Application Note

Common forms of cell culture contamination and how to avoid them

Seeing beyond

Author: Dr. Benjamin-Maximilian Schwarz
Carl Zeiss Microscopy GmbH, Germany

Date: April 2023

Solid research and scientific progress rely on dependable research results. Good practice and peer review mechanisms ensure that the imparted knowledge meets an assured quality standard. However, contamination of biological cell cultures continues to jeopardize these standards. According to studies by the U.S. Food and Drug Administration (FDA), 35% of all cell cultures worldwide are contaminated with mycoplasma alone – just one of many cell culture contaminants. It seems as though biological cell culture contamination is accepted as an unpleasant but unavoidable norm in lab environments. The truth, however, is that contamination causes economic damage amounting to hundreds of millions of dollars each year. In this article, you will learn about what kinds of contamination to expect in your daily lab work and what you can do to prevent them.

The most common lab contaminants

Contaminations by bacteria, fungi, and yeasts are among the most frequent in cell cultures, as they surround us everywhere and at all times. The fact that they face no competition for nutrients under perfect culture conditions removes all impediments to unhampered growth.

Bacteria

Bacteria occur in various cell forms. They can be round (*cocci*), rod-shaped (*bacilli*), and spiral-shaped (*spirilla*). Under the microscope, bacterial contaminations can be identified by their morphology, a diameter between 0.5 and 1 µm, and a length of up to 20 µm, and active or passive movement (Brownian molecular motion) between the cells in the cell culture. Bacteria can occur in isolation, in chains of two cells or more, or in clusters.

Look for:

- Moving black dots and rods between the cells or a shimmering shiny surface in the cell-free space
- Objects moving fast in any direction
- Turbidity of the phenol red culture medium and a medium discolored deep yellow (highly acidic) suddenly overnight (aerobic growth)
- Turbidity of the phenol red culture medium and a medium discolored deep pink (highly alkaline) suddenly overnight (aerobic growth)

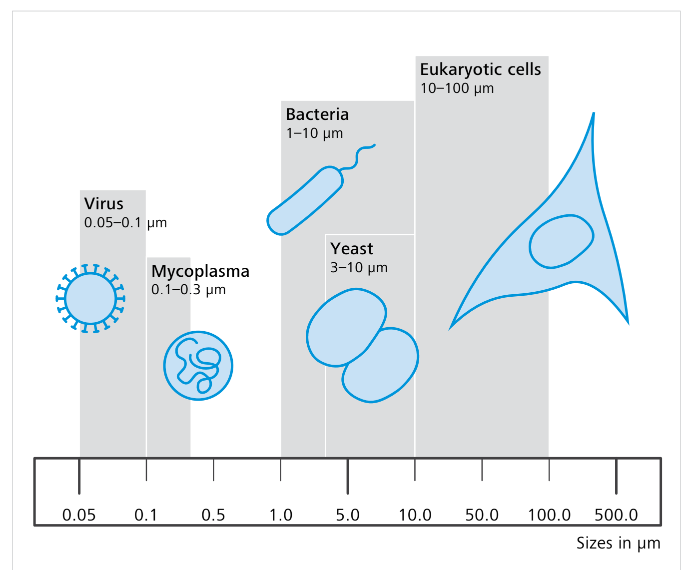


Figure 1 *Size comparison of various types of contamination in biological cell cultures*

