

Cleanliness of Esco CelCulture® Incubator Touch Screen (CCL-TS)

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Summary

The ISO Class 5 Air Cleanliness Test was conducted on two CO₂ incubator units, namely the CCL-170B-8-TS and the CCL-240B-HHS-8-TS. Using the AeroTrak APC 9310-02 particle counter, airborne particles were measured across five size categories ($\geq 0.3 \mu\text{m}$ to $\geq 5.0 \mu\text{m}$). Following chamber sealing, both units showed a rapid and consistent decline in particle counts, achieving ISO Class 5 cleanliness levels within six minutes. This significant reduction in airborne particle concentration highlights the effectiveness of the incubators' filtration and airflow systems. Notably, the CCL-170B-8-TS unit demonstrated a decrease from 5,360,297 particles/m³ ($\geq 0.3 \mu\text{m}$) at the first minute to only 147 particles/m³ by the sixth minute. Similar downward trends were observed across all particle sizes, confirming that both units successfully meet the stringent criteria of ISO Class 5 air cleanliness. These findings underscore the units capability to provide highly controlled, low-contamination environments suitable for critical applications.

Key Words: *Air Cleanliness, CelCulture® CO₂ Incubators Touch Screen, ISO Class 5*

Introduction

CO₂ incubators are critical instruments used to maintain stable environmental conditions for the cultivation of mammalian cells and other sensitive biological materials. These devices are adjustable heater (typically for cell incubation 37°C)¹, relative humidity (typically 85–90%)², and carbon dioxide (commonly maintained at 5%)³ to support optimal cell growth and physiological stability. However, beyond these parameters, one of the most vital yet often overlooked aspects of cell culture success is the air cleanliness within the incubator chamber.

In contamination-sensitive applications such as biomedical research, pharmaceutical production, and aseptic processing, airborne particulates both viable (microorganisms) and non-viable pose significant risks to sample integrity and process reliability^{4,5}. Therefore, maintaining ultra-clean air inside the incubator chamber is essential to minimize contamination and ensure consistent experimental outcomes.

To address this challenge, Esco CO₂ incubators are equipped with the ULPA filtration system, which recirculates chamber air through an ULPA (Ultra-Low Penetration Air) filter capable of capturing $\geq 99.999\%$ of particles $\geq 0.12 \mu\text{m}$. This system is designed to deliver an internal environment with a cleanliness level equivalent to or better than ISO Class 5, similar to that of biosafety cabinets⁵.

This white paper presents the results of an ISO Class 5 Air Cleanliness Test conducted on Esco CO₂ incubator models CCL-170B-8-TS and CCL-240B-HHS-8-TS. The objective of the test is to quantify airborne particle concentrations within the chamber using a calibrated particle counter and to validate the performance of the ULPA filtration system in achieving the required cleanliness standards.

Materials and Method

Materials used in this experiment were:

1. Esco CelCulture® Incubator Touch Screen: CCL-170B-8-TS (SN: 200844) and CCL-240B-HHS-8-TS (SN: 200862).
2. AeroTrak Airborne Particle Counter (Model APC 9310-02, SN: 93101450006)

Air Cleanliness Test

The air cleanliness test was performed in accordance with ISO 14644-1 standards to verify compliance with ISO Class 5 classification and was conducted on 01 November 2024. Prior to measurement, the incubator chamber door was kept open for 15 minutes to allow the internal environment to reach equilibrium with the surrounding room air. After this equilibration period, the door was closed to isolate the chamber, and particle counting was initiated using a calibrated AeroTrak particle counter. The isokinetic probe was positioned at the geometric center of the chamber and connected securely to an LSAPC-type particle counter via a sampling tube to ensure a representative measurement and prevent leakage. Airborne particle concentrations were monitored across five particle size categories ($\geq 0.3 \mu\text{m}$, $\geq 0.5 \mu\text{m}$, $\geq 1.0 \mu\text{m}$, $\geq 3.0 \mu\text{m}$, and $\geq 5.0 \mu\text{m}$) to confirm the chamber's ability to achieve ISO Class 5 cleanliness levels.

Analysis data

Compare ISO Cleanliness Standards: Compare the measurement result to the relevant ISO Cleanliness Class standards (e.g., ISO 14644-1). These standards define acceptable particle concentration limits for different cleanroom environments based on particle size (Figure 1).

