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Evaluating contamination control for open-vessel cultivation in the Multitron shaker with HEPA filtration

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Abstract

This study demonstrates the performance of the Multitron 4 incubator shaker with integrated HEPA filtration system for contamination-free cultivation of bacterial (*E. coli*) and mammalian (CHO) cells. The system maintains Class 10 (ISO 4) air quality within 3 minutes of door closure by continuously filtering airborne particles ≥ 0.3 microns, minimizing contamination risk without requiring decontamination cycles. Results from air exposure, surface monitoring, and open-vessel cultivation confirm that the Multitron 4 supports clean, uninterrupted cell growth while reducing downtime and improving workflow efficiency.

Keywords
Contamination control, HEPA filtration, Multitron, incubator shaker, CHO, *E. coli*, suspension culture

Introduction

Orbital shakers are widely used for bacterial and mammalian cell cultivation, providing the necessary mixing and environmental control for reliable growth. However, mitigating contamination risks during cultivation can be a challenge—especially when working with open systems. Many incubator shakers rely on manual decontamination steps, which can disrupt workflows and increase the risk of contamination between runs.

The Multitron 4 incubator shaker addresses this issue by incorporating an internal HEPA filtration system. This system continuously filters the air inside the chamber, helping to maintain a clean environment without interrupting active cultures or requiring time-consuming shutdowns for cleaning.

This study investigates the performance of the Multitron 4 with HEPA filtration in supporting contamination-controlled cell culture environments. The ability of the system to minimize airborne risk and to avoid decontamination-related downtime is highlighted by using open vessels without sterile barrier for bacterial and mammalian systems, an especially challenging use case.

Objective

This study aims to demonstrate the effectiveness of the Multitron 4 with the HEPA filtration system for maintaining a clean environment during open-vessel cultivation.

- Assess the HEPA filtration system performance
- Confirm contamination control in exposed media and internal surfaces during active use
- Compare bacterial and mammalian cell growth in open versus closed cultivation setups

Methods and materials

Equipment configuration

Experiments were performed using the INFORS HT Multitron 4 incubator shaker, equipped with:

- Internal HEPA filtration system
- CO_2 control
- Bi-directional humidification system
- 25 mm orbital diameter

Airborne contamination monitoring (Settle Plates)

To evaluate airborne contamination during shaker operation, Tryptic Soy Agar (TSA) settle plates were placed inside the Multitron 4 chamber and left uncovered for 3 hours with the HEPA filtration system running. Plates were then incubated as follows:

- 48 hours for 30 °C for bacterial growth
- 72 hours at 20 °C for yeast and mold detection

A positive control was exposed to the lab environment under the same conditions. Negative controls consisted of plates prepared at the same time but not opened prior to incubation.

Surface cleanliness assessment (TVC Swabs)

Surface monitoring was assessed using Total Viable Count (TVC) Envirocheck® swabs, featuring:

- Side 1: Nutrient agar
- Side 2: Nutrient agar with 0.05% Triphenyl Tetrazolium Chloride (TTC), which turns red in the presence of colony forming units (CFU)

Swabs were applied to internal surfaces of the shaker chamber and incubated at 37°C for 48 hours. Additional samples were collected using sterile Milli-Q® water swabs streaked onto TSA agar to cross-validate cleanliness across multiple surface types.

Contamination control in liquid media

To evaluate how well the Multitron 4 maintained a contamination-free environment for open vessels, the following steps were taken:

1. 25 mL LB broth was dispensed into uncapped 50 L falcon tubes
2. Tubes were placed into the Multitron 4 for 24 hours at 37 °C, 230 min^{-1}, and 80 % relative humidity
3. Tubes were then capped and held at room temperature for another 24 hours to observe for potential microbial growth

Bacterial cell cultivation

Escherichia coli (E.coli) was cultured in the Multitron 4 in 250 mL Erlenmeyer flasks without lids, containing either Terrific Broth (TB) or Lysogeny Broth (LB), with a working volume of 50 mL. Cultures were maintained under the following conditions:

- 37 °C
- 350 min^{-1}
- 80 % relative humidity

Both covered and uncovered flasks were used to compare the growth environment. Optical density (OD) measurements were taken at defined intervals to assess bacterial proliferation under clean air conditions.

Mammalian cell cultivationn

Chinese Hamster Ovary (CHO) cells were cultured in the Multitron 4 in 50 mL spin tubes, without lids, filled with 20 mL of serum-free EX-CELL® medium (Sigma Aldrich). Tubes were maintained under the following conditions:

- 36.5 °C
- 5 % CO_2
- 230 min^{-1}
- 80% relative humidity

Open and closed tubes were compared across 72 hours of cultivation. Cell density and viability were monitored using the NOVA FLEX2 Cell Analyzer.

Results

System cleanability

To test the cleanability of the system, three locations within the Multitron 4 were selected as swab locations and sampled after a period of non-sterile maintenance work on the instrument (Figure 1). Each swab location was composed of a different material type to control for any differences in cleanability. The locations were sampled using both TVC Envirocheck® and a sterile swab saturated with sterile Milli-Q® water which was then streaked on TSA agar.