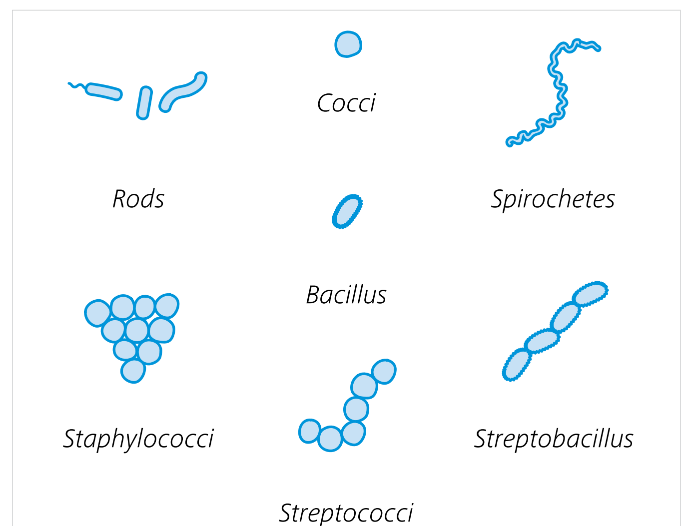


Figure 2 *Morphology of bacteria*

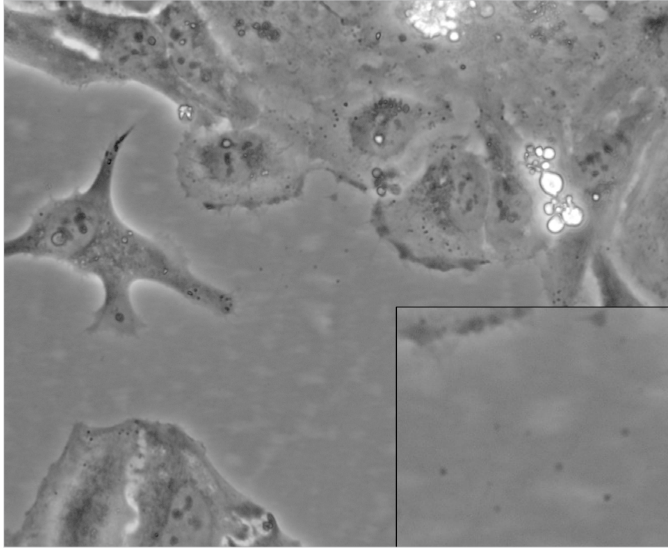


Figure 3 *U2OS cells, an early stage of bacterial contamination; acquired with ZEISS Axiovert 5 digital*

Figure 4 *ZEISS Axiovert 5 digital*

Detection:

Due to their size, bacterial contaminations are easy to detect using conventional light microscopy. With inverted microscopes, 100–400× magnification, and phase contrast, it is easy to integrate a contamination check into your existing lab routine.

Common sources of contamination:

When it comes to preventing contamination, every additional touch and lab utensil is one too many. The less complex your cell handling procedures, the lower the risk. After all, the most common sources of bacterial contamination are:

- Contaminated reagents and media
- Lab personnel / frequently changing users
- Non-sterile lab utensils (water baths, culture flasks, etc.)
- Already contaminated cell isolates

Prevention:

In order to detect bacterial contamination as quickly as possible or to prevent it altogether, it is advisable to use bright field microscopy and contrast techniques to check the cell culture as often as possible and at short intervals. Perform all work in a sterile and standardized manner. Effectively sterilize work surfaces and utensils, and regularly change all room ventilation filters, the clean bench, and pipette aids. If contamination occurs despite these measures, antimycotic agents will help to protect important cell cultures.

In case of contamination:

If local contamination takes place, disinfect all working utensils and dispose of culture reagents and media. You can also eliminate local contamination using antibiotics, if necessary. However, exercise caution when using the research results. In case of heavy contamination, dispose of the cells. If contamination has already spread to multiple cultures, dispose of the cells and disinfect the cell culture equipment and incubator as well as the facilities. Replace reagents and culture media.

Fungi

Fungi are often overlooked during routine microscope checks. This is because they often occur very locally, floating on the surface in the culture, where they can often be mistaken for filter fibers at an early stage. Fungi can be identified as grayish or greenish objects floating on the surface of the culture medium or as a local cord-like hyphal network below the surface.

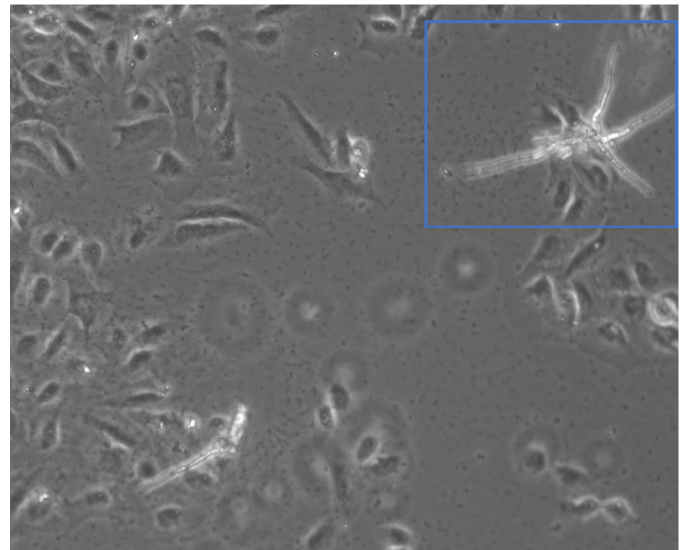


Figure 5 *A contaminated cell culture, marked in blue: initial stage of a fungal-mycelial network*

Look for:

- Turbidity of the phenol red culture medium
- Phenol red culture medium suddenly discolored pink (highly alkaline) overnight
- Structures floating in the cell culture flask
- Visible hyphal formation

Detection:

Trained eyes can very easily identify fungi without the use of a microscope even at an early stage of contamination. But light microscopy can help to confirm or rule out fungal contamination with certainty. Inverted microscopes that feature phase contrast are ideal for this purpose.

