

Tue 12/16/03 2:05 PM

A Biosafety Discussion List [BIOSAFTY@MITVMA.MIT.EDU]; on behalf of; Scott, Rick  
[SCOTTWI@MAIL.ECU.EDU]  
[BIOSAFTY@MITVMA.MIT.EDU](mailto:BIOSAFTY@MITVMA.MIT.EDU)  
fungal contaminated cell culture...

I am helping a PI who is suffering from a fungal contamination problem. He is culturing murine 3T3 cells on DMEM media supplemented with either calf or fetal calf serum. The problems are showing up usually about 2-3 weeks into the cycle, other than that- no obvious pattern. I recently rescanned the supply and exhaust HEPA on their biosafety cabinet. The BSC runs 24/7. The incubator has a new filter in it, and they keep it wiped out religiously. Between the limited ideas I have for them, and the things they have already tried, we're a bit short on ideas. They are doing a lot of things right. I know this is a bit like saying, "my car is squeaking, what's wrong with it?" So- I am not asking for a definitive answer, just ideas.

I read one website that cited dust mites as a major culprit of fungal contamination. ?

Thanks for any help you can offer,

Rick Scott  
East Carolina University

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A Biosafety Discussion List [BIOSAFTY@MITVMA.MIT.EDU]; on behalf of; Vic D'Amato  
[vdamato@MASIMAX.COM]  
[BIOSAFTY@MITVMA.MIT.EDU](mailto:BIOSAFTY@MITVMA.MIT.EDU)  
Re: fungal contaminated cell culture...

Scott;

I had a similar problem with mold contamination in media, which was definitely incubator-related. We changed out the water reservoirs, water lines, and the tygon tubing lines for the CO2. This seemed to solve the problem in one case. For another incubator, we had to resort to decontaminating the incubator using formaldehyde, which seemed to solve the problem.

Good luck.

Victor J. D'Amato, CIH, CSP  
Deputy Director, Environmental Health and Safety Services  
MasiMax Resources, Inc.  
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Reston, Virginia 20190

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A Biosafety Discussion List [BIOSAFTY@MITVMA.MIT.EDU]; on behalf of; Melinda Young  
[melinday@BART.RPRC.WASHINGTON.EDU]

[BIOSAFTY@MITVMA.MIT.EDU](mailto:BIOSAFTY@MITVMA.MIT.EDU)

Re: fungal contaminated cell culture...

Have you spoken to the incubator vendor. Does it have a small fan to recirculate the air? I recall one particular model which had problems with the propellers on the fan supporting fungal growth...they changed them out ones constructed of another material.

It also helps to check humidity of lab...we have found in our area if you have high humidity you could have many spores in the room air and it only takes 1 to tag along on a glove, etc.

Melinda

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A Biosafety Discussion List [BIOSAFTY@MITVMA.MIT.EDU]; on behalf of; Joseph P. Kozlovac  
[jkozlovac@NCIFCRF.GOV]

[BIOSAFTY@MITVMA.MIT.EDU](mailto:BIOSAFTY@MITVMA.MIT.EDU)

Re: fungal contaminated cell culture...

If the incubator is more than a few years old you may want to check the CO2 monitor port. In some of the older models of incubators this line was not equipped with a filter and thus you had a piece of tubing running from the outside of the incubator to the inside which would develop condensation in the line with subsequent mold growth. Every time the door was open and closed you had the potential to contaminate the incubator interior. Might be a long shot but something you can easily check out.

The only other suggestion I would have is look at the technique of the folks doing the cell culture. Are they working appropriately within the BSC, are they using good sterile technique, etc. In my experience it contamination issues are typically a result of bad technique rather than a Bad HEPA filter.

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A Biosafety Discussion List [BIOSAFTY@MITVMA.MIT.EDU]; on behalf of; Moravek, Paula  
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[BIOSAFTY@MITVMA.MIT.EDU](mailto:BIOSAFTY@MITVMA.MIT.EDU)

Re: fungal contaminated cell culture...

