Application note

CTS Series CO_2 incubators

Compliance testing demonstrates a CO_2 incubator merits certification for use in grade A/B environments

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Keywords

cell therapy, cell therapy manufacturing process, cell therapy systems, CTS, ISO Class 5, GMP Grade A/B cleanroom, ISO 14644 standard, EU GMP, contamination prevention, contamination control strategies, air quality requirements, cleanroom compatible CO_2 incubator, GMP compliance certification, ISO Class 5 suitability, particle control system, particulate management, third – party validation and certification, HEPA filtration system, stainless steel exterior, electropolished stainless steel interior, validated disinfection procedures, compatibility with cleanroom disinfectants, IP54 compliant electronics.

Introduction

As the central equipment in a cell therapy or cellular genetic engineering manufacturing process, a cleanroom compatible CO_2 incubator plays a huge role in contamination control, thus maintaining a high quality and safe therapy for human patients. This is accomplished by designing the CO_2 incubator to capture emitted particulates through an onboard HEPA filtration system. Other features that contribute to cleanroom compatibility include CO_2 incubator ease of use, stainless steel exterior, electropolished interior and shelving, in-chamber HEPA filtration to further protect precious cultures, validated disinfection and cleaning procedures, including compatibility with the STERIS dry, non-condensing VHP® process, and IP54 compliant electronics (1).

Since a final cell-based product is inherently limited in the amount and ways that it can be purified, particulate management remains a primary concern in the cell therapy manufacturing setting. Non-microbial particulates were the reason for 22% of U.S. FDA recalls of sterile injectables from 2008-2012 (2) and the second leading cause of recalls from 2009 to 2019 (3). Particles present in a sterile injectable can represent a variety of dangers for a patient from an unintended immune response to a pulmonary embolism. And since the equipment itself is responsible for an estimated 15% of particle generation in a cleanroom (4), controlling particulate emissions from equipment is extremely advantageous (1).

For these reasons, Thermo Scientific™ Heracell™ Vios™ CR and Thermo Scientific™ Forma™ Steri-Cycle™ CR CO_2 Incubators Cell Therapy Systems (CTS™) Series represent an important innovative design for use in ISO Class 5 and GMP Grade A/B cleanrooms. These CO_2 incubators are certified cleanroom compatible by an independent industry specialist (5,6) in accordance with ISO 14644-14 (9). Together with the optimal conditions for cell growth and outstanding parameter recovery and uniformity demonstrated by earlier Heracell VIOS and Forma Steri-Cycle models, this unique combination of leading technologies represents an unprecedented advance for cell and gene therapy applications. The innovative technology (see Figure 1) is proven capable of significantly reducing particle emissions during normal operation and during the Thermo Scientific™ Steri-Run™ automated 180 °C sterilization cycle. Thus, Heracell Vios CR and Forma Steri-Cycle CR incubators are highly suitable for use in an ISO Class 5, Grade A/B cleanroom, without costly customizations or structural adaptations required.

The process of incorporating new equipment into a cleanroom environment can be very challenging due to stringent air quality requirements. Therefore, it is important for the equipment to be tested and validated by the manufacturer to provide proof of compatibility with ISO Class 5. For these incubators, the validation methodology (summarized in Figure 2) was followed as per ISO 14644-14:2016 (9) and VDI 2083 Part 9.1 (8) and performed by an independent industry specialist. Both standards provide step-by-step instructions to assess the suitability of equipment for use in cleanrooms and associated controlled environments, with respect to airborne particle cleanliness as specified in ISO 14644-1:2015 (7). Here, we explain the validation methodology and the resulting certification.

Methods:

Test subjects

Models of both 165 L and 255 L sizes were tested, representing Heracell Vios CR and Forma Steri-Cycle CR CO_2 incubators.

Figure 1. Graphic illustration of the particle capture technology incorporated into Heracell Vios CR and Forma Steri-Cycle CR CO_2 incubators. Particulate generated during operation of the unit are collected from inside the incubator casing and directed to the back of the unit (red arrows), where they are captured by the H13 type HEPA filter. Cleaned, filtered air is released from the HEPA filter (A: thin wavy blue arrows) and cleanroom air – which also helps to cool