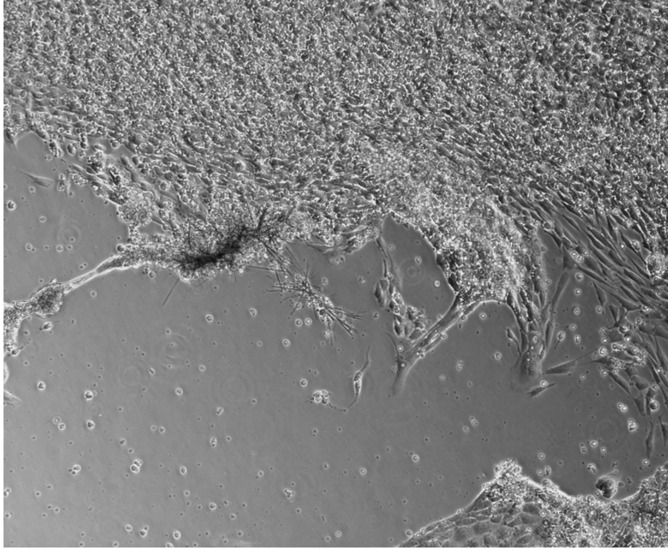


Figure 6 *Eukaryotic cells contaminated and killed by an unidentified fungus; courtesy of Katherine L. McCoy, Sana Biotechnology, USA*

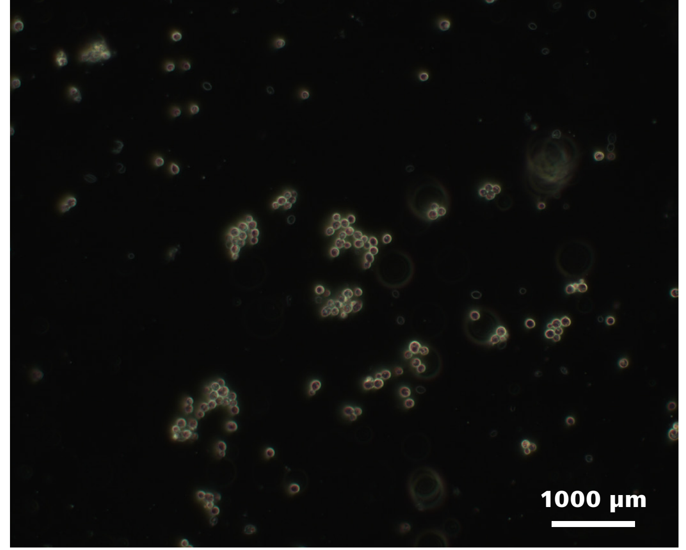


Figure 7 *Bacteria in probiotic food*

Common sources of contamination:

- Contaminated filters (workbench, culture flasks, pipettes / pipette aids)
- Unsterile work practices
- Contamination through ambient air (vents and open doors)
- Reagents and liquids contaminated with spores

Prevention:

The best precautions to permanently protect cell culture from fungal contamination are standardized, sterile workflows and regular checks. Antibiotics may prevent contamination to a great extent but may also cause the cells to behave in physiologically abnormal ways. Since fungal spores can spread quickly through the air in a room without being noticed, regularly replacing filters, keeping workbenches and pipettes sterile, and closing doors and windows are an absolute must. Avoid contamination bridges in the form of wet filters on culture flasks and pipettes and unclean work practices at all costs. Regularly dust and disinfect all lab surfaces. Ideally, all microscope work should be carried out under sterile conditions at a sterile workbench. It is also important to never put your hands above or work over open cell culture flasks.

In case of contamination:

If you detect fungal contamination in a cell culture, remove the culture container from the incubator as quickly and carefully as possible and check for further contamination in other culture flasks. If you do not find any additional contamination, it is still advisable to replace all cell culture filters and to passage all remaining cells as soon as possible. Since fungal contamination in cell cultures is usually very local, it should be sufficient to disinfect all surfaces in the cell culture lab and clean the incubator. Dispose of used/opened culture media and reagents.

