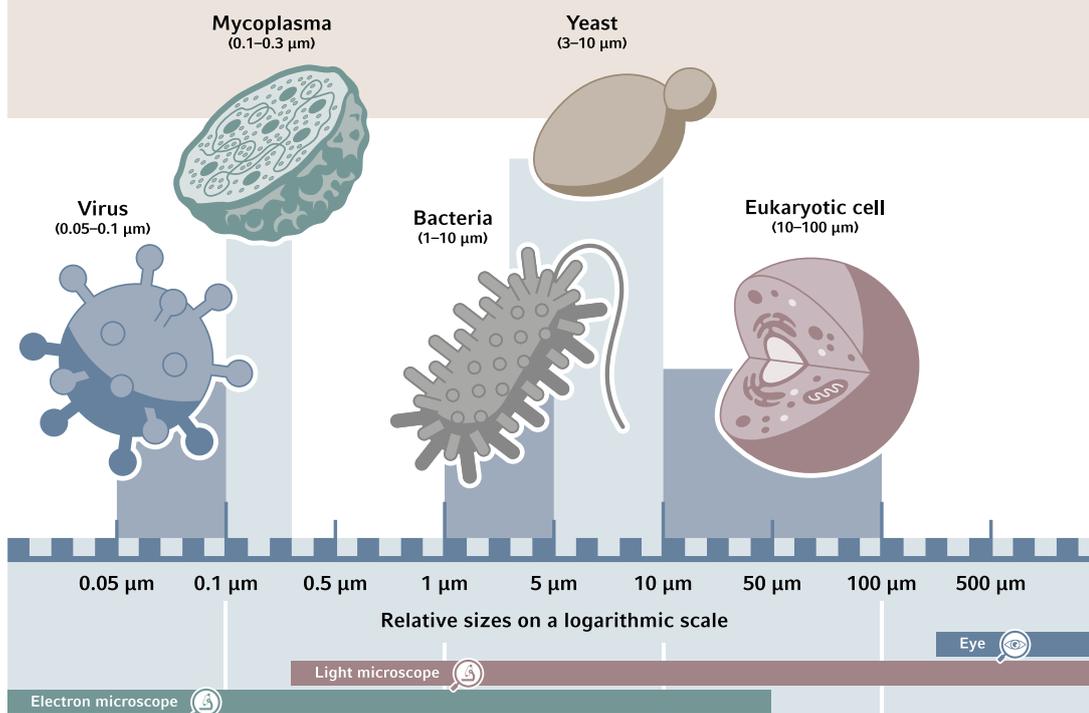


# How to Remove Contamination in a Cell Culture Lab

## Executive Summary

Contamination is one of the worst fears for scientists working in a cell culture laboratory. Biological contamination in cell culture is nearly always associated with experimental repeats,

project delays, and sometimes even with lab closures or the loss of irreplaceable cells – wasting valuable time and precious resources.



**Figure 1:** Cell culture contaminants vary in size from around 100 μm down to 50 nm, making it difficult to detect the smallest ones (viruses and mycoplasma) with conventional light microscopy.

[Click here for microscopic/macrosopic images of different contamination types](#)

There are a wide range of biological entities that commonly contaminate *in vitro* cell cultures. These include:

- > Bacteria
- > Fungi
- > Viruses
- > Yeasts
- > Mycoplasma
- > Eukaryotic cells

All these contaminants manifest themselves in very different ways and as a result require different strategies for prevention, detection, and removal. Fungi, bacteria, and yeasts rapidly grow alongside the cells and their effects are easy to detect macroscopically and microscopically. Mycoplasma and viruses infect cells and, due to their size, are difficult to detect directly. Eukaryotic contamination often consists of fast-growing cells that are visible under a microscope, but can be hard to distinguish from the cultured cells and can gradually take over a culture [1].

Sources of contamination in the lab are equally wide ranging. In some cases, contaminants are already present in cells when they are brought into the lab; this is most often the case with hard-to-detect contaminants such as eukaryotic cells and mycoplasma. Cell culture media and other solutions are another source – in particular when prepared and sterilized in-house, used by multiple people, or used for extended periods of time. Consumables could potentially harbor contaminants, highlighting the importance of using only sterile consumables in cell culture. And finally, lapses in aseptic technique can contribute.

