



ReCO₂ver™ CO₂ Incubator

Air Cleanliness and Recovery Testing

Aaron Johnson
Engineering Test Supervisor

Jennifer Pachas
Laboratory Technician

Kara Held, Ph.D.,
Science Director



Contents



ReCO₂ver™ CO₂ Incubator Air
Cleanliness and Recovery Testing 1

Contents 2

Introduction 3

Materials and Equipment 4

Phase 1 4

Phase 2 7

Results and Conclusions 8

References 8

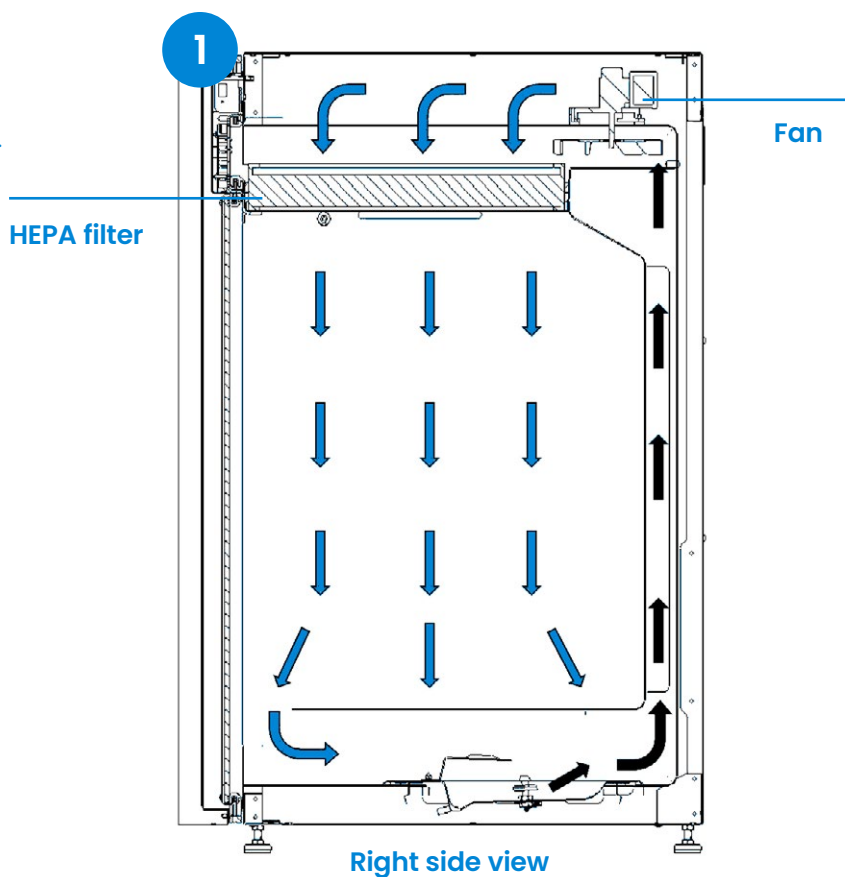
INTRODUCTION

Providing a clean air environment for cultures during incubation is critical to preventing contamination. Some manufacturers equip their incubators with a HEPA filter and claim to provide ISO Class 5 (Class 100) clean air conditions inside the chamber. However, the HEPA filters supplied are typically too small, and the airflow pattern inside the chamber too turbulent, to clean the air within a reasonable amount of time, putting cultures at risk.

To address this problem, ReCO₂ver™, the CO₂ incubator from Baker, was designed with an expansive, full-face HEPA filter and uniform downward airflow to provide better-than-ISO-Class 4 (Class 10) clean air conditions (at 0.5 µm and larger) within just 60 seconds (**Figure 1**). This paper describes the tests performed to determine the level of air cleanliness ReCO₂ver™ provides (Phase 1), as well as to determine how quickly the unit recovers to better-than-ISO Class 4 conditions following a 30-second door opening (Phase 2).

Figure 1

Full-face HEPA filter and uniform downward airflow.



MATERIALS AND EQUIPMENT

Materials	Serial #	Tempered
1. ReCO ₂ ver™ Plus CO ₂ Incubator (Ultrasonic Humidification System, 110V)	112093	The Baker Company Sanford, ME
2. Discrete Particle Counter (DPC) with a particle size discrimination capability of 0.5 µm (Model CI 450T)	068042	CLIMET Instruments Redlands, CA
3. Analog stopwatch capable of reading minutes and seconds	N/A	N/A

PHASE 1

Air Cleanliness Test

The purpose of this test was to demonstrate that the ReCO₂ver™ CO₂ incubator provides an internal air cleanliness of better-than-ISO Class 4 (at 0.5 µm and larger) according to ISO 14644-1:2015: Cleanrooms and Associated Controlled Environments (1).

