# ATTAINING RAPID ISO 5 AIR QUALITY IN A $CO_2$ INCUBATOR

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## Abstract

Caron's in-chamber HEPA filtration system rapidly provides cleanroom-quality air within a $CO_2$ cell culture incubator, preventing environmental contaminants from settling on internal surfaces and culture vessels. Unlike competing contamination reduction technologies, properly executed HEPA filtration systems provide highly consistent results, and along with good aseptic technique, are key to reducing culture contamination and resulting sample loss and wasted time.


## Introduction

HEPA air filtration has long been the technology used to prevent work area contamination within Biological Safety Cabinets (BSC's), as well as within clean-room environments. Over the last two decades, HEPA filtration has also emerged as the most common contamination control technology within $CO_2$ incubators. Prior to this point, users could only rely on good aseptic technique and regular manual chamber cleaning with liquid disinfectants, a time-consuming, unpleasant, and downtime-inducing procedure, to delay the onset of incubator chamber contamination.

One of the most common source of incubator contamination is the laboratory air. Cell cultures require regular access for feeding, harvesting, and inspection. Air exchange into the incubator occurs at this point, introducing dust and other airborne particulates into the chamber. These particulates often contain biologicals, such as mold spores, bacteria, and viruses, which can eventually settle on interior surfaces and culture vessels and begin to grow.

Initially, incubator manufacturers only provided in-line filtration for incoming gasses and air exchange ports, preventing ingress of contaminants during unit operation. This approach, however, only works until the incubator door is opened. Most laboratory environments have little to no specialized air filtration, and have air quality levels similar to that of offices and light manufacturing environments.

| Current ISO 14644-1 Class | Former Federal Standard 209E Class* | Operating Environment |
|---|---|---|
| 4 | Class 10 | Sterile fill/packaging |
| 5 | Class 100 | Biological Safety Cabinets, medical device packaging |
| 6 | Class 1,000 | Medical device manufacturing, Computer chip fabrication |
| 7 | Class 10,000 | Biomedical research |
| 8 | Class 100,000 | Circuit board assembly |
| 9 | Room air | Offices, laboratories |

**Superseded by ISO 14644 in 2001, but still used as a general reference.*

The next air quality advancement was to introduce a higher capacity HEPA filter into the incubator, which, in conjunction with a forced air circulation system, cleaned chamber air on a continuous basis. While potentially effective, these systems initially had no provable efficacy. A major innovation, and turning point in market acceptance, occurred when manufacturers began establishing documented performance criteria for their in-chamber filtration systems. By listing both resulting chamber air quality, either in terms of the former Federal Standard 209E (ie. Class 100) or current ISO 14644-1 (ie. ISO 5), specifying a time to performance (typically 5-10 minutes), and documenting materials and procedures used, manufacturers finally provided their incubator customers with the same level of performance assurance that had long been available for BSC's and critical clean-room environments.

For the current ISO 5 classification, maximum allowable particulates are categorized by total maximum concentration for several discrete particle sizes:

| Particle Size (Microns) | Maximum allowable concentration (in Parts Per Cubic Meter, (PPCM)) |
|---|---|
| 0.3 | 10,200 |
| 0.5 | 3,520 |
| 1.0 | 832 |
| 5.0 | 0 |

Several manufacturers have attempted to obtain the same continuous contamination control effect with UV irradiation systems, located in the airstream, but outside of the main chamber culture area. While this technology can be used for decontamination of air and water, it lacks HEPA filtration's ability to quickly remove particulates from the airstream, and has reduced efficacy inactivating biologicals when particulate or humidity levels are high. HEPA air filtration remains the airborne contamination control gold standard for BSC's, clean rooms, and $CO_2$ incubators.

## Background

Caron's chamber HEPA air filtration system, as configured within the Wally™ $CO_2$ incubator, operates continuously when the unit doors are closed.

Caron's filter configuration was determined through both theoretical and empirical test data:

- The system was tested with a background particulate level exceeding 1,000,000 Parts Per Cubic Meter (PPCM). By challenging the system with a defined elevated background level, Caron assured that the system will perform in a wide range of lab environments.
- Prior testing proved that a HEPA filter of the size and format selected, working within a ducted system and sealed chamber, can reliably and rapidly remove particulates down to a usefully low level.

## Materials and Methods

- 7410-5-1 Wally $CO_2$ incubator
- HEPA filter, Caron rectangular pleated HEPA filter, captures up to 99.97% of airborne particles at 0.3 microns.
- Discrete Particle Counter (DPC), Lighthouse Worldwide Solutions, Handheld 3016, part number 402997216-1

## Particle Count Settings

- 0.3, 0.5, 1.0 & 5.0 Micron sizes monitored
- 60 second sample time, no delay, no hold
- Cumulative mode, normalized to $m^3$

## Pre-Test – Establish Background Particle Level Baseline

1. Close the incubator doors and place the Discrete Particle Counter *outside* the incubator and directly in front of the closed doors. The isokinetic probe should be approximately 10 cm from the incubator front.
2. Log data for 1 hour.
3. Average 0.5 micron sized particles and verify background level exceeds 1,000,000 PPCM

## Background Baseline Test Results

Background noise was typically 2,500,000 PPCM with spikes up to 4,500,000 when activity was nearby. This is well in excess of the 1,000,000 PPCM minimum baseline for an effective HEPA filter test.