In the fight against contamination, researchers place a lot of emphasis on prevention, as “prevention is better than cure”. Many of these preventative measures are now standard operating practice in cell culture labs, for example autoclave sterilization, use of pre-made and pre-sterilized culture media, aseptic technique training, regular mycoplasma testing,

and the addition of antibiotics in cell media. The latter however, is becoming less prevalent in modern cell culture as scientists increasingly recognize the effect that antibiotics have on cell growth and behavior – leading them to switch to antibiotic-free media.

However, even in labs with the most stringent preventative measures, there is still a small risk of contamination affecting cell culture. If this happens, it is important to act quickly and to act in accordance with the protocols that are in place for these situations. Although these protocols may vary considerably between labs, most of the actions that lab users should take fall into the four areas discussed here:

- > Cleaning up
- > Determining the extent of contamination
- > Finding the cause
- > Moving forward

## Cleaning up

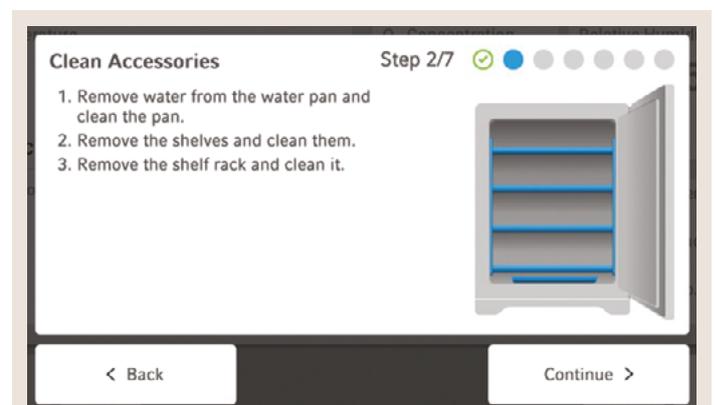
In order to prevent contamination from spreading around the lab or recurring, carrying out a thorough clean-up is the most urgent course of action after it has been discovered. Bacteria, yeasts, and fungal spores spread rapidly and often go unnoticed until they infect other cultures and even other labs.

An essential aspect of cleaning up is re-tracing your steps. Even fast-growing contaminants take time to grow to detectable levels, which means that when a contaminant is visible to the naked eye or under a microscope, it is likely that it has been present in the culture for more than 24 hours. For this reason, it is important to quickly identify any areas, media, reagents, and people that have been in contact with the infected culture in the past days.

Firstly, infected cultureware (multiwell plates, T-flasks, Petri dishes) should be autoclaved or disinfected and then discarded. Autoclaving all affected cultureware is recommended as it is a safe, reliable way of making sure no traces of contamination remain in the cultureware. It can then be disposed via the correct disposal routes for biological samples.

Secondly, affected incubators and biosafety cabinets should be cleaned. When cleaning an incubator, all other cultures should be removed beforehand, though to avoid potential further contamination, it is recommended that the cultures are not moved to other incubators. If possible, move all cultures to a biosafety cabinet temporarily and return them to the same incubator when cleaning is completed.

It is important to choose a disinfectant that is effective against the contaminant in question and is compatible with the materials it is used on. In many cases, an alcohol-based disinfectant (70% ethanol or isopropanol) used for routine cleaning is suitable, but certain cases may require alternative cleaning agents (see table 1). Additionally, some incubators have automatic disinfection cycles, for example using high temperatures, which help to decontaminate incubators quickly and easily following contamination (figure 2). In some incubators it is even possible to program automatic reminders to ensure regular cleaning of the device.



**Figure 2:** Modern CO<sub>2</sub> incubators can have clear step-by-step instructions for standardized cleaning procedures to be carried out before sterilization at 180 °C. To further reduce the probability of user errors the process should be sensor supported.

