Reducing Biosafety Risks with Use of a Barrier Isolator for Cell or Virus Production

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Abstract

Virus production poses increased risks to laboratory workers above and beyond those of routine laboratory work, which by itself contains biohazards. Three cases of laboratory acquired infections (LAI) were publicly reported in 2016. In all three cases, no route of infection was noticed; no needlesticks, animal bites, ingestion or splashes. This makes inhalation of droplets the most likely route of infection. Airborne droplets are generated by routine open operations when a liquid film is broken or when energy is added to a solution. Even working in a BSC, people are exposed to airborne droplets when their arms or other objects interrupt the laminar air flow in close proximity to the face. Particles that remain airborne in the workspace can settle out on a worker's gloves and sleeves to get carried to the face outside of the BSC. The Xvivo system barrier isolator provides a physical barrier between critical processing spaces and the operator. It also actively removes particles from the processing spaces. The continuously recirculating atmosphere cleaner (CRAC) system recirculates the internal atmosphere through HEPA filters. Using the null hypothesis that the CRAC system would make no difference in the persistence of 0.5 micron particles in the processing chamber, we tested routine operations performed in cell and virus production including pipetting liquids, opening tubes of fluid and centrifugation. Real-time particle monitoring showed that with the CRAC in operation, generated particles were rapidly cleared from the critical processing spaces. With the CRAC off, particles generated from these routine laboratory operations remained airborne. We concluded that the barrier isolator with the CRAC system was able to help prevent exposure of the operator to potentially infectious particles by rapidly eliminating them from the atmosphere.

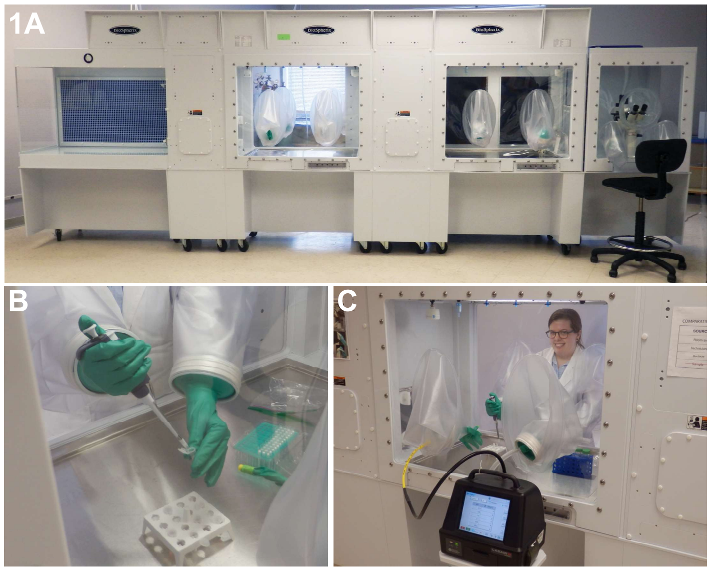
Background

- **Even with modern BSCs, lab workers continue to contract LAI[1-3]. Routine laboratory operations put infectious particles into the air which can spread through the air or settle on gloves[4].**
- **BSCs are not tested in realistic conditions. Under more realistic conditions, they do not perform well enough to protect the worker at manufacturer-recommended airflow rates[5].**
- **The Cytocentric Xvivo System puts an extra layer of protection between infectious materials and personnel, reducing the risk of infection. The Continuously Recirculating Air Cleaning (CRAC) Sytem actively removes particles from the internal atmosphere by HEPA filtration, reducing risk even more.**

Objectives

- **Perform routine laboratory operations like pipetting and opening fluid-containing tubes with and without the CRAC System in operation**
- **Monitor the atmosphere airborne throughout the chamber and near the hand for particles**

Experimental Design



Experimental Design. (Fig. 1A) Routine cell thawing operations were performed in the Cytocentric XVIVO System, which contains an integrated particle monitor that records particles broadly circulating in the processing chamber. During the thaw procedure, materials like sterile gauze and tubes were prepared in the chamber, the medium was equilibrated with the chamber oxygen by pouring back and forth between 50ml tubes, the thawed cells were transfered to a centrifuge tube. The tube was transferred to the centrifuge (in its own chamber to the rear of the XVIVO System, not shown). In the processing chamber, the medium was poured off and the pellet resuspended in fresh medium. The cells were sampled for counting and then dispersed toT-75 flasks for culture. In addition, some manual processes were performed to record particle levels near the hand while **(B)** micropipetting 0.2ml DMEM medium (Sigma, USA) into 0.5ml microtubes (shown), pipetting 10ml DMEM into 50ml conical tubes, pouring 25ml medium between 50ml tubes. **(C)** Local particles were recorded using a Lasair particle counter with the sampling tube sealed into the chamber and located near the laboratory worker's gloves during manual operations **(B).**

References

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Results

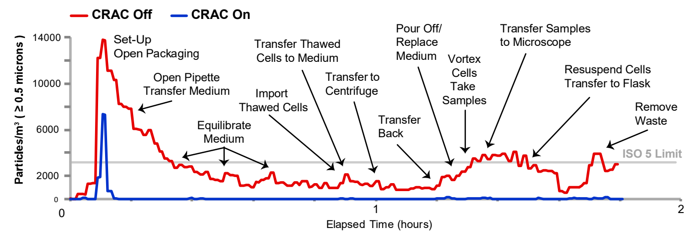

Figure 2. The CRAC System Controls Airborne Particles During a Routine Cell Thawing Process. A routine cell thawing process was performed with the Continuously Recirculating Air Cleaning (CRAC) System on or off. The CRAC rapidly removed particles from the Processing Chamber Air that otherwise would have remained aloft.

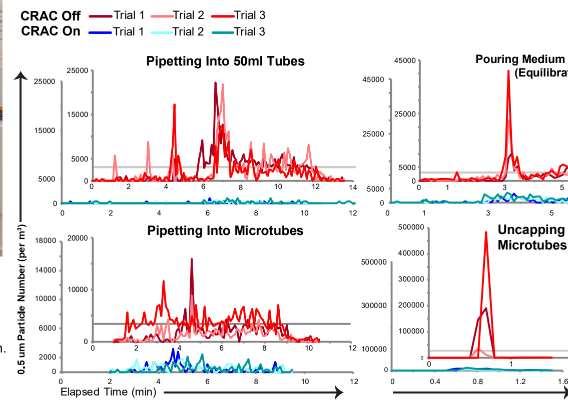

Figure 3. The CRAC System Controls Particles Even Close to the Hands. The Particle counter intake was set close to the hands during routine cell handling. With the CRAC System on, particles were kept below ISO 5 levels, even during uncapping.

Conclusions

- **Routine manual laboratory processes generate high levels of potentially infectious particles; actions that break a fluid film or add energy to a cell or virus supension**
- **The XVIVO Sytem with the CRAC System in operation rapidly removes particles from the cell handling space, reducing the risk of LAI to laboratory workers**