A couple of suggestions...

Sometimes the trays and/or shelves that culturists use to shift their cultures on are either:

1. Not cleaned often enough (soap & water first, then wrapped and autoclaved OR chemically sterilized before use)
2. Not made of cleanable materials (i.e.: cardboard, rusty metal edges, "rolled" metal edges that can't be really cleaned, etc.))

If carts are used to transport TC cultures and/or culturing materials, check UNDER the shelves and see if there is cardboard glued to it (it's there for sound dampening--but spores & other dirt stick). Also make sure the carts get a good cleaning/  
surface disinfecting every once in a while, especially if carts are used as an auxiliary work surface.

Paula Moravek  
Chemistry & Biochemistry Department

Biosafety Officer - Environmental & Occupational Safety  
Worcester Polytechnic Institute, GH128  
Worcester, MA 01609

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A Biosafety Discussion List [BIOSAFTY@MITVMA.MIT.EDU]; on behalf of; Steve Brown [sabrow1@UKY.EDU]

[BIOSAFTY@MITVMA.MIT.EDU](mailto:BIOSAFTY@MITVMA.MIT.EDU)

Mold contamination

One of our labs recently had a similar problem. I traced it back to contaminated pipet-aids. One had mold growing in the nosepiece and a second one had a moldy cotton plug from a pipette stuck in it. It has been my experience that most labs rarely (never) clean their pipet-aids. Its a good idea to always have a clean one available for use when culture media is drawn up into a handpiece.

Steve Brown

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A Biosafety Discussion List [BIOSAFTY@MITVMA.MIT.EDU]; on behalf of; Richard Fink [rfink978@HOTMAIL.COM]

[BIOSAFTY@MITVMA.MIT.EDU](mailto:BIOSAFTY@MITVMA.MIT.EDU)

Re: fungal contaminated cell culture...

Hi Rick,

I agree with the others - most likely the incubator but I had one case of contamination that was traced to the BSC - under the work surface. I know, theoretically what is under the work surface should not be able to contaminate the work surface itself. However in reality it was. Removed the work surface and thoroughly cleaned the bottom and plenum space (all very, very dirty). Contamination ended.

Another thought is to clean the incubator with a quat - very fungistatic in high dilution and leaves a residue. If the TC cells are very sensitive, they may not like the quat either.

Richie Fink  
Biosafety Officer  
Wyeth BioPharma  
Andover, MA

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A Biosafety Discussion List [BIOSAFTY@MITVMA.MIT.EDU]; on behalf of; Judy Pointer [JPointer@SALUD.UNM.EDU]

[BIOSAFTY@MITVMA.MIT.EDU](mailto:BIOSAFTY@MITVMA.MIT.EDU)

Re: fungal contaminated cell culture...

You've gotten lots of good possible answers Scott. I couldn't resist adding my experience. I think I told this story years ago to the group.

I had a fungal contamination problem, once. After months and months of cleaning, etc. I just happened to notice one day when I opened the incubator first thing in the morning, that water droplets had formed

on its ceiling AND one of them just happened to fall before my very eyes. It landed just beside the rim of one of the plates and it seemed to bounce back up into the space between the plate's rim and it's top. The mysterious contamination more often happened in plates versus flasks. I started using plates only when I had to. When I did, I would first gently suck out any bubbles of trapped media, before I even opened the plate and then I would wipe the plate rim with an alcohol dipped sterile gauze pad before I changed the media. I still cleaned the incubator everyday, but I think the change in technique was the solution because I never had another case of the dreaded FUNGAL INFECTION!

Judy Pointer UNM

Wed 12/17/03 6:37 AM

A Biosafety Discussion List [BIOSAFTY@MITVMA.MIT.EDU]; on behalf of; [pr18@COLUMBIA.EDU](mailto:pr18@COLUMBIA.EDU)  
[BIOSAFTY@MITVMA.MIT.EDU](mailto:BIOSAFTY@MITVMA.MIT.EDU)

Re: fungal contaminated cell culture...