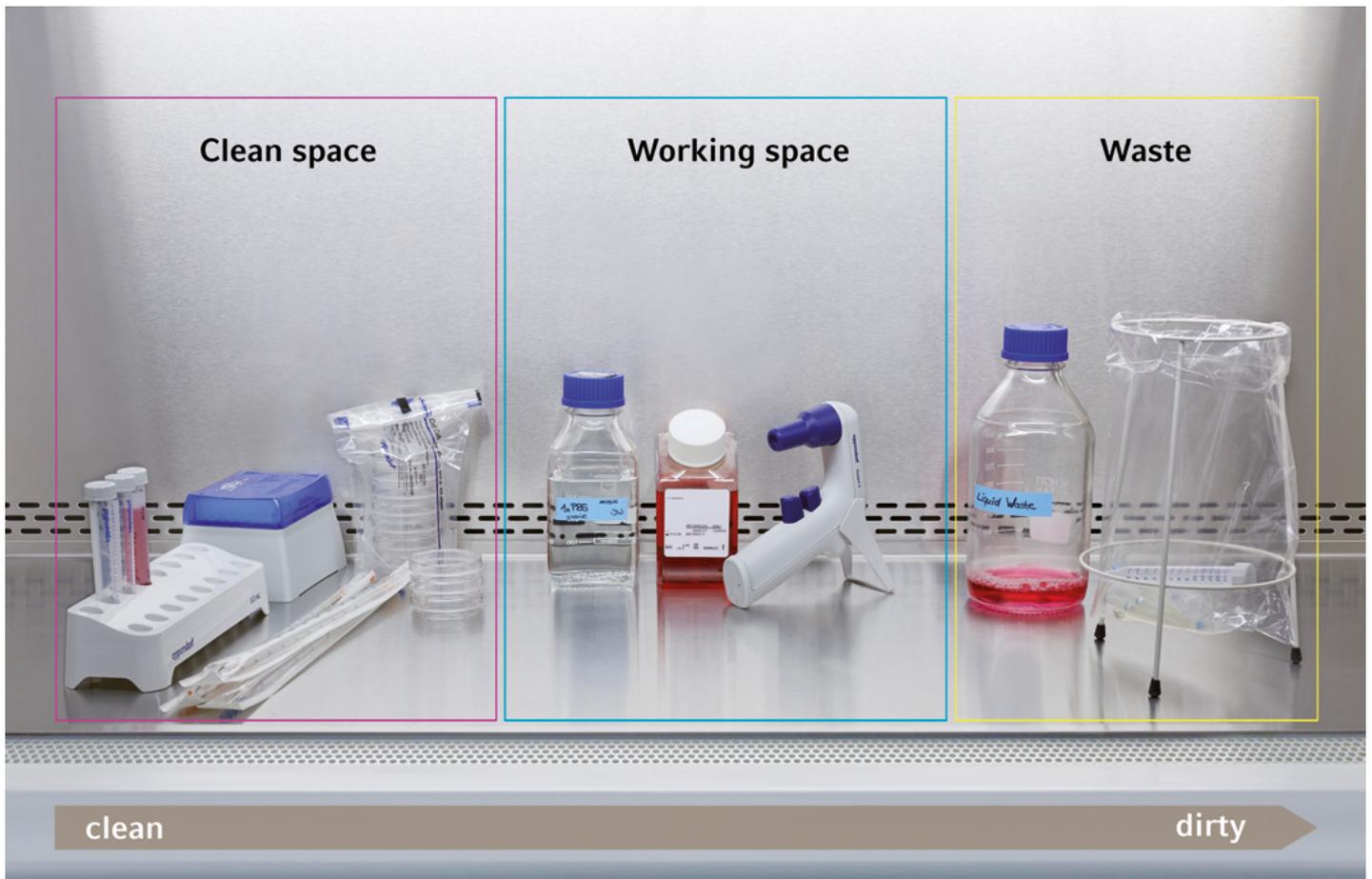
[See here for more information](#)

