Case Study – Decontamination of a PC2 Cell Culture Laboratory in a University setting.

Brett Cole, B.Sc (Hons) M. Occ. Hyg. & Tox. CBP[1], **Benedikt Heyn**[1]
And **Mark A Czarneski,** BSc MSc (Eng)[2]

1. Biosafety Pty Ltd – Hygiene Consulting & Services, PO Box 101 Ferntree Gully Victoria Australia 3156
2. ClorDiSys Solutions Inc, PO Box 549 Lebanon New Jersey USA 08333

Hygiene Consulting
Decontamination
Contamination Control

COMMERCIAL POSTER

ABSTRACT

The Australian Standards and Regulators do not require PC2 (Physical Containment Level 2) Laboratories to undergo routine fumigation or gaseous decontamination as part of their preventative maintenance programs. This is due to the category of biological samples being handled, that is, Risk Group 2 (Standards Australia, 2010). However, quite often PC2 laboratories have contamination issues that can require gaseous decontamination.

A University had a contamination issue in a PC2 laboratory cell culture facility. The cause of the contamination was investigated and it was revealed that biological contamination from the Heating and Ventilation System (HVAC) was contaminating the cell cultures during laboratory operations. A hygiene approach was taken to investigate the cause and appropriate rectification works were recommended. This included the addition of filtration fitted to the supply air to the PC2 laboratory and the laboratory cleaning and fumigation using Chlorine dioxide gas to decontaminate any remaining potential biological contamination. More rigid housekeeping and gowning procedures were also implemented to reduce future contamination from occurring.

The process involved coordinating rectification works, remediation and gassing the facility to ensure that the laboratory was remediated and minimise the chance of future contamination of the cell culture processes. The data and results indicated that the project was successful.

INTRODUCTION

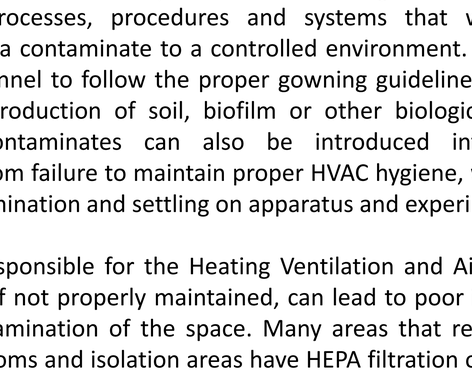
Contamination is an ever present concern in controlled environments, with potentially severe consequences for facilities and the staff that work in them. Ensuring sterility within these environments is a process that begins during the construction of a facility and will in most instances continue up until its decommissioning.

The causes of contamination vary as wildly as the different forms of contamination itself. Commonly however, it is through the improper appliance of everyday processes, procedures and systems that will lead to the introduction of a contaminate to a controlled environment. For example, the failure of personnel to follow the proper gowning guidelines of a facility can lead to the introduction of soil, biofilm or other biological contaminates. Additionally contaminates can also be introduced into a controlled environment from failure to maintain proper HVAC hygiene, which can lead to airborne contamination and settling on apparatus and experiments.

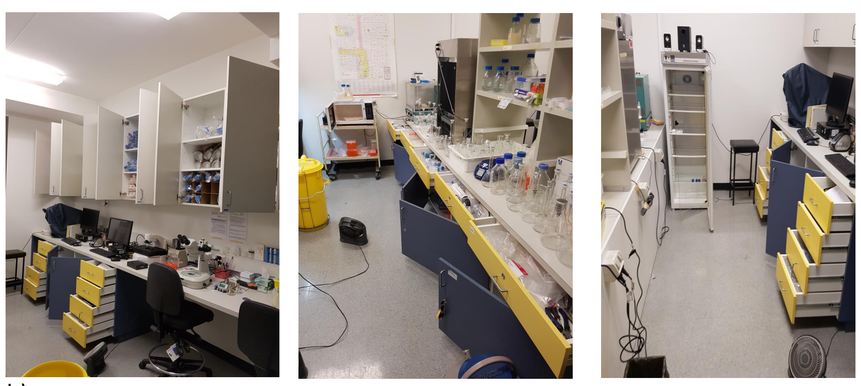
The HVAC is responsible for the Heating Ventilation and Air-Conditioning of any space and if not properly maintained, can lead to poor indoor air quality (IAQ) and contamination of the space. Many areas that require critical IAQ such as cleanrooms and isolation areas have HEPA filtration or similar fitted to the HVAC system. PC2 laboratories are considered potentially "dirty" zones and therefore rarely require HEPA filtration. Cell Culture facilities can sometimes fall in between these two requirements with regards to containment (net air flow inwards) and air cleanliness (ISO14644-1).

