CONTROLLED Environments® MAGAZINE
CONTAMINATION CONTROL FOR LIFE SCIENCES AND MICROELECTRONICS
NOVEMBER/DECEMBER • 2011 VOL. 14 • NO. 10

Thermal Decontamination:

The Key to Effective Culture

When culturing various organisms it is vital that they do not become cross-contaminated compromising their viability.

Verena Ruckstuhl

Contamination is a major concern for any microbiologist. When culturing various organisms (bacteria, yeast, or fungi) for use in a multitude of downstream applications, it is vital that potentially contaminating microorganisms are removed from the incubation environment. If cultures do become infected with microorganisms, or cross-contaminated with foreign cells, the viability of the culture is significantly compromised. In order to prevent this from having a negative impact on subsequent experimental steps and resulting data, contaminated cultures are commonly destroyed. As such, time and money are wasted as the entire preparation and culture must begin again, using fresh organisms and reagents. However, since sources of contamination are ubiquitous and difficult to identify, they are often especially hard to completely eliminate. As cell culture research is taking an increasingly prominent position in the development of therapeutics and vaccines, for example, it is vital that laboratories across the pharmaceutical, medical, food, research, and clinical sectors are employing incubation techniques with proven, trustworthy decontamination methodologies.

> As incubators are commonly shared, one user's culture can have a negative effect on the next user's culture...

THE MICROBIOLOGICAL INCUBATOR

Designed for laboratory use, microbiological incubators maintain optimal conditions for the effective culture of prokaryotic and/or eukaryotic cells. They provide advanced control over temperature to ensure that organisms proliferate and grow in a fast and efficient manner. As incubators are commonly shared between multiple users, it is often the case that if the internal chamber is not properly cleaned, one user's culture can have a negative effect on the next user's culture, through the cross-contamination of cell types. Furthermore, microorganisms can be introduced through regular door openings, contact with skin/hair/ clothes, or poor cleaning routines. Bacteria and fungi (including yeast and

Microorganism	Colony forming unit (CFU) applied to each contamination site
Aspergillus brasiliensis (conidiospores)	1st run:, total of 1.2 x 10^8 2nd run: total of 0.9 x 10^8
Bacillus atrophaeus (spores)	1st run: total of 4.8 x 10^8 2nd run: total of 4.9 x 10^8
Pseudomonas aeruginosa	1st run: total of 5.4 x 10^8 2nd run: total of 4.7 x 10^8
Staphylococcus aureus	1st run: total of 4.7 x 10^8 2nd run: total of 4.2 x 10^8

Table 1: Microorganisms and CFU applied to contamination sites.

molds) are the easiest contaminants to detect. They are also ubiquitous to the environment and can colonize extremely fast. Other contaminating microorganisms, such as mycoplasma and viruses, are more challenging to identify, yet can cause significant damage to the culture and, on occasion, the user. Therefore, the ideal microbiological incubator would incorporate an effective decontamination method, to ensure that even trace amounts of potentially detrimental contaminants are removed from the incubation environment. This ensures that precious cultures are protected and integrity is maintained.

Here, we discuss a third party test (performed by IBFE Institut für Biotechnische Forschung und Entwicklung, Germany),[1] in which the 140ºC dry heat decontamination cycle of an incubator is tested for its effectiveness in eliminating a spectrum of contaminating microorganisms.

METHODS

A microbiological incubator was tested to assess the efficiency of its built-in 140°C thermal decontamination cycle. Cell suspension was added directly to Phosphate Buffered Saline (PBS), diluted and plated on Tryptic Soy Agar (TSA), to determine the total colony forming units of the applied suspension. This allowed determination of the efficacy of the recovery of the cells from the surface after the experiment.