Yeasts

Yeasts are single-celled fungi that reproduce by fission or budding. This method of reproduction results in the distinctive features that can be seen under a phase-contrast microscope. Yeasts have round to oval shapes and often occur alone, as groups of two, or as chains in the medium between the cells in the cell culture. Their diameter of 3–4 µm also makes it easy to detect yeast contamination using light microscopy.

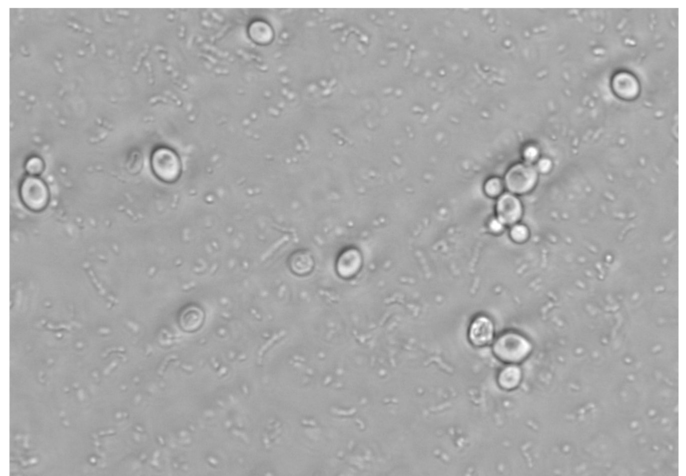


Figure 8 *A cell culture contaminated by yeast and bacteria*

Look for:

- Strong turbidity of the culture medium
- As the medium often discolors late in the course of contamination, looking for discoloration is not helpful for early detection

Detection:

Yeasts are easy to detect using phase contrast and under the microscope at 100× magnification and higher. Even at early stages of contamination, the round bubble-shaped yeast cells are clearly visible under an inverted cell culture microscope.

Common sources of contamination:

- Contamination usually occurs through ambient air (e.g., drafts in the cell culture lab)
- Yeasts can settle in air filters, where they are released into the ambient air over a long period of time
- Contamination often occurs through dust and dander on lab clothing

Prevention:

- Standardized workflows
- Sterile work practices and regular disinfection
- Avoid frequently opening doors
- Wash work clothes regularly or only wear your lab coat in the cell culture lab
- Do not put your hands above open cultures
- Use penicillin and streptomycin

In case of contamination:

In case of local contamination, disinfect all working utensils and dispose of culture reagents and media. You can also suppress local contamination using antibiotics, if necessary. However, eradication is rarely successful. As such, exercise caution when using the research results. In case of heavy contamination, dispose of the cells immediately. If contamination has already spread to multiple cultures, dispose of the cells and disinfect the cell culture equipment and incubator as well as the facilities. Replace reagents and culture media. Optimize your work practices to ensure sterile work.

Time is of the essence when combating the contamination of cell cultures, as the earlier you can detect and eradicate the contamination, the less likely it is to have serious consequences. This is why mycoplasma and viral contamination is one of the most serious hazards for cell cultures.

Infected cell lines do not always exhibit visible damage or mutations in the culture. Due to their small size, it is almost impossible to detect mycoplasma using light microscopy, while viruses can only be identified using special PCR assays.

Mycoplasma

With a diameter of around 0.3 µm, mycoplasma is the smallest bacteria. Furthermore, mycoplasma lacks a proper cell wall and therefore has no characteristic shape. As mycoplasma is mostly parasitic, it represents a major threat to any cell culture. Mycoplasma does not cause a change in pH in the medium, nor does it result in detectable changes in morphology or toxicology in eukaryotic cells in the early stages of contamination. This means there are hardly any early signs of contamination. However, even in low numbers, mycoplasma can cause, among other things, metabolic disorders, nucleic acid synthesis defects, chromosomal alterations, and altered transfectivity.

Important:

As there are no "external signs" of early contamination, it is generally advisable to test the cell culture for mycoplasma at regular intervals as a precautionary measure (every 1–2 months).

Detection:

Various tests allow you to reliably and unambiguously detect contamination. Hoechst 33258 or DAPI staining of a control culture is suitable as a quick first check. To do this, separate an aliquot of the main culture and treat it with DNA-staining reagents. In the case of more advanced contamination, fluorescence microscopy (approx. 400× magnification and higher) can be used to detect accumulated signals along the cells' plasma membrane. However, in order to verify mycoplasma contamination, a PCR test with specific primers for the 16S RNA sequences of the most common mycoplasma strains is recommended. Using this method, you can detect contamination at an early stage and treat it before the damage spreads to the entire cell culture. Given the high relevance for the integrity of the cell culture, a variety of different assays and service providers can be used to detect mycoplasma.

