vent	5	100
430825	150	Rectangular	Canted	Vent	5	50
431080	175	Rectangular	Canted	Vent	5	50
431082	225	Rectangular	Canted	Vent	5	25
10020	1,720	Corning HYPERFlask®	-	-	4	4
10030	1,720	Corning HYPERFlask®	-	-	1	4

Costar® Multiple Well Plates, Tissue Culture-treated

Cat. No.	Description	Plate Type	Qty/Pk	Qty/Cs
3516	6-well	Clear	1	50
3513	12-well	Clear	1	50
3526	24-well	Clear	1	50
3548	48-well	Clear	1	100
3596	96-well	Clear	1	50
3595	96-well	Clear*	1	50
3599	96-well	Clear	1	100
3916	96-well	Black	20	100
3917	96-well	White	20	100
3603	96-well	Black/Clear-bottom	1	48
3610	96-well	White/Clear-bottom	1	48

*Low evaporation lid

Corning Dishes, Tissue Culture-treated

Cat. No.	Dish Style* (mm)	Approx. Height (mm)	Growth Area (cm²)	Qty/Pk	Qty/Cs
430165	35	10	8	20	500
430166	60	15	21	20	500
430167	100	20	55	20	500
430599	150	25	148	5	60
431110	245	25	500	4	16

*Dish Style (mm) 35 mm = 34.4; 60 mm = 52.1 mm; 100 mm = 83.9 mm; 150 mm = 139.1 mm. Square dishes have interior bottom dimensions 224 mm x 224 mm.

Corning Cell Scrapers

Cat. No.	Description	Blade Length (cm)	Handle Length (cm)	Qty/Pk	Qty/Cs
3008	Cell lifter	1.9	18	1	100
3010	Scraper, small	1.8	25	1	100
3011	Scraper, large	3	39	1	100

Corning Cell Strainers

Cat. No.	Description	Qty/Pk	Qty/Cs
431750	40 µm, blue	1	50
431751	70 µm, white	1	50
431752	100 µm, yellow	1	50

Corning Cryogenic Vials

Cat. No.	Capacity (mL)	Style	Self-standing	Qty/Pk	Qty/Cs
430659	2	Round bottom, internal thread	Yes	50	500
430488	2	Round bottom, external thread	Yes	50	500

Falcon® Cell Culture Flasks, Tissue Culture-treated

Cat. No.	Surface Area (cm²)	Flask Style	Neck Style	Cap Style	Qty/Pk	Qty/Cs
353018	25	Rectangular	Canted	Vent	20	100
353136	75	Rectangular	Canted	Vent	5	60
355001	150	Rectangular	Canted	Vent	5	40
353112	175	Rectangular	Straight	Vent	5	40
353138	225	Rectangular	Canted	Vent	5	30
353143	525	3-Layer	-	Vent	2	12
353144	875	5-Layer	-	Vent	1	8

Falcon Cell Culture Plates, Tissue Culture-treated

Cat. No.	Description	Plate Type	Qty/Pk	Qty/Cs
353046	6-well	Clear	1	50
353043	12-well	Clear	1	50
353047	24-well	Clear	1	50
353078	48-well	Clear	1	50
353072	96-well	Clear	1	50

Falcon Cell Culture Dishes

Cat. No.	Dish Style	Approx. Height (mm)	Growth Area (cm²)	Qty/Pk	Qty/Cs
353001	35	10	11.78	20	500
353002	60	15	21.29	20	500
353003	100	20	58.95	20	200
353025	150	25	156.36	10	100

Falcon Cell Strainers

Cat. No.	Description	Qty/Pk	Qty/Cs
352340	40 µm, blue	1	50
352350	70 µm, white	1	50
352360	100 µm, yellow	1	50

Falcon Cell Scrapers

Cat. No.	Description	Qty/Pk	Qty/Cs
353085	1.8 cm TPE blade with 18 cm polystyrene handle	1	100
353086	1.8 cm TPE blade with 25 cm polystyrene handle	1	100
353089	3.0 cm TPE blade with 25 cm polystyrene handle	1	100
353087	3.0 cm TPE blade with 40 cm polystyrene handle	1	100

Corning CoolCell® Containers

Cat. No.	Description	Capacity (Vials)	Exposed Vial Tops	Qty/Pk	Qty/Cs
432000	CoolCell, purple	12	No	1	1
432001	CoolCell LX, purple	12	Yes	1	1
432002	CoolCell LX,. green	12	Yes	1	1
432003	CoolCell LX, orange	12	Yes	1	1
432004	CoolCell LX, pink	12	Yes	1	1
432138	CoolCell LX, 4 colors (purple, green, orange, pink)	12	Yes	--	4
432005	CoolCell 5 mL LX, purple	12	Yes	1	1
432006	CoolCell FTS30, purple	30	Yes	1	1
432007	CoolCell FTS30, orange	30	Yes	1	1
432008	CoolCell FTS30, green	30	Yes	1	1
432009	CoolCell FTS30, pink	30	Yes	1	1
432010	CoolCellSV2	12	Yes	1	1
432011	CoolCell SV10	6	Yes	1	1

Corning Vacuum Filtration Systems

Cat. No.	Membrane	Funnel/Bottle Volume (mL)	Pore Size (µm)	Qty/Cs
431153	PES	150/150	0.22	12
431096	PES	250/250	0.22	12
431097	PES	500/500	0.22	12
431098	PES	1,000/1,000	0.22	12