Figure 1. Testing locations indicated inside the Multitron 4.

After sampling the instrument before cleaning, the Multitron 4 was then cleaned with bleach (allowed 10-minute contact time) followed by 70 % ethanol. The sample locations were resampled after cleaning and samples were incubated at the conditions specified above. Bleach and ethanol are suitable sanitizing and cleaning agents for the interior of the Multitron 4, providing cultivation conditions with low contamination risks (Table 1).

TSA Agar Results			TVC Enviroche ck® Results			
Multitron 4 test locations	Before cleaning	After cleaning	Before cleaning		After cleaning	
			Side 1	Side 2	Side 1	Side 2
1 (Side wall)	—	—	—	—	—	—
2 (Tube rack)	+	—	—	—	—	—
3 (Door window)	+	—	++	+	—	—

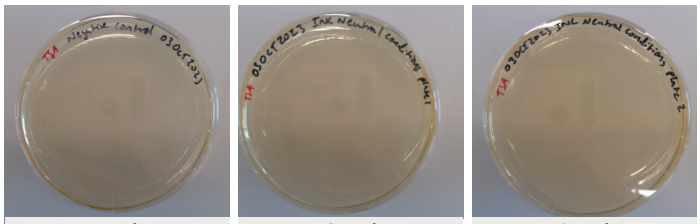
Table 1. Results of the cleanability test (CFU, Colony Forming Units). —: No growth, +: 1-2 CFU, ++: 3-4 CFU, +++: >5 CFU, TNC: CFU too numerous to count.

Internal environment air quality monitoring

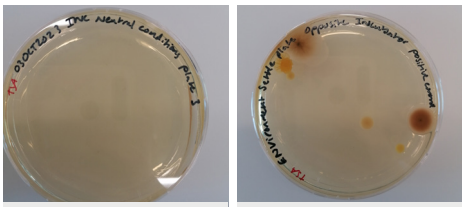
TSA settle plates were employed to monitor viable air particles within the Multitron 4 while the HEPA filtration system was in continuous operation. TSA settle plates were placed within the Multitron 4 and maintained for 3 hours without lids. A positive control consisted of an additional settle plate maintained for the same period in the laboratory environment.

After the settle time was completed, Total Viable Count (TVC) Enviroche ck® samples were taken of the surface directly in front of the settle plates to check for surface contamination. The results of the settle plates showed no growth except for the positive control (Figure 2A).

Settle plate results:



Negative control	Test location: 1 (side wall)	Test location: 2 (tube rack)
—	—	—

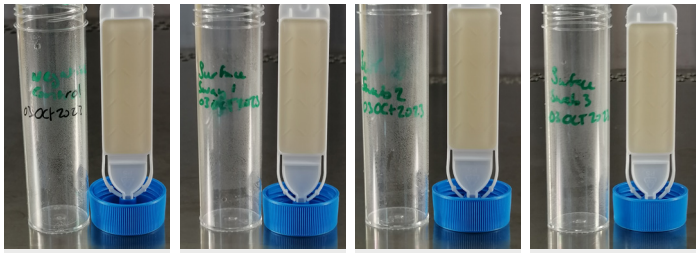


Test location: 3 (door window)	Positive control
—	+++

Figure 2 (A). Settle plate results following 3 hour settling time within the Multitron 4.

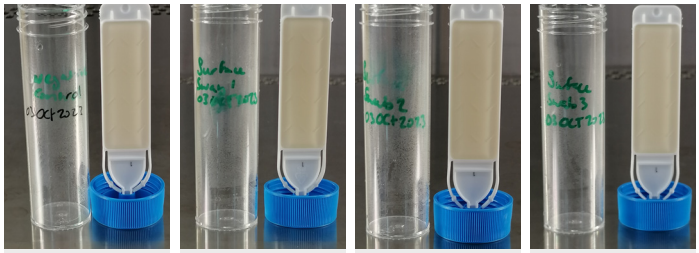
The Enviroche ck® slides taken directly after settling corroborated this finding, as no growth was seen on any slide (Figure 2B). These findings indicate that the Multitron 4 can maintain a contamination-free environment, which is necessary for cultivation of cells in open vessels.

Side 1 Enviroche ck® results:



Negative control	Test location: 3 (door window)	Test location: 3 (door window)	Test location: 3 (door window)
—	—	—	—

Side 2 Enviroche ck® results:



Negative control	Test location: 3 (door window)	Test location: 3 (door window)	Test location: 3 (door window)
—	—	—	—

Figure 2 (B). Enviroche ck® results following the end of the settling period.

Media exposure test

Maintenance of uncapped falcon tubes containing LB medium in the Multitron 4 did not lead to measurable growth of contaminating species. After maintaining the tubes at room temperature for another 24 hours, still no growth was observed (Figure 3).

