# ENABLING IPSC PRODUCTION IN A SCALABLE, MODULAR ENVIRONMENT

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## ABSTRACT

**Production of iPS is increasingly automated[1] so protecting automation from microbial contamination is critical. The Xvivo System is a closed cell incubation and handling environment charged with sterile-filtered dry gases (O2, CO2, N2) under controlled temperature and relative humidity (RH). Previously, we showed that low RH in the cell processing chamber can kill all but spore-forming microbes[2]. Here, we extend those findings to test if microbes dried onto surfaces remain viable to contaminate cultures. We dried 5 problem microbes onto coupons and placed them on all chamber surfaces, including the ceiling, and repeated this for 8 runs. For a total of 37 weeks, we performed media runs, mimicking a 10-day adherent cell expansion protocol, using a permissive microbial broth. We removed coupons at intervals to check microbe viability. All 369 medium samples across 21 independent media runs were negative for microbial growth. Positive controls were positive, and negative controls were negative. At an RH maintained below 50%, the dried-on microbes were effectively sequestered and did not contaminate the mock cultures opened in the same chamber. Controlling the RH of the cell processing environment, and no antibiotics, controlled even massive microbial risks to cell cultures. We concluded that the closed atmosphere of the Xvivo System can help reduce microbial risks to cell cultures.**

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## The Xvivo GMP System®

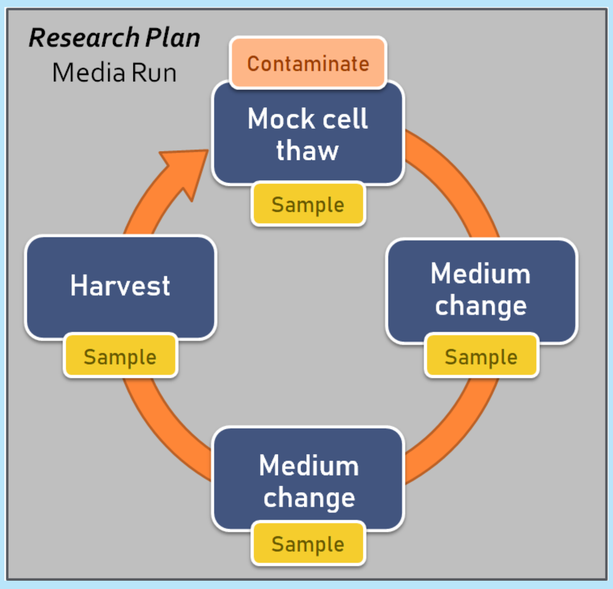
### Closed Cell Incubation and Processing

- Cytocentric® - Constant Controlled $O_2$, $CO_2$, Temp for Cells
- Low $O_2$ for iPS Generation, High $O_2$ for Differentiation
- Modular, Scalable, and Movable
- Reduced Operating and Maintenance Costs
- Compressed, Dry Gases - No Room Air
- Constant HEPA Filtration - ISO5/Class A
- Mitigates Microbial Risk in a Unique Way



