Aseptic Culture Conditions to Reduce Risks of Metabolic Stress from Antibiotics

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ABSTRACT

Antibiotic use can skew metabolic data, inducing HIF-1 alpha, disrupting the electron transport chain, and causing oxidative damage in cultured cells. Yet researchers still use antibiotics to reduce the risk of microbial contamination that comes with handling cells in room air biological safety cabinets. We have cultured cells in antibiotic-free medium in the Xvivo System for 8 years without a single culture contamination. The cell processing chamber completely separates the cell handling space from room air and continuously HEPA-filters the atmosphere. Gas levels can be set for unbroken physiologically relevant oxygen and CO_2 levels. We decided to test the aseptic nature of the cell handling space by performing routine cell culture practices with a highly permissive color-changing bacterial broth. Surrogate cell cultures were incubated in flasks, harvested and centrifuged in simulated cell culture operations. We filled glass vials with the surrogate end cell product, then sealed and incubated them for up to14 days. We sampled probable risk surfaces with bacterial touch plates after cell handling and before any cleaning. In 3 separate trials with appropriate positive and negative controls, we saw no evidence of contamination of the surrogate cell cultures or the cell handling environment. We concluded that the cell handling environment can be kept aseptic throughout routine cell culture operations. This, in turn, allows for antibiotic-free cell culture without adding risk of microbial contamination and helps provide better care for the mitochondria as well as the cells in

BACKGROUND

- **Mitochondria are related to bacteria**
- **Antibiotics can negatively affect mitochondria[1,2]**
- **Microbial contamination is a major concern for researchers handling cells in the room air**
- **The Xvivo System provides a closed, HEPA-filtered environment that separates the cell handling and incubation space from room air**

OBJECTIVES

- **Perform 3 trials with a specific cell culture protocol in the Xvivo System, using highly permissive microbial TSB growth medium as surrogate cell cultures**
- **Sample the Xvivo System internal environment with contact plates to assess microbial contamination of work surfaces**

REFERENCES

1. Elliott, R. L.; Jiang, X. P., The adverse effect of gentamicin on cell metabolism in three cultured mammary cell lines: "Are cell culture data skewed?". PloS one 2019, 14 (4), e0214586.
2. Kalghatgi, S.; Spina, C. S.; Costello, J. C.; Liesa, M.; Morones-Ramirez, J. R.; Slomovic, S.; Molina, A.; Shirihai, O. S.; Collins, J. J., Bactericidal antibiotics induce mitochondrial dysfunction and oxidative damage in mammalian cells. Science translational medicine 2013, 5 (192), 192ra85-192ra85.

EXPERIMENTAL DESIGN

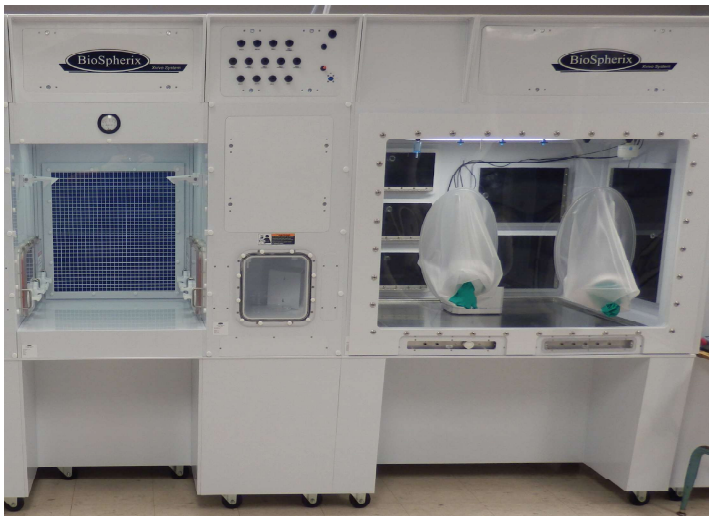


Figure 1. Experimental Design. Routine cell processes (cell thawing, media change, and cell harvest) were mimicked using TSB media (BioMerieux, St. Louis, MO). All operations were performed inside the Xvivo System including centrifugation (not pictured), incubation of the mock cultures between processes, and harvest of the surrogate cell product into sterile vials. Surfaces in the Xvivo System were sampled using contact plates after each process. Three full trials were performed. Materials were surface disinfected with SporKlenz (Steris, Mentor, OH) in the Laminar Flow Hood (left) and moved into the Process Chamber (right). All gases were medical-grade, tanked, and continuously HEPA-filtered.