Built specifically to conduct work with risk category 2 microorganisms, PC2 laboratories have to comply with both the Australian/New Zealand standard for safety in Microbiological laboratories and the OGTR (Office of the Gene Technology Regulator) certification guidelines for a level 2 physical containment facility . This particular laboratory was PC2 compliant however had contamination issues on the media and cell culture.

Following the hierarchy of controls (see Figure 1.1), the potential source of contamination was investigated, the facility was isolated and work ceased as a precaution. The room was isolated and live cell cultures were stored in two incubators. The challenge here was that these live cultures had to remain in the incubators whilst the hygiene works were conducted. This meant that they had to be isolated in the space during the gassing phase of the project.



a)



b)

Figure 1.1 **a) The hierarchy of controls**
b) Images of the inside of the PC2 Cell Culture Facility.

METHODS

The decontamination of the PC2 cell culture facility of 72m^3 volume involved a stepwise approach to achieve success. Following the concept of the hierarchy of controls and good hygiene practice (GHP), the following steps were identified:

Control level	Description
Elimination	Not possible as process is critical to research
Substitution	Not possible as process is critical to research
Engineering Controls	Fitting additional filtration to supply air Cleaning of laboratory Fumigation of laboratory to create sterile background
Administrational Controls	Restricted access Improved Gowning Procedure Better Good Laboratory Practice (GLP)
Personal Protective Equipment (PPE)	Better sterile gowning and entry procedures

The first phase involved fitting a Filtration system to the supply air followed by manual cleaning of the facility to remove organic load and dust. Gassing with chlorine dioxide (ClorDiSys Solutions Inc) followed by preventative administrational measures to reduce future contamination. The additional challenge of this project was the presence of active cultures that had to remain in the laboratory whilst the decontamination process was undertaken.

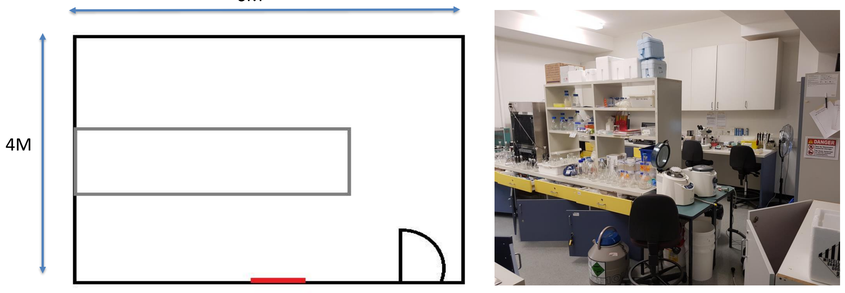


Figure 1.2 – The floor plan of the cell culture Laboratory, with the red area indicating the suspected contaminated supply air.

Another challenge associated with the fumigating the cell culture laboratory was ensuring that the concentrations of ClO2 could be held at levels high enough to ensure kill, in a space that was not engineered to undergo a gaseous decontamination cycle. Additionally there was also the presence of live cultures that needed to be taken into consideration during the fumigation cycle. These were sealed in the incubators and left running whilst gassing was conducted.

The process of fumigation could then be carried out in a time efficient manner. Once technicians had arrived on site, the process of preparing the laboratory began Immediately with the relative humidity of the lab being raised to between 65%-75% and fans being laid out to ensure even dispersal of the ClO2 gas. As the HVAC system could not be used in this instance to safely purge the Laboratory of Chlorine Dioxide, carbon scrubbers were setup within the room and in a manner which allowed them to be externally activated when required.

Figure 1.3 – The Cell culture laboratory after setup for fumigation had been completed. The air filter fitted on the supply air can be seen in the centre with the filter after 1 week operation. The sealed incubator containing live cultures can be seen on the left.

The gassing method followed the standard ClorDiSys Solutions Inc (New Jersey, USA) Chlorine dioxide gas generation method whereby Chlorine dioxide gas is generated outside the target area and is then injected into the building until the internal Chlorine dioxide gas concentration reached 1mg/L required for a Log 6 reduction. The gas level in the lab was maintained until the exposure (concentration x time) reached 720 ppm hours and residing at a relative humidity (RH) of 65-75%. To ensure the efficacy of the process, standard biological indicators (*G. stearothermophilus* – Tyvek on Tyvek – NAMSA Labs) were used to determine that a Log 6 reduction had indeed been reached in all areas of the laboratory. Once the required exposure was reached in all gas sample locations, carbon scrubbers were activated to purge the ClO2 from the laboratory until the concentration drops to 0.1 ppm. This is the 8-hour safety level which in addition to the odour threshold level (Safe Work Australia, 2011) ensured that the laboratory would be safe to enter after that time. All biological indicators were removed and returned to a third-party laboratory for analysis. All other equipment was then removed from the site.