Disinfectant class	Advantages	Disadvantages	Effect on other materials
Alcohols	<ul style="list-style-type: none"> <li>&gt; Short contact times for effective kill rates</li> <li>&gt; Economical and simple to prepare</li> <li>&gt; Low toxicity</li> </ul>	<ul style="list-style-type: none"> <li>&gt; Flammable</li> <li>&gt; Not effective against spores and fungi</li> <li>&gt; Poor penetration of organic material</li> </ul>	<ul style="list-style-type: none"> <li>&gt; Non-corrosive to metals but may damage plastics and rubber</li> </ul>
Hypochlorites (and halogen-releasing agents)	<ul style="list-style-type: none"> <li>&gt; Effective against most microorganisms</li> <li>&gt; Compatible with detergents</li> </ul>	<ul style="list-style-type: none"> <li>&gt; Toxic</li> <li>&gt; Limited activity against fungi.</li> <li>&gt; Rapidly inactivated by organic matter</li> <li>&gt; Inactivated by anionic detergents.</li> <li>&gt; Solutions decompose rapidly and should be replaced frequently</li> </ul>	<ul style="list-style-type: none"> <li>&gt; Corrosive to metals and rubber</li> <li>&gt; Will stain and bleach fabrics and surfaces</li> </ul>
Phenolics	<ul style="list-style-type: none"> <li>&gt; Effective against most microorganisms</li> <li>&gt; Strong anti-fungal agent</li> </ul>	<ul style="list-style-type: none"> <li>&gt; Toxic skin irritant</li> <li>&gt; Not effective against some non-enveloped viruses</li> <li>&gt; Inactivated by anionic detergents, hard water and salts</li> <li>&gt; Strong odor</li> </ul>	<ul style="list-style-type: none"> <li>&gt; Slightly corrosive to metals</li> <li>&gt; Can damage many plastics</li> </ul>
Quaternary ammonium compounds	<ul style="list-style-type: none"> <li>&gt; Effective against most microorganisms</li> <li>&gt; Low toxicity</li> </ul>	<ul style="list-style-type: none"> <li>&gt; Not effective against spores and some non-enveloped viruses</li> <li>&gt; Long contact times required for effective kill rates</li> <li>&gt; Inactivated by organic matter and anionic detergents</li> <li>&gt; Activity reduced by hard water/salts</li> </ul>	<ul style="list-style-type: none"> <li>&gt; Minimally corrosive</li> <li>&gt; Can stain surfaces</li> </ul>
Peroxygen compounds	<ul style="list-style-type: none"> <li>&gt; Effective against most bacteria, viruses and fungi</li> <li>&gt; Some activity against spores</li> </ul>	<ul style="list-style-type: none"> <li>&gt; Irritant</li> <li>&gt; Working solutions decompose and should be replaced every few days</li> </ul>	<ul style="list-style-type: none"> <li>&gt; Corrosive but less than hypochlorites</li> </ul>

**Table 1:** Comparison of the benefits and drawbacks of commonly used lab disinfectants (adapted from [2]).

When biosafety cabinets are used correctly and cleaned effectively after each use, there is a low risk of contamination spreading between cultures of different users (figure 3). However, if there is any indication that contaminants still remain in a cabinet – for example repeated infections from the same cabinet – additional, more

thorough cleaning with alcohols or other disinfectants is highly recommended. Fumigation of biosafety cabinets, although recommended as part of routine maintenance, is not required for contamination control unless in exceptional circumstances [3].



**Figure 3:** Researchers can reduce the risk of contamination during cell culture work by organizing their workspace in a way that separates clean and dirty materials.

Finally, it is necessary to clean any other equipment or tools that may carry contamination, such as water baths and pipettes. Although in modern cell culture, most materials that come into contact with cells and media are single-use, it is good to check that any potentially contaminated objects are discarded, autoclaved (if possible), or otherwise disinfected.



Find a cleaning schedule here

## Saving irreplaceable cultures

The clear majority of cases of contamination will require the affected culture to be autoclaved and discarded immediately. However, there may be cases where cultures are irreplaceable. In this situation, researchers might be prepared to invest significant time and resources in attempting to 'save' a contaminated culture. Whether this is possible depends on the nature and the extent of the contamination. Also, it is important to realize that these methods are likely to have a significant, and often permanent, effect on the cells in question, for example the activation of stress-signaling cascades [4].

When a cell culture has become contaminated with a fast-growing eukaryotic cell type, for example the HeLa or HEK293 cell line, limiting dilution might be an option. Diluting and plating cells at low, or even single-cell density

in 96-well plates can help to obtain very small but pure cultures, which can then be expanded and tested, for example using short tandem repeat (STR) analysis).