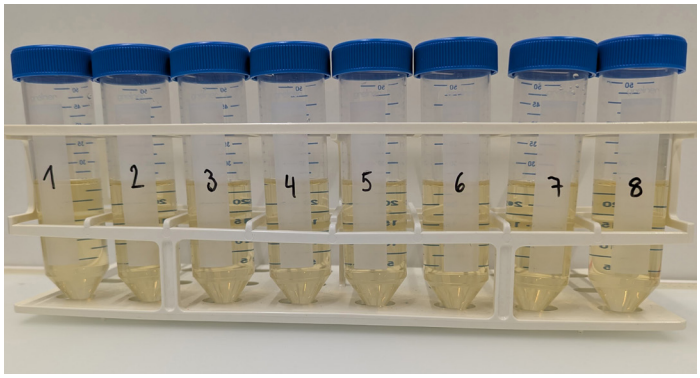


Figure 3. Tubes 1-8 contain LB media after being maintained uncapped in the Multitron 4 at 37 °C for 24h and an additional 24 capped at room temperature.

Bacterial cultivation

For bacterial cultivation, Erlenmeyer flasks containing TB or LB medium were inoculated with overnight cultures of *E. coli*. Three flasks per media type were left uncapped during the cultivation, one each was capped as a reference in a closed system. The cell growth observed was comparable in the capped and uncapped cultivation systems, indicating contamination-free and robust growth conditions in the uncapped Erlenmeyer flasks (Figure 4).

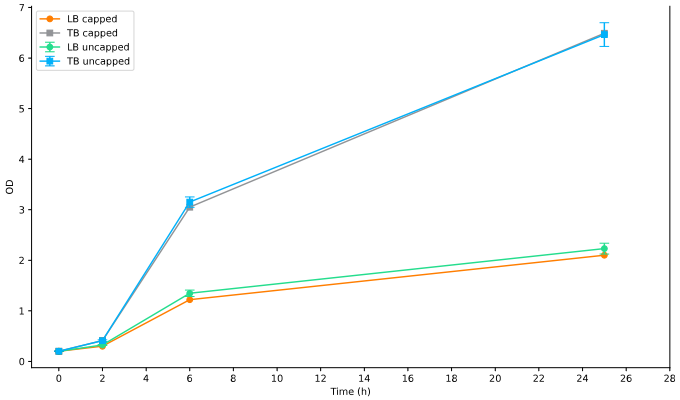


Figure 4. Growth of *E. coli* cultures in TB and LB medium in capped and uncapped Erlenmeyer flasks. Error bars represent the standard error of the mean (n=3).

Mammalian cell cultivation

For mammalian cell cultivation, 50 mL spin tubes containing EX-CELL® medium were cultivated with CHO cells at an initial cell concentration of 0.25 10^6 cells per mL. Three tubes were left uncapped during the cultivation, three tubes were capped as a reference in a closed system. Cell growth as well as cell viability over 72h of culturing was comparable in the capped and uncapped cultivation systems (Figure 5) and no contamination was observed.

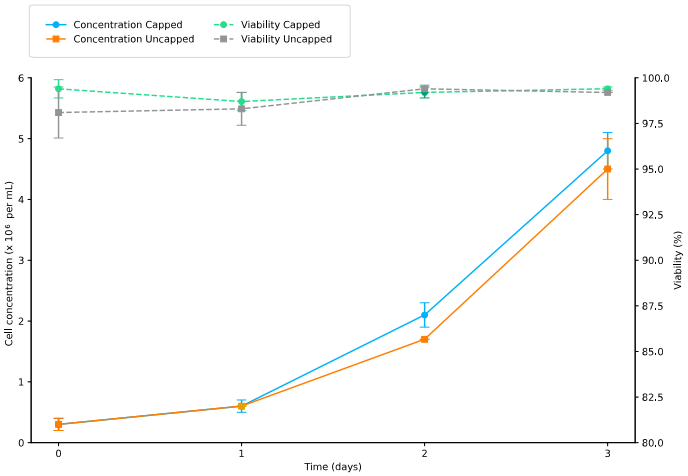


Figure 5. Cell growth and viability of CHO cells in open and closed cultivation systems in the Multitron 4. Error bars represent the standard error of the mean (n=3).

Conclusion

The data presented in this study confirms that the Multitron 4 incubator shaker with the integrated HEPA filtration system effectively maintains a clean air environment suitable for sensitive bacterial and mammalian cell cultures—even in open vessels. Air quality measurements, surface cleanliness data, and successful cultivation of CHO and *E. coli* cells all demonstrate the system's ability to prevent contamination under continuous use.

Unlike systems that rely on time-consuming shutdowns for decontamination cycles, the Multitron 4 supports uninterrupted workflows, making it a valuable tool for high-throughput labs and time-sensitive processes. By maintaining Class 10 (ISO 4) air conditions within minutes and continuously recirculating filtered air, the system enables reproducibility, protects culture integrity, and reduces operational downtime—meeting the needs of modern, contamination-conscious researchers.

Learn more at
infors-ht.com/multitron

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