Corning Bottle Top Vacuum Filtration Systems

Cat. No.	Membrane	Volume (mL)	Neck Size (mm)	Pore Size (um)	Qty/Cs
431161	PES	150	45	0.22	48
431118	PES	500	45	0.22	12
431174	PES	1,000	45	0.22	12

Corning Syringe Filters

Cat. No.	Diameter (mm)	Pore Size (µm)	Housing Material	Membrane Material	Sterile	Inlet/ Outlet	Packaging	Qty/Cs
431219	28	0.2	SFCA	AC	Yes	LL/LS	Indiv.	49
431222	25	0.2	RC	PP	Yes	LL/LS	Indiv.	50
431224	25	0.2	NY	PP	Yes	LL/LS	Indiv.	50
431229	20	0.2	PES	AC	Yes	LL/LS	Indiv.	50

Reagents

Cat. No.	Description	Size	Qty/Cs
25-950-CQC	Dimethyl Sulfoxide	250 mL	1
354253	Cell Recovery Solution	100 mL	1
354216	Calcein AM fluorescent dye, 500 µg (10 x 50 µg)	500 mg	1
354217	Calcein AM fluorescent dye	1 mg	1
354218	DiIC12(3) fluorescent dye	100 mg	1
25-900-CI	Trypan Blue Solution, 0.4% (w/v) in PBS, pH 7.5 ± 0.5	100 mL	1
354235	Dispase	100 mL	1

Sera

Cat. No.	Description	Size	Qty/Cs
35-010-CV	Fetal Bovine Serum, regular	500 mL	1
35-015-CV	Fetal Bovine Serum, premium	500 mL	1

Corning Media

Cat. No.	Description	Size	Qty/Cs
10-013-CV	DMEM [+] 4.5 g/L glucose, L-glutamine, sodium pyruvate	500 mL	6
10-017-CV	DMEM [+] 4.5 g/L glucose, L-glutamine [-] sodium pyruvate	500 mL	6
15-013-CV	DMEM [+] 4.5 g/L glucose, sodium pyruvate [-] L-glutamine	500 mL	6
15-090-CV	DMEM/F12 50:50 mix [-] L-glutamine	500 mL	6
10-092-CV	DMEM/Ham's F-12 50/50 mix [+] L-glutamine, 15 mM HEPES	500 mL	6
16-405-CV	DMEM/Ham's F-12 50/50 mix [+] L-glutamine [-] phenol red	500 mL	6
15-016-CV	Iscove's Modification of DMEM [+] 25 mM HEPES, [-] ß-thioglycerol, ß-mercaptoethanol, L-glutamine	500 mL	6
10-016-CV	Iscove's Modification of DMEM [+] L-glutamine, 25 mM HEPES [-] ß-thioglycerol, ß-mercaptoethanol	500 mL	6
10-080-CV	Ham's F-12 Medium [+] L-glutamine	500 mL	6
10-060-CV	Medium 199 (Mod.) [+] Earle's salts, L-glutamine	500 mL	6
10-022-CV	MEM alpha medium [+] Earle's salts, ribonucleosides, deoxyribonucleosides, L-glutamine	500 mL	6
15-012-CV	MEM alpha medium [+] Earle's salts [-] ribonucleosides, deoxyribonucleosides, L-glutamine	500 mL	6
10-010-CV	MEM [+] Earle's salts, L-glutamine	500 mL	6
10-009-CV	MEM [+] 1.5 g/L sodium bicarbonate, NEAA, L-glutamine, sodium pyruvate	500 mL	6
15-010-CV	MEM [+] Earle's salts [-] L-glutamine	500 mL	6
17-305-CV	MEM [+] Earle's salts [-] L-glutamine, phenol red	500 mL	6
10-040-CV	RPMI 1640 [+] L-glutamine	500 mL	6
15-040-CV	RPMI 1640 [-] L-glutamine	500 mL	6
17-105-CV	RPMI 1640 [-] L-glutamine, phenol red	500 mL	6
10-041-CV	RPMI 1640 [+] L-glutamine, 25 mM HEPES	500 mL	6
15-041-CV	RPMI 1640 [+] 25 mM HEPES [-] L-glutamine	500 mL	6

Corning Buffers

Cat. No.	Description	Size	Qty/Cs
21-040-CV	Phosphate-Buffered Saline (PBS), 1X without calcium and magnesium	500 mL	6
21-020-CV	Hanks' Balanced Salt Solution (HBSS), 1X with calcium and magnesium	500 mL	6
21-023-CV	HBSS, 1X with calcium and magnesium without phenol red	500 mL	6
21-021-CV	HBSS, 1X without calcium and magnesium	500 mL	6
21-022-CV	HBSS, 1X without calcium, magnesium, and phenol red	500 mL	6
21-030-CV	Dulbecco's Phosphate-Buffered Saline (DPBS), 1X with calcium and magnesium	500 mL	6
21-031-CV	DPBS, 1X without calcium and magnesium	500 mL	6
25-015-CI	Corning glutagro™ supplement	100 mL	1
25-021-CI	Trace Elements A, 1,000X	100 mL	1
25-022-CI	Trace Elements B, 1,000X	100 mL	1
25-023-CI	Trace Elements C, 1,000X	100 mL	1
25-030-CI	MEM Amino acids, 50X	100 mL	6
25-025-CI	MEM Nonessential amino acids, 100X	100 mL	6
25-800-CR	Insulin-Transferrin-Selenium (ITS)	10 mL	1

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