RESULTS

The following results were obtained from the biological indicator data, concentration of chlorine dioxide (CD) gas and ongoing from the study. Figure 2 (a) shows the post decontamination biological indicator results. Figure 2 (b) shows the concentration and exposure of chlorine dioxide (CD) gas. from each of the three stages gas sampling points and it can be seen that in all locations that the minimum exposure of 720 ppm-hrs was achieved

Biological indicator	Status after Fumigation
A1 A2	No Growth No Growth
B1 B2	No Growth No Growth
C1 (control) C2 (control)	Growth Growth

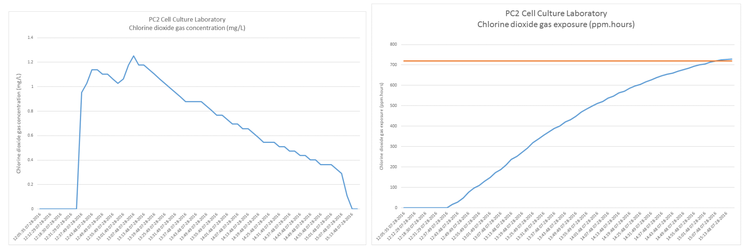


Figure 2 (a) Biological Indicator results and b) Gas concentration and exposure.

DISCUSSION

At all gas sample locations a minimum of 720 ppm-hrs exposure to Chlorine dioxide gas was achieved. There was no growth in exposed BI's confirming the gassing cycle was successful.

During the fumigation cycle, consideration had to be given towards the live cell cultures still within the laboratory. These were contained within sealed incubators, which then had additional sealing measures applied to prevent intrusion by the chlorine dioxide gas.

The ongoing monitoring of cell cultures by the facility staff has shown that the repeated cases of contamination have ceased after the cleaning, fumigation and preventative measures were commenced.

During the packing down process of the decontamination cycle, a new pre-filter was placed over the HVAC supply air grill, which had been determined as a probable source of contamination. After a period of a week the temporary fixture was removed with a more permanent housing for the filter being put into place. Over the duration of a week there were significant levels of particulate build up on the filter which suggested that it was highly likely that this was a source of contamination within the laboratory.

With the addition of better administrational control measures, the facility has implemented restricted access and better gowning procedures to help prevent re-contamination occurring through the transition of personnel throughout the facility.

CONCLUSION

From the data obtained from this study, the Chlorine dioxide gas concentrations in the monitored areas all exceeded the minimum level of 720ppm-hrs required to successfully perform a log 6 decontamination cycle. The post-decontamination concentrations for each stage were all negative supporting successful biological decontamination. After the hygiene measures herein were undertake, the ongoing cell culture contamination monitoring indicated that the facility remained contamination free. Given the aforementioned post-decontamination results, it is feasible to assume that the decontamination of the Cell culture laboratory was successful.

ACKNOWLEDGEMENTS

The author's would like to acknowledge the assistance of Mr. Mark Czarneski from ClorDiSys Solutions for technical advice and all others who assisted with this project. We would also like to acknowledge the input and support of the staff at the Research Facility and for providing ongoing reports.

This poster is presented as a case study of a decontamination project performed by Biosafety Pty Ltd. Biosafety Pty Ltd. acknowledge that this was a commercial project and this case study is presented as such.

REFERENCES

Luftman, H., Regits, M., Lorcheim, P., Lorcheim, K., & Paznek, D. (2008). Validation Study for the Use of Chlorine Dioxide Gas as a Decontaminant for Biological Safety Cabinets. *Applied Biosafety*, Vol. 13, No. 4, pp. 199-212.

Ryan, J. (1994). *Understanding and Managing Cell Culture Contamination - Technical Bulletin.* Lowell MA USA: Corning Incorporated.

Safework Australia. (2011). *Occupational Exposure Limits for Airborne Contaminants in the Workplace.* Canberra ACT: Safework Australia.

Standards Australia. (2008). *AS 2476 General Fumigation Procedures.* Sydney NSW: Standards Australia.

Standards Australia. (2010). *AS/NZS 2243.3 Safety in Laboratories - Microbiological Safety and Containment.* Sydney NSW: Standards Australia.