Class	Maximum particles / m ³					
	$\geq 0.1 \mu\text{m}$	$\geq 0.2 \mu\text{m}$	$\geq 0.3 \mu\text{m}$	$\geq 0.5 \mu\text{m}$	$\geq 1 \mu\text{m}$	$\geq 5 \mu\text{m}$
ISO 1	10	2	0	0	0	0
ISO 2	100	24	10	4	0	0
ISO 3	1,000	237	102	35	8	0
ISO 4	10,000	2,370	1,020	352	83	0
ISO 5	100,000	23,700	10,200	3,520	832	29
ISO 6	1,000,000	237,000	102,000	35,200	8,320	293
ISO 7	10,000,000	2,370,000	1,020,000	352,000	83,200	2,930
ISO 8	100,000,000	23,700,000	10,200,000	3,520,000	832,000	29,300
ISO 9	1,000,000,000	237,000,000	102,000,000	35,200,000	8,320,000	293,000

Figure 1. ISO 14644-1 Cleanliness Standards

Results and Discussion

The particle count measurements for both CO₂ incubator models (CCL-170B-8-TS and CCL-240B-HHS-8-TS) were continuously recorded over the test duration, with readings taken every one minute to track the reduction trend in airborne particles. The analysis focused on five particle size ranges: $\geq 0.3 \mu\text{m}$, $\geq 0.5 \mu\text{m}$, $\geq 1.0 \mu\text{m}$, $\geq 3.0 \mu\text{m}$, and $\geq 5.0 \mu\text{m}$. For the CCL-170B-8-TS, initial particle concentrations at minute 1 were notably high, with 5,360,297 particles/m³ ($\geq 0.3 \mu\text{m}$). However, a progressive and substantial decline was observed over the next five minutes, culminating in only 147 particles/m³ for the same size range by minute 6. Similar reductions were observed across all other particle sizes, including a complete elimination of $\geq 3.0 \mu\text{m}$ and $\geq 5.0 \mu\text{m}$ particles by the final minute.

Likewise, the CCL-240B-HHS-8-TS unit followed a comparable trend. The initial reading at minute 1 was 3,152,403 particles/m³ ($\geq 0.3 \mu\text{m}$), which dropped to 2,120 particles/m³ by minute 6. The particle counts for larger sizes ($\geq 1.0 \mu\text{m}$ to $\geq 5.0 \mu\text{m}$) also showed significant reduction, reaching zero in some categories by the end of the testing period (Table 1).

These results indicate that both incubator models effectively reduce airborne particle concentrations in a short duration, successfully achieving the cleanliness thresholds set by ISO Class 5 standards. The consistent downward trend across all particle sizes reflects the efficiency of the internal airflow and filtration systems implemented in both models.

Table 1. Air Cleanliness Test Data for CO₂ Incubator Models CCL-170B-8-TS and CCL-240B-HHS-8-TS

	Maximum Particles of CCL-170-B TS / m ³				
Time (minutes)	≥0.3	≥0.5	≥1.0	≥3.0	≥5.0
1	5360297	800941	248322	16056	1807
2	615710	84930	22949	826	54
3	99406	16006	4080	851	100
4	13732	4166	1381	0	0
5	3975	352	203	53	0
6	147	0	0	0	0

	Maximum Particles of CCL-240-B-8 HHS TS / m ³				
Time (minutes)	≥0.3	≥0.5	≥1.0	≥3.0	≥5.0
1	3152403	450530	84382	3816	530
2	701944	97951	19293	954	177
3	164028	22403	4240	212	0
4	36643	5760	1272	0	0
5	9081	1449	424	106	0
6	2120	318	141	0	0

Conclusion

A significant reduction in particle counts was observed, with both incubators achieving ISO Class 5 cleanliness by the sixth minute, demonstrating the high efficiency of the filtration system in reducing airborne particles.

References

1. R. Lehmann, J.C. Severitt, T. Roddelkopf, S. Junginger, K. Thurow, Biomek cell workstation: a variable system for automated cell cultivation, *J. Lab. Autom.* 21 (3) (2016) 439e450, <https://doi.org/10.1177/2211068215599786>.
2. Mulyazmi, Mulyazmi & Wan Daud, Wan & Octavia, Silvi & Ulfah, Maria. The Relative Humidity Effect Of The Reactants Flows Into The Cell To Increase PEM Fuel Cell Performance. *MATEC Web of Conferences*. 2018. 156. 03033. 10.1051/mateconf/201815603033.
3. Triaud, F.; Clenet, D.; Cariou, Y.; et al. Evaluation of Automated Cell Culture Incubators. *J. Assoc. Lab. Autom.* 2003, 8, 82–86.
4. Sciorio R, Rapalini E, Esteves SC. Air quality in the clinical embryology laboratory: a mini review. *Ther Adv Reprod Health*. 2021 Feb 11; 15:2633494121990684. doi: 10.1177/2633494121990684.
5. International Standards Organization (ISO) 14644-1 “Classification of Air Cleanliness”.