Common sources of contamination:

- Human contamination (clothing, head hair, dander, coughing and sneezing, speaking in the direction of the laminar flow bench)
- Frequently changing lab personnel
- A lack of or insufficiently sterile work practices
- Cultures from manufacturers and cooperation partners that are already contaminated
- Sera, media, and reagents that are already contaminated

Prevention:

Since human interaction with cell cultures is one of the main factors of contamination, it is crucial to take action to prevent this. In terms of sterile work practices, it is very important to always wear a clean lab coat and, if possible, a mask. Avoid engaging in conversations in the cell culture lab, and do not work if you have cold symptoms. New gloves that have been thoroughly disinfected are essential as is footwear designed for working with cell cultures.

Do not store new cell lines, media, and sera for too long before use, and check them for preexisting contamination. Open and close all lab utensils, such as cell culture flasks, in as sterile a manner as possible. Sterilize all lab utensils before storage, and disinfect them again before placing them under the laminar flow bench. Prepare cell cultures as quickly and efficiently as possible to reduce unnecessary exposure to ambient air.

Regularly maintain and disinfect all sterile benches (e.g., with UV light). Do not overfill incubators to avoid transferring any preexisting mycoplasma contamination from one culture to another.

In case of contamination:

If you detect mycoplasma in a cell culture, discard the affected culture flask as soon as possible. If the culture is very important or very expensive, you can also attempt to eradicate the contamination with various antibiotics (e.g., Plasmocin). Concomitant treatment with tetracycline and Baytril is recommended. Treatment may take several weeks or months. However, it will not always be successful and increases the chance of further cell lines being infected. This is because mycoplasma can spread rapidly via contamination bridges, water vapor in incubators, or water baths, and cause considerable damage. To close the contamination window, disinfect the entire cell culture lab and all media and sera in addition to discarding and replacing reagents. You should also replace any open consumables.

Conclusion:

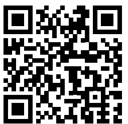
Contamination is a serious problem, but it is controllable. The earlier contamination is detected, the less damage is done. Light microscopes from ZEISS can be perfectly integrated into existing lab routines for detection, so that you stay one step ahead of contamination. To help with routine checks, simple microscopy methods can be integrated directly into everyday workflows. Using inverted microscopes, such as Axiovert 5 and Primovert, it is possible to detect the most common contaminations quickly, reliably, and – most importantly – at a low cost. Combined with the Labscope modules AI Cell Confluency and AI Cell Counting from ZEISS, this easily and quickly increases the reliability of cell cultures through shorter dwell times outside the incubator.

Once a contamination has been successfully identified, depending on the type of pathogen, you should stop all experiments, postpone student work, and decontaminate or replace all lab utensils and consumables. In the worst case, you may need to question results you have already published and revise any submitted manuscripts. In industry, production batches may also fall victim to contamination and have to be recalled for quality reasons. After all, research and production with contaminated cells do not generate reliable results. Contaminated cells can deviate too much from healthy cultures in terms of their morphological, metabolic, and epigenetic properties.

Interestingly, university cell cultures are increasingly affected by contamination. At industry labs, on the other hand, numbers are falling. It stands to reason that constantly changing cultures and a revolving group of new users are conducive to the contamination of cell cultures. The number one risk factor for contamination is human error and a lack of training in sterile work practices.

The key to reducing the risk of contamination lies in user training, short reaction times, and, consequently, regular checks on the culture. The longer a contamination goes undetected, the greater the damage it will cause.

Cover photo
Talaromyces sp. nov. extended depth of field imaging to study the morphology of growing fungi. Acquired with ZEISS Axio Zoom.V16 and ZEISS Axiocam 512 color. Courtesy of C. Visagie, Forestry and Agricultural Biotechnology Institute, University of Pretoria, South Africa



Carl Zeiss Microscopy GmbH
07745 Jena, Germany
microscopy@zeiss.com
www.zeiss.com/cell-culture

Not all products are available in every country. Use of products for medical diagnostic, therapeutic or treatment purposes may be limited by local regulations.
Contact your local ZEISS representative for more information.
EN_41_013_292 | CZ 04-2023 | Design, scope of delivery and technical progress subject to change without notice. | © Carl Zeiss Microscopy GmbH