If a culture is contaminated with mycoplasma, there are ways of trying to remove it from the cultured cells. Although commonly used antibiotics such as penicillin and streptomycin do not kill mycoplasma, there are antibiotics that can be used, for example tetracyclines, macrolides, and quinolones. Most antibiotic formulations optimized for mycoplasma decontamination however suffer from high cytotoxicity and treatment typically needs to be carried out for several weeks [5].

Other types of contamination such as bacteria and fungi quickly release toxins into the culture medium, which may permanently alter the behavior and metabolism of any surviving cells. This means that the reliability and reproducibility of any results obtained with a previously contaminated culture are questionable. However, several manufacturers offer antibiotic and antifungal agents that, in combination with extensive washing and potentially re-plating, could remove early-stage infections. Nevertheless, due to the negative effect of cell contamination and the potential for further spreading, this approach is rarely recommended.

[Click here for more information about STR analysis](#)

DNA Double Helix



## Determining the extent of contamination

After clean-up, or potentially even during clean-up, it is essential to assess the extent of the contamination beyond what is immediately obvious. Communication among lab members plays a key role in this. Whenever any lab member discovers contamination, the responsible lab manager(s) must be informed. They can help inform other lab users that might be affected and advise on further actions. This is particularly important in large communal facilities.

When determining the extent of contamination, it is important to be aware that one type of contamination can affect cultures at different speeds and at different times. This means that when contamination has appeared in one culture, there is a risk that the same contaminant is already present in other cultures as well, e.g. if the same culture medium is used for multiple flasks or cell lines – even if it is not yet visible.

For this reason, all potentially affected cultures must be examined thoroughly to avoid low-level contaminants being overlooked and quarantined if possible. These measures should continue for several days to account for slow-growing organisms. In the case of mycoplasma detection, standard microscopic inspection of live cells is unlikely to reveal the contamination because they are too small, which means that screening for example using PCR is necessary.

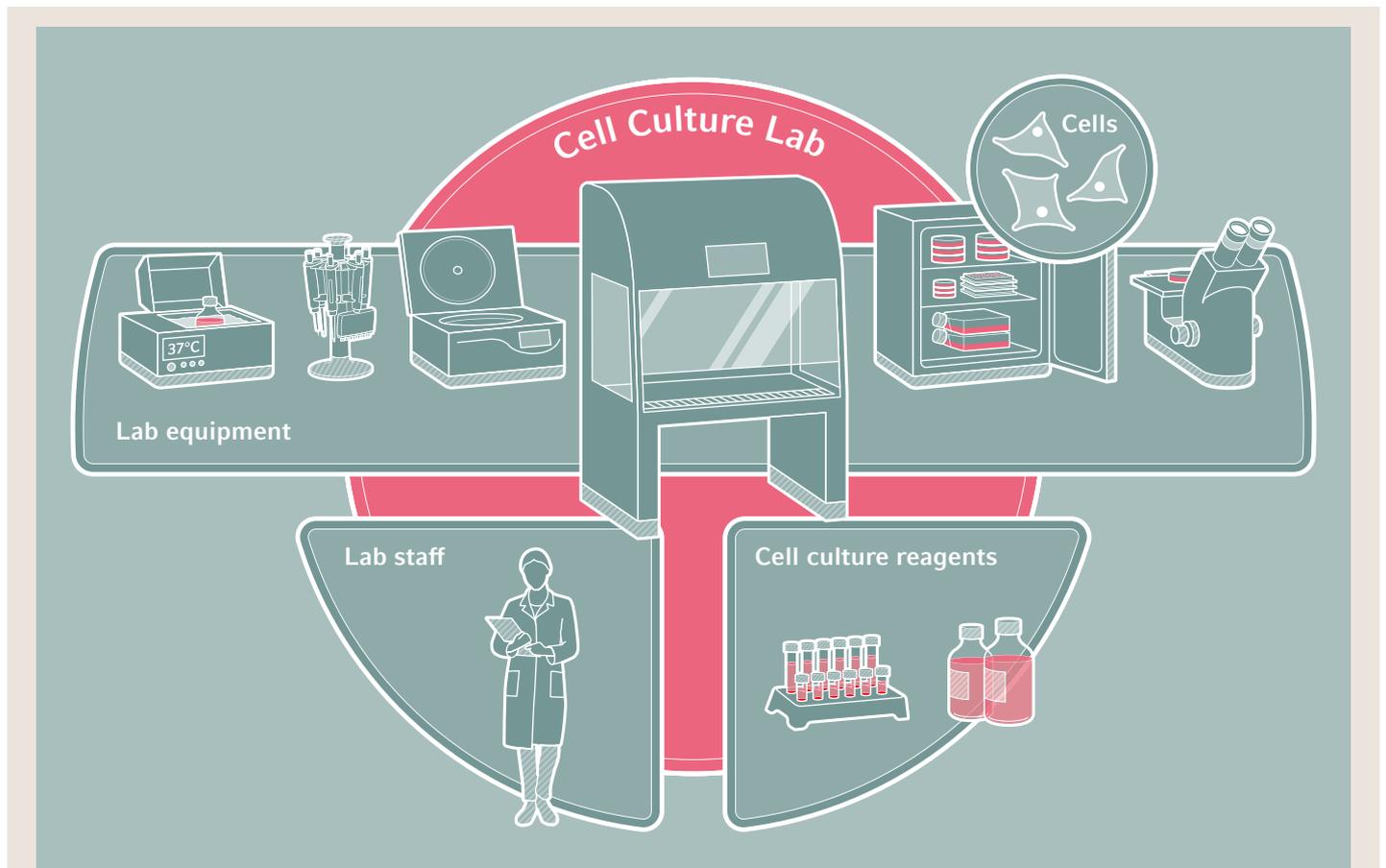
[Find a list of mycoplasma detection methods here.](#)



