Cell Culture Contamination: How to Stay Sterile When Contaminants are Everywhere

Aseptic culture technique is something we've all learned and practiced, but few have mastered. There are so many variables at play when seeding, growing, and maintaining cells in culture that it's not uncommon for accidental culture contamination to occur. Whether you exclusively culture mammalian cells, or you traffic in yeast, insect, bacterial, or plant cells, your cultures are prone to contamination. The best way to prevent contamination from corrupting your data is to understand how a variety of contaminants make their way into your culture, and to enact processes to prevent those routes of contamination.

Bacteria, Fungus, and Yeast

When you think of cell culture contamination, you're probably most familiar with these three culprits. We've all left our seemingly healthy cultures in the incubator on a Friday evening, only to be shocked by bright yellow media or a fuzzy film overtop on Monday morning. Bacteria, fungus, and yeast can all wreak havoc on aseptic cultures.

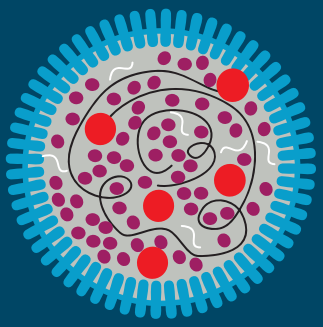
Sterile technique is meant to avoid this type of contamination, but several factors in your laboratory might be working against you. To begin, most scientists share their incubator, meaning that you're not entirely in control of the conditions in which your cells grow. Anything from dripping media to accidental jostling can lead to the introduction of unwelcome microbes. The same is true of shared equipment like biological safety cabinets (BSCs; AKA "hoods") and microscopes.

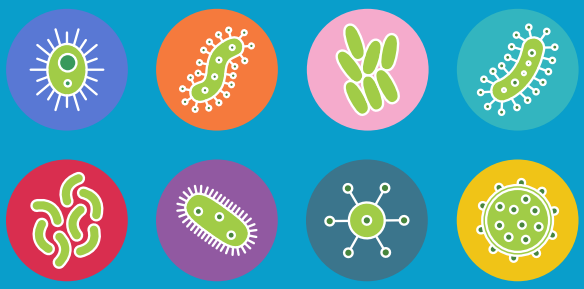
Human error is a major source of contamination. Unchanged gloves, dropped media-bottle caps, and accidentally using nonsterile pipettes can lead to contamination. Those missteps can be avoided with attention and practice, but can you keep your eyelash from landing on your gloved hand while splitting cells? Can you stop your skin cells from being sucked into the BSC? Humans have evolved to coexist with these microorganisms, so we're all walking, talking contamination factories.

Mycoplasma

Mycoplasma, a bacterium, deserves its own section since it is so sneaky and insidious, it can completely alter the behavior of your cultures without ever being detected. The small cells lack a cell wall, so they escape the actions of most antibiotics. Their presence is an added stress on your cells, which may alter their behavior. Even worse, you may sequence a gene or identify a protein in your culture that leads you to new conclusions, only to discover – often, too late – that your finding was due to mycoplasma contamination.

Mycoplasma is the bane of cell-culture facilities, and it can be difficult to eradicate once it has made its way into your cell-line stocks. Once you've cleaned up and replaced your cells, take precautions to avoid reinfection of your cultures. Did you know that humans play host to several species of mycoplasma? So, don't cough or sneeze near an open BSC!





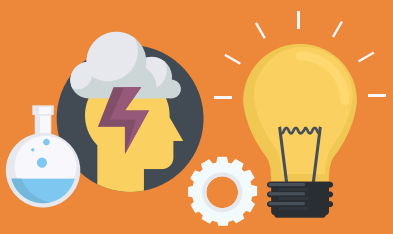
Other Cells

Culture contamination with non-native cells is a common and persistent problem. In fact, according to ATCC, up to 36 % of cell lines may be contaminated with unexpected cells.[1] These cells compete with the original cells for nutrients and they may overtake the original cells. Depending on the interloper, there may be no overt signs of this culture coup, only detected when suspicions lead to the thawing of a new vial of cells and leaving scientists to wonder how long their cultures have been compromised. Along with all media, supplements, and the frozen stocks, piles of data are often discarded.

Where do these rogue cells come from? Whenever multiple cell types are being cultured in the same incubator, fed and split in the same BSC, and monitored with the same equipment, there is a chance for cell cross-contamination. Being fastidious about changing tips/pipettes, working with only one cell line at a time, judicious use of ethanol spray, and making sure that equipment (micropipettes, serological pipettes, BSC surfaces, microscope stages, plate imagers, etc.) don't harbor any hitchhiking cells is the only manual way to forestall an invasion.

Process Makes Perfect

The old saying, "practice makes perfect" holds true in several arenas, but practice can't prevent all cases of cell culture contamination. Instead, you'll need to adopt a process that ensures that your aseptic cultures stay that way. The best way to prevent cross-contamination and environmental contamination is to use a self-contained liquid handling system to seed and feed your cells. The precision of these platforms helps to prevent aerosol formation, splashover, tip reuse, and culture perturbation. Beckman offers several Biomek liquid handlers that can be set up within an enclosure to prevent dust-borne contamination. Committing to preventing contamination takes planning and effort, but the savings in time and wasted reagents can make the process well worth your while.



References: 1. P. Hughes et al., "The cost of using unauthenticated, over-passaged cell lines: how much more data do we need?," BioTechniques, 43:575-586, 2007.

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