We had a similar problem in which the PI was doing everything right but was still getting contamination-this includes careful cleaning of the incubator. It stopped when they had their incubator formaldehyde-gas deconned,

Paul RubockQuoting "Scott, Rick" <SCOTTWI@MAIL.ECU.EDU>:

Wed 12/17/03 10:33 AM

A Biosafety Discussion List [BIOSAFTY@MITVMA.MIT.EDU]; on behalf of; Matthew S Philpott  
[mphilp1@LSU.EDU]

[BIOSAFTY@MITVMA.MIT.EDU](mailto:BIOSAFTY@MITVMA.MIT.EDU)

Re: fungal contaminated cell culture...

Probably the incubator or BSC is the source. Removable parts should be thoroughly cleaned and autoclaved if possible at least weekly. Once a cell line becomes contaminated, it is likely to remain so. Sometimes you can passage the cultures repeatedly in the presence of nystatin or ketokonazole and "cure" them, sometimes not.

Matt Philpott  
LSU

Wed 12/17/03 10:55 AM

A Biosafety Discussion List [BIOSAFTY@MITVMA.MIT.EDU]; on behalf of; Hauck, Philip  
[philip.hauck@MSSM.EDU]

[BIOSAFTY@MITVMA.MIT.EDU](mailto:BIOSAFTY@MITVMA.MIT.EDU)

Re: fungal contaminated cell culture...

I had personal experience with this.....the contaminated BSC, especially the plenum under ther front grastes/grill. The PI I worked for was notorious for working over the front grate. After bombing the incubator twenty times in a row, I pulled the grates (Old Baker Cabinet....what was underneath was appalling. I had to spray down and scrub the surface...thats why you had a ball-cock-drain valve on the old Sterilgards...to flood it drain it, and air wash it. Once done, no more contamination. Caveat....make sure you are not working with pathogens, or else decon the unit first, then access the area under the grates.

Phil Hauck

Wed 12/17/03 11:37 AM

A Biosafety Discussion List [BIOSAFTY@MITVMA.MIT.EDU]; on behalf of; Kyle G Boyett [kboyett@UAB.EDU]  
[BIOSAFTY@MITVMA.MIT.EDU](mailto:BIOSAFTY@MITVMA.MIT.EDU)

Re: fungal contaminated cell culture...

Rick, All of the advice that you have been given is very good and valid. One more thought. We encounter situations like this from time to time also and we recommend to the PI that he/she explore every aspect of where the cultures travel and look for contamination there also. On several occasions we discovered the contamination in the water bath that was being used. Hope this helps.

Kyle

Kyle G. Boyett  
Asst. Director of Biosafety  
Safety Short Distribution List Administrator  
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Wed 12/17/03 12:04 PM

A Biosafety Discussion List [BIOSAFTY@MITVMA.MIT.EDU]; on behalf of; Barbara Ernise  
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fungal contaminated cell culture...

ick,

My experience with fungal contamination was extremely personal. I finally went fungus free when I started wearing gloves at all times in tissue culture. Check also that lab coats are clean and cleaned frequently and that wrists are covered by the coat and/or gloves. Barb Ernise Children's Hospital, Boston

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A Biosafety Discussion List [BIOSAFTY@MITVMA.MIT.EDU]; on behalf of; Ward, Connie B  
[Connie.Ward@MED.VA.GOV]

[BIOSAFTY@MITVMA.MIT.EDU](mailto:BIOSAFTY@MITVMA.MIT.EDU)

Re: fungal contaminated cell culture...

Just a note: When water droplets form on the ceiling of your incubator, it means the water in the jacket is low. If you top it off this problem will disappear.

Connie Ward  
Biosafety Officer  
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