## Finding the cause

After all measures to control the immediate spread of contamination have been taken, the sometimes-difficult task begins of trying to find the cause. Although many instances of contamination are one-off incidents, for example as a result of improper aseptic technique, many others are caused by an external source, which has the potential to infect other cultures in the future. For this reason, attempts at finding a source should be swift, thorough, and should involve a team rather than an individual.

A good first question to ask is: Is there a pattern? If some cultures are affected while others are not, this might be a clue that leads to the potential source(s), which could be a small, easily overlooked detail (figure 4). If the issue occurs in cultures across the lab, a systemic cause is more likely, for example faulty ventilation, filters, autoclaves, etc.



**Figure 4:** Cell culture contamination can come from many different sources. Looking for a pattern can help in identifying the source.

Another useful question to ask is: What's changed? Labs, in particular research labs, may lack standardization and SOPs for certain procedures, so checking lab journals carefully for recent changes to methods, suppliers, and equipment is another good way to find a cause.

Regardless of whether media and reagents are the cause, it is recommended to discard low-value stock solutions that have been used for the affected cultures. This is because it is also possible that the contamination has already spread from the culture into a stock solution. For higher-value stock solutions, thorough sterility testing should be carried out to ensure they are safe to use. This can be done by incubating samples in a T-flask and placing the flasks in a quarantined incubator (figure 5).

Testing of cell stocks is another routine procedure that can be used to find a cause for contamination, especially when a culture has recently been revived from liquid nitrogen stocks. Testing can be done by thawing and plating another vial from the same stock and checking whether contamination is present. If this is the case, culture should be restarted from an earlier passage or from a newly purchased stock. In the rare event that a supplier is the likely cause of contamination, the supplier in question should be contacted at the earliest opportunity to notify them of the issue encountered. When contacting a supplier, it is important to obtain as much information about the affected product as possible (exact product name, product code, batch number, etc.) so that any manufacturing or logistics issues can be resolved as quickly as possible. To ensure that batch numbers are always traceable, it is important that when aliquoting solutions the batch number is noted on each aliquot.

### Moving forward

After all traces of contamination have been cleaned up and the cause has been identified, a final step that can help in preventing future outbreaks is checking whether any changes need to be made moving forward. When the problem is likely to have originated from a reagent or consumable, it is useful to check that all items are purchased from a reputable supplier, that they are sterile, cell culture grade items, and that the items are shipped appropriately.

When contamination is likely to have occurred due to an operator error, examining and updating existing protocols could help in avoiding future issues. It is important to be aware that errors can occur anywhere and that simple modifications to protocols, for example adding warnings to critical steps, can help prevent errors. In addition, user training helps to improve aseptic working and reduce contamination.



**Figure 5:** Quarantine incubators are a good way to limit the spread of contamination, while saving potentially unaffected cells and solutions.