RESULTS

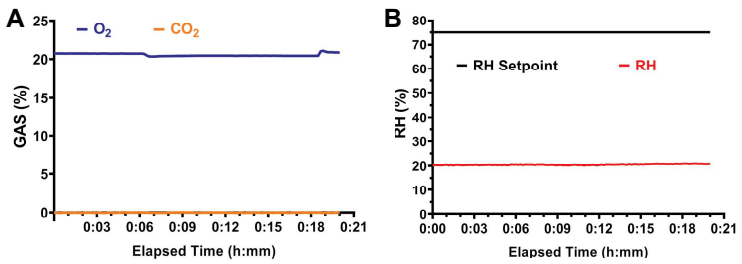
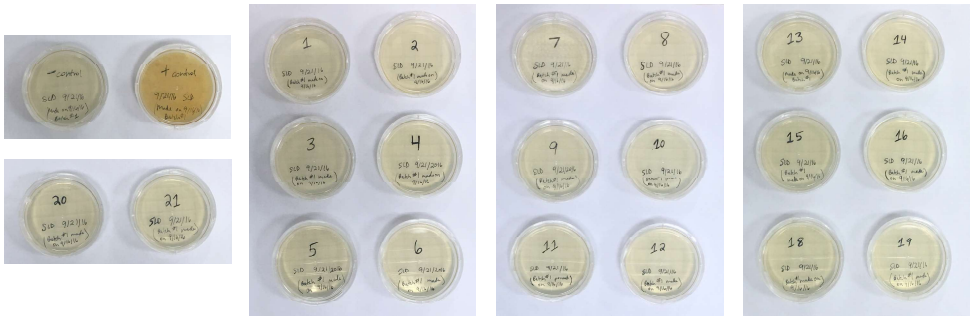


Figure 2. Process Chamber Readings (**A**) During cell handling processes in the Process Chamber, the O_2 and CO_2 levels were monitored and controlled full-time to mimic room air conditions. (**B**) The relative humidity (RH) in the Process Chamber was also constantly monitored and able to be controlled.

A

B



C

	Micro-scope Chamber	Process Chamber 1	Process Chamber 2	Process Chamber 3	Incubators	Buffer Chambers	Negative Control	Positive Control
Trial 1	<1 CFU	<1 CFU	<1 CFU	<1 CFU	<1 CFU	<1 CFU	<1 CFU	~90%
Trial 2	<1 CFU	<1 CFU	<1 CFU	<1 CFU	<1 CFU	<1 CFU	<1 CFU	~85%
Trial 3	<1 CFU	<1 CFU	<1 CFU	<1 CFU	<1 CFU	<1 CFU	<1 CFU	~95%

Figure 3. TSB Media & Contact Plate Results (**A**) The vials of TSB media were incubated for 14 days after harvest and observed for contamination on Day 7 (left photo) and Day 14 (right photo) of one representative trial. Positive control vials were turbid yellow (far left in photos) and positive for microbial growth. Test vials from all three trials were pink and clear and negative for microbial growth. (**B**) A total of twenty surfaces in the Xvivo System were sampled with contact plates during each trial (Trial 1 contact plates shown). Each trial had positive and negative control plates as well. (**C**) All tested surfaces and negative control plates were negative for microbial growth in all three trials. Positive control plates were positive for microbial growth in all three trials. In the Process Chambers and Microscope Chamber multiple sites were sampled including multiple spots on the floor, the gloves, and the air (using an air sampler). In the Incubators and the Buffer Chambers one spot on the floor was sampled.

CONCLUSION

The Xvivo System provides a closed, aseptic cell incubation and handling environment that allows for antibiotic-free cell culture.