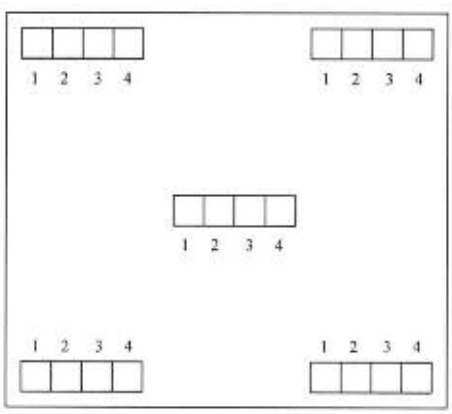

Figure 1: Sites of contamination for the testing of the thermal decontamination efficiency with various test microorganisms.

In order to test the effect of the surfaces on cell inactivation, two surfaces of the incubator interior (the bottom stainless steel panel and the inner surface of the glass door) were contaminated, as detailed in Table 1 above.

- Cell suspensions were dried on the surfaces for six hours at 35ºC.
- The organisms were recovered from the surfaces using sterile cotton plugs.
- Following vortexing and ultrasonication, appropriate dilutions were spread on TSA agar and colony forming units (CFU) were consequently determined after 24 and 48 hours of incubation.

To test the efficacy of the decontamination routine on cell inactivation, 55 areas of the interior of the unit (right, bottom, top, left, back, interior of glass door, exterior of glass door, steel door, shelf 1, shelf 2, shelf 3) were contaminated with each of the microbial suspensions listed in the table.

The location of the contamination is represented in Figure 1.

- The decontamination program was run until completion (140ºC, 6 hours).
- The microorganisms were recovered using microbiological detection plates—replicate organism detection and counting (RODAC), with TSA agar as a growth medium. This method is more sensitive than the cotton plugs for detection of very low CFU levels on surfaces.
- After an incubation period of 24 and 48 hours, the number of CFU were determined.
- The entire protocol was performed twice, to ensure reliability of the resulting data.

RESULTS

After running one cycle of the thermal decontamination (140ºC for 6 hours), the study found that none of the microorganisms were recoverable from any of the artificially contaminated surfaces. This demonstrates that an inactivation of more than 99.99999% (>7 log) occurred. Results from the second decontamination cycle used in the study presented identical inactivation rates. No viable microorganism cells were recoverable from any part of the surface.

The comparative drying process of 6 hours at 35ºC used in the study did not have much of an impact on the spores (*B. atrophaeus*) and conidiospores (*A. brasiliensis*). The CFU levels of *P. aeruginosa* and *S. aureus* were influenced the most, reflecting their need for humid environmental conditions. However the inactivation was below 1 log and negligible, compared to the above demonstrated effectiveness of the 140ºC decontamination cycle.

CONCLUSION

Decontamination is a pivotal part of any culturing protocol to eliminate contamination from various microorganisms. By incorporating an effective decontamination cycle into a microbiological incubator, such as the 140ºC decontamination cycle of the incubators, users can be confident that potentially contaminating microorganisms present are effectively inactivated. Certified by an accredited microbiological institute (IBFE Institut für Biotechnische Forschung und Entwicklung, Germany), this routine eliminates the need for separate autoclaving of interior fittings, freeing up valuable time for other experimental protocols. In addition, this level of confidence in the integrity of the cultured organisms ensures their viability for downstream use. Users can therefore be confident in the knowledge that their resulting data is reliable and reproducible, while being of the highest possible quality.

References

1. Report # 1420710-2a: Determination of the Efficiency of the Thermal Decontamination in a Microbiological Incubator (Heratherm IMH180-S/41112744). Dr. Heiko Ewen, IBFE Institut für Biotechnische Forschung und Entwicklung.

PFCTHTINCarticle_NA_11_2011

Verena Ruckstuhl *studied Biological Process Engineering and is an applications specialist for Thermo Fisher Scientific. She is responsible for the biological R&D lab at the Langenselbold site in Germany. With two years of experience in the life science industry and as a certified disinfector, one of her functions is to develop disinfection methods for laboratory equipment. While working as a lecturer at disinfection seminars she enjoys sharing experiences and can be contacted at verena.ruckstuhl@thermofisher.com.*