## EXPERIMENTAL DESIGN - THE CONTAMINATION CHALLENGE

2A




B



C

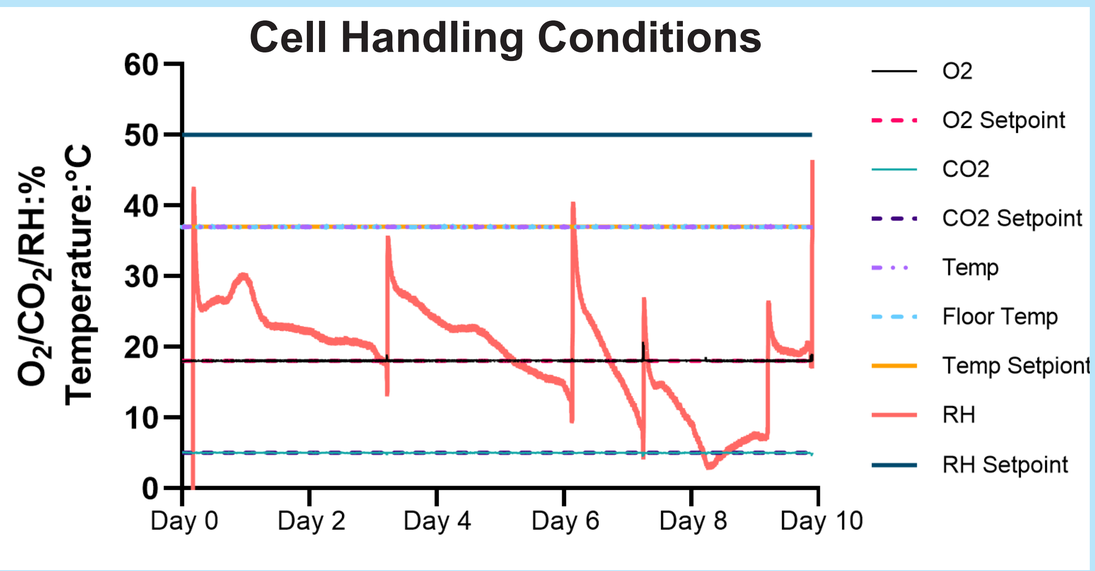



**Figure 2. Experimental Setup.** We simulated a massive contamination event on the first day of a 10-day cell expansion operation. (A) The Xvivo System was used for full-time environmental control. Media runs were performed, 10 days long with handling (removal, centrifugation, replacement). Days (0, 3, 7, 10) were overlapped for a total of 21 runs over 34 weeks. 1.25e8 microbes and a set of "missed spots" (steel coupons) were spiked with a total of 3.75e8 CFU each run. We removed coupons at intervals to test for continued viability. Fresh microbes were introduced Day 0 of each run. (B) Coupons loaded with microbes were taped on surfaces surrounding the mock cell processing. The coupons were left in the chamber as if a massive contamination event had happened and disinfection was not performed the first day of the process. Coupons were removed at intervals to test for microbial viability. Five microbes were used: *A. brasiliensis* (Ab), *B. subtilis* (Bs), *C. albicans* (Ca) *P. aeruginosa* (Pa), and *S. Aureus* (Sa). (B) Microbes accumulated in the chamber for the first 9 media runs, then decreased as coupons continued to be removed for analysis. (C) Typical cell processing conditions in the Xvivo System are below the RH needed for microbial growth (>60%). Limit controls are routinely set at 50%RH so that the Xvivo automatically infuses fresh dry gases to keep RH down.

## No Contamination

**Table 1. All media were negative for microbial growth in every run.** Color-changing highly permissive bacterial broth was used to simulate cell media. All 369 samples across 21 independent media runs were negative for microbial growth (pink). All positive controls were positive for microbial growth (yellow). All negative controls were negative (pink).

| Run: | | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 | 14 | 15 | 16 | 17 | 18 | 19 | 20 | 21 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| CCM ctrls | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + |
| D0 | Waste | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| D3 | F1 | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| | F2 | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| | F3 | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| | Waste | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| D7 | F1 | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| | F2 | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| | F3 | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| | Waste | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| D10 | F1 1 | - | - | - | - | - | - | - | - | - | | | | | | | | | | | | |
| | F1 2 | - | - | - | - | - | - | - | - | - | | | | | | | | | | | | |
| | F1 3 | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| | F1 4 | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| | F1 Ctrl | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + |
| | F2 1 | - | - | - | - | - | - | - | - | - | | | | | | | | | | | | |
| | F2 2 | - | - | - | - | - | - | - | - | - | | | | | | | | | | | | |
| | F2 3 | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| | F2 4 | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| | F2 Ctrl | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + |
| | F3 1 | - | - | - | - | - | - | - | - | - | | | | | | | | | | | | |
| | F3 2 | - | - | - | - | - | - | - | - | - | | | | | | | | | | | | |
| | F3 3 | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| | F3 4 | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| | F3 Ctrl | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + |

## REFERENCES

1. Bohrer, L.R., et al., Automating iPSC generation to enable autologous photoreceptor cell replacement therapy. J Transl Med, 2023. 21(1): p. 161.
2. He, Y., et al., Temperature and Relative Humidity Control to Reduce Bioburden in a Closed Cell Processing and Production System without Disinfectants. BioPharm International, 2021.

## CONCLUSION

## FULL-TIME CONTROL OF CELL HANDLING CONDITIONS WITH THE XVIVO SYSTEM CAN REDUCE MICROBIAL RISKS TO CELLS DURING MANUFACTURE