Establishing the Number of Sample Locations

Using the formula $NL = \sqrt{A}$, the minimum number of sample locations required was calculated. NL is the minimum number of sample locations rounded up to a whole number, and A is the area of the clean zone in square meters.

- ReCO₂ver™ useable interior volume= 5.92 ft³ or 0.168 m³
- $NL = \sqrt{0.168}$
- $NL = 0.409$, or rounded up to 1 sample location

The sample location that was chosen is the geometric center of the incubator chamber, requiring all four shelves to be moved to the highest available locations for DPC introduction (**Figure 2**).

According to ISO14644-1, where only one sample location is required, a minimum of three single sample volumes must be taken.

Figure 2

DPC location in ReCO₂ver™.



Establishing the Number of Sample Locations

For this experiment, an ISO Class 4 air cleanliness classification was selected. The formula $V_s = 20 / C_{n,m} \times 1000$ was used to determine the single sample volume at the location specified above. V_s is the minimum single sample volume; $C_{n,m}$ is the class limit or number of particles per cubic meter (PPCM) allowed (**Figure 3**) and 20 is the defined number of particles that could be counted if particle concentration were at the class limit.

- $V_s = 20 / 352 \times 1000$
- $V_s = 56.8$ liters required

In order to validate that the ReCO₂ver™ incubator meets the ISO 4 air cleanliness level, a single sample volume of 57 liters is required. Three consecutive samples must be taken at the incubator's geometric centerline and the average particle concentration cannot exceed 352 PPCM.

Figure 3

ISO 14664-1 Table.

Maximum particle counts for each size and air classification (particles per cubic meter, PPCM).

For ISO Class 4 conditions, no more than 352 PPCM 0.5µm or greater can be detected.

ISO Class	0.1µm	0.2µm	0.3µm	0.5µm	1µm	5µm
1	10	2				
2	100	24	10	4		
3	1,000	237	102	35	8	
4	10,000	2,370	1,020	352	83	
5	100,000	23,700	10,200	3,520	832	29
6	1,000,000	237,000	102,000	35,200	8,320	293
7				352,000	83,200	2,930
8				3,520,000	832,000	29,300
9				35,200,000	8,320,000	293,000

Testing Procedure

1. The ReCO₂ver™ incubator was turned ON and allowed to warm up for at least 10 minutes, with setpoint of 37°C, 0% CO₂, 0% Relative Humidity (RH).
2. During this warm-up, the DPC was set up outside the incubator and used to measure the background particle level at ≥0.5 µm recorded in **Figure 4**. Temperature, humidity and CO₂ levels were neither controlled nor monitored during these tests.
3. Next, the four stainless steel shelves of the ReCO₂ver™ incubator were moved to the highest available position to allow room for DPC unit. The DPC was then placed on the stainless steel false floor of the incubator at its geometric center. The DPC power cord was routed through the right sidewall cable port and left unsealed.
4. The DPC was turned ON and the particle counter background noise count rate (zero count) was performed according to manufacturer's procedures to purge instrument of particles.

5. Next the glass door of the incubator was opened and the isokinetic probe was mounted on top of the DPC using a small piece of flexible tubing.

The DPC was programmed to the following parameters according to ISO 14644-1:2015 to verify an air cleanliness level of ISO Class 4. NOTE: The DPC was programmed to sample in particles per cubic foot (PPCF) to minimize testing time and all particle counts were normalized and are displayed in PPCM.

- Channel ≥0.5 micron in concentration mode
- Sample volume = 2 cubic feet (56.8 liters)
- Initial delay of 2 minutes (to allow for door closing and air circulation within chamber)
- Start delay of 0 seconds (time delay between samples)
- Samples taken: 3 (at geometric center of chamber), repeated 7 times

PHASE 2

Air Cleanliness Recovery Time Test

1. The ReCO₂ver™ incubator was turned ON and allowed to warm up for at least 10 minutes, with setpoint of 37°C, 0% CO₂, 0% Relative Humidity (RH).
2. During this warm-up, the DPC was set up outside the incubator and used to measure the background particle level at ≥0.5 µm recorded in **Figure 4**. Temperature, humidity and CO₂ levels were neither controlled nor monitored during these tests.
3. Next, the four stainless steel shelves of the ReCO₂ver™ incubator were moved to the highest available position to allow room for DPC unit. The DPC was then placed on the stainless steel false floor of the incubator at its geometric center. The DPC power cord was routed through the right sidewall cable port and left unsealed.
4. The DPC was turned ON and the particle counter background noise count rate (zero count) was performed according to manufacturer's procedures to purge instrument of particles.