Find out more information about CO<sub>2</sub>- incubators for isolation and quarantine here.

These working practices make routine use of antibiotics unnecessary and ensure that cultured cells closely represent a natural phenotype or disease state.

Accurate, detailed record keeping is of vital importance to any lab, however tracing the cause of contamination can sometimes reveal gaps in record keeping. The aftermath of a contamination event can be a suitable time to ensure that the record keeping of all lab users is precise and standardized across the lab.

Despite potential benefits in preventing contamination, the routine use of antibiotics in cell culture is not recommended. Many antibiotics have been shown to affect both cell proliferation and metabolism [6]. When bacterial contamination is a recurring issue, the limited use of antibiotics could contribute to reducing the risk of contamination. However, preventing cell culture contamination should normally be achieved by training and following good laboratory practice.

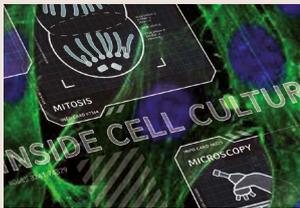
## Summary

Contamination forms a continuous risk to every cell culture lab. Although prevention is the key to reducing the burden of contamination, acting fast, thoroughly, and systematically when it does occur is instrumental in limiting its consequences.

Every lab will have different procedures for dealing with contamination, but in general the steps outlined here form a good starting point for minimizing disruption and helping labs to operate efficiently.

## References

1. Neimark J. Line of attack. *Science* 2015; 347: 938–940.
2. Geraghty RJ et al. Guidelines for the use of cell lines in biomedical research. *British Journal of Cancer* 2014: 1–26.
3. Kennedy DA and Collins CH. Microbiological Safety Cabinets: Selection, Installation, Testing and Use. *British Journal of Biomedical Science* 2000; 57(4): 330–7.
4. Ji Y et al. Mycoplasma Infection of Cultured Cells Induces Oxidative Stress and Attenuates Cellular Base Excision Repair Activity. *Mutation Research* 2019; 845.
5. Uphoff CC and Drexler HG. Eradication of Mycoplasma Contaminations. *Methods in Molecular Biology* 2005; 290: 25–34.
6. Varghese DS et al. Effects of Aminoglycoside Antibiotics on Human Embryonic Stem Cell Viability During Differentiation *In Vitro*. *Stem Cells International* 2017.



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# Standard Operating Procedure/ Dealing with Contamination

## Contacts

<b>Name</b> _____	<b>Name</b> _____
<b>Tel.</b> _____	<b>Tel.</b> _____
<b>Email</b> _____	<b>Email</b> _____

### Step 1: Cleaning up

- > Re-trace your steps (identify potentially affected areas, media, reagents, people)
- > Autoclave and discard affected well plates, T-flasks, Petri dishes, etc.
- > Clean and sterilize incubators, biosafety cabinets, water baths, pipettes, etc.

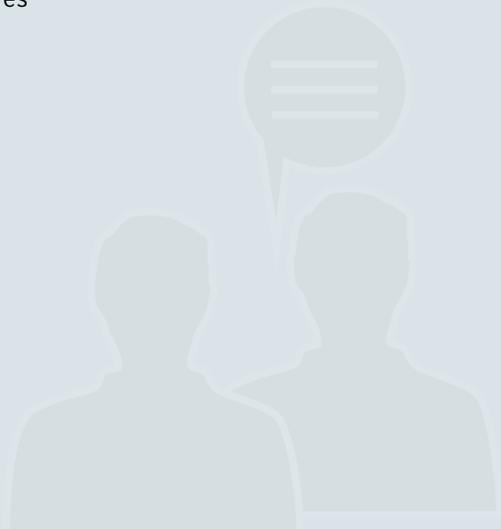
**Notes:**



### Step 2: Determining the extent

- > Inform other lab users and managers
- > Carry out additional checks on potentially affected cultures

**Notes:**



### Step 3: Finding the cause

- > Think: "is there a pattern?" or "what's changed?"
- > Discard or test potentially affected stocks (e.g. media, supplements, cell stocks)

**Notes:**



### Step 4: Moving forward

- > Contact potential sources
- > Think: 'are any changes necessary?' (e.g. protocols, training, record keeping)

**Notes:**