## Test - Recovery time with 99.97% efficiency HEPA filter

1. Open the doors, remove the filter housing, and place & seat a new *99.97% efficiency HEPA* filter into the housing, and snap firmly into place as shown in the User's Manual
2. Configure the DPC, then center and place the instrument on the bottom shelf of the incubator. This locates the isokinetic probe in the general vicinity of the incubator's geometric center.
3. Turn the incubator 'on'
4. Run for 10 minutes minimum.
5. Open *both* doors a full 90 degrees for 30 seconds.
6. Turn on the DPC, and immediately close the doors. Begin particulate count as soon as the doors are closed.
7. Record data for 60 minutes with door closed.
8. Repeat steps #4 through #6 an additional 2 times.

## Recovery Time Test Data

### RUN #1 –PPCM

| Time (min) | Particle Size (Microns) 0.3 | 0.5 | 1.0 | 5.0 |
|---|---|---|---|---|
| 1 | 3287827 | 451326 | 129959 | 10241 |
| 2 | 1971283 | 295233 | 80871 | 7769 |
| 3 | 8476 | 1766 | 706 | 0 |
| 4 | 1766 | 353 | 0 | 0 |

ISO5 reached in under 3 minutes

| | | | | |
|---|---|---|---|---|
| 5 | 0 | 0 | 0 | 0 |
| 6 | 1413 | 353 | 353 | 0 |
| 7 | 1766 | 353 | 353 | 0 |
| 8 | 0 | 0 | 0 | 0 |
| 9 | 353 | 0 | 0 | 0 |
| 10 | 706 | 0 | 0 | 0 |
| 11 | 3178 | 0 | 0 | 0 |
| 12 | 353 | 0 | 0 | 0 |
| 13 | 353 | 0 | 0 | 0 |
| 14 | 0 | 0 | 0 | 0 |
| 15 | 353 | 0 | 0 | 0 |
| 16 | 1413 | 353 | 0 | 0 |
| 17 | 1413 | 0 | 0 | 0 |
| 18 | 353 | 0 | 0 | 0 |
| 19 | 3178 | 353 | 0 | 0 |
| 20 | 0 | 0 | 0 | 0 |

## RUN #2 - PPCM

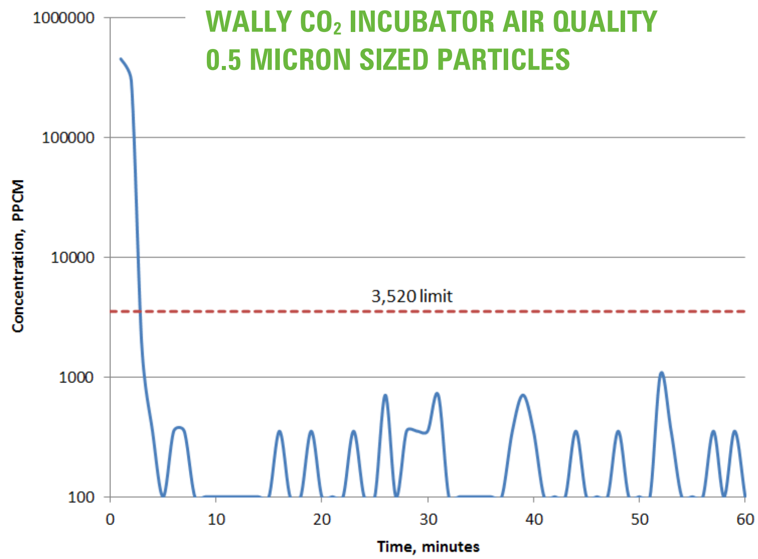
| Time | Particle Size (Microns) | | | |
|---|---|---|---|---|
| (min) | 0.3 | 0.5 | 1.0 | 5.0 |
| 1 | 3739859 | 550914 | 186816 | 17658 |
| 2 | 215422 | 29665 | 8476 | 1059 |
| 3 | 2825 | 0 | 0 | 0 |
| 4 | 353 | 0 | 0 | 0 |
| 5 | 2119 | 0 | 0 | 0 |

ISO5 reached in under 3 minutes

## RUN #3 - PPCM

| Time | Particle Size (Microns) | | | |
|---|---|---|---|---|
| (min) | 0.3 | 0.5 | 1.0 | 5.0 |
| 1 | 2965754 | 298059 | 116540 | 17658 |
| 2 | 655800 | 54385 | 20483 | 2472 |
| 3 | 3885 | 0 | 0 | 0 |
| 4 | 0 | 0 | 0 | 0 |
| 5 | 0 | 0 | 0 | 0 |

ISO5 reached in under 3 minutes




Following a 30 second door opening, the HEPA air filtration system returned the incubator interior to a cleanliness of ISO5 within 3 minutes. This test was repeated two additional times with the same results of ISO5 cleanliness in less than 3 minutes.

## Conclusions

Caron's in-chamber HEPA air filtration system provides reproducible air quality performance for low maintenance cell culture.

Not only does the Wally unit achieve an air quality level equivalent to that found in BSC's and high-end clean rooms, but it attains this level in a short period of time.

## Bibliography and References

ISO TC 209
14644-1:2015, Cleanrooms and associated controlled environments - - Part 1: Classification of air cleanliness by particle concentration
Geneva, 2015-12-09

B. Call, US Test & Balance Corporation
Airflow in the 300mm Semiconductor Factory
Semiconductor Fabtech, 8th Edition 105-107, 2004

US FDA, HHS
Sterile Drug Products Produced by Aseptic Processing –
Current Good Manufacturing Practice

Pharmaceutical CGMP's, September 2004

NSF International
Biosafety Cabinetry: Design, Construction, Performance
And Field Certification
NSF International Standard Adopted April 28, 2008,
Revised October 2008

Schaefer, James W., M. A. Barris, and Dr. B. Y. H. Liu.
"Filter Media Design for High Purity Air Applications."
The Journal of Environmental Sciences, July/August 1986



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