5. Next the glass door of the incubator was opened and the isokinetic probe was mounted on top of the DPC using a small piece of flexible tubing.

The DPC was programmed to the following parameters according to ISO 14644-1:2015 to verify an air cleanliness level of ISO Class 4. NOTE: The DPC was programmed to sample in particles per cubic foot (PPCF) to minimize testing time and all particle counts were normalized and are displayed in PPCM.

- Channel ≥0.5 micron in concentration mode
- Sample volume = 2 cubic feet (56.8 liters)
- Initial delay of 2 minutes (to allow for door closing and air circulation within chamber)
- Start delay of 0 seconds (time delay between samples)
- Samples taken: 3 (at geometric center of chamber), repeated 7 times

Testing Procedure

Measuring Background Particle Levels

Per ISO 14644-1:2015 (1) a minimum sample volume of 2 liters was taken to determine the background particle level. With the DPC located outside the ReCO₂ver™, the background particle levels were measured at ≤0.5 micron and larger for a time period of one minute.

Establishing the Recovery Time

Next, the incubator's glass door was opened and the DPC was reprogrammed to take 20 consecutive 6-second samples in concentration mode with a 2-second initial delay, reporting in PPCM. Again, no sample delay or waiting period between samples was programmed into the DPC. Five complete test cycles were performed.

- Channel ≥0.5 micron in concentration mode
- Sample volume = 0.2 cubic feet (equaling 6-second samples)

- Initial delay of 2 seconds prior to sampling to allow for door closure
- Start delay of 0 seconds (time delay between samples)
- Samples taken: 10 samples in concentration mode, reported in PPCM

The DPC and time-keeping device were started simultaneously and then the incubator glass door was closed, initiating incubator fan operation.

Air cleanliness recovery is defined as three consecutive counts that are less than or equal to the baseline particle level. Total recovery time is defined as the period of time beginning when the incubator fan turns on and ending when air cleanliness recovery is achieved.

RESULTS AND CONCLUSION

The ReCO₂ver™ incubator demonstrates an air cleanliness level of better than ISO Class 4 as determined by the 3 samples required by ISO14644-1:2015 (1, **Figure 4**).

(NOTE: The maximum particle counts for an ISO Class 4 space are no more than 352 PPCM when measuring particles $\geq 0.5 \mu\text{m}$.) Additionally, ReCO₂ver™ demonstrates an average total air cleanliness recovery time of less than 60 seconds (**Figure 5**).

Figure 4

Air Cleanliness Phase 1 Test Results.

Testing Sample Number	Pre-testing Background Level	Maximum Allowed Particles for Class 4 (PPCM)	Average Actual Particles Measured (PPCM)	ISO Classification Achieved
1	Greater than 1,000,000 PPCM (above ISO Class 7)	352	94	ISO Class 4
2		352	121	
3		352	115	

*Particles $\geq 0.5 \mu\text{m}$

Figure 5

Air Cleanliness Phase 2 Recovery Time Test Results.

Testing Sample Number	Pre-testing Background Level	Elapsed Time After Door Closing (Seconds)	Measured Particle Level (PPCM, $\geq 0.5 \mu\text{m}$)		
1	Greater than 1,000,000 PPCM (above ISO Class 7)	6	493688	286044	455822
2		12	212622	97066	195911
3		18	59377	33955	97600
4		24	20622	19733	40533
5		30	8000	12088	8177
6		36	3911	3200	2844
7		42	1955	266	1422
8		48	1777	1066	533
9		54	355	177	355
10		60	0	0	0

REFERENCES

ISO 14644-1:2015: Cleanrooms and associated controlled environments
- Classification of air cleanliness, International Organization for Standardization, Case Postale 56, CH-1211, Geneve 20, Switzerland, www.iest.org

CAG-002-2006 CETA Compounding Isolator Testing Guide, Controlled Environment Testing Association, 1500 Sunday Drive, Suite 102, Raleigh, NC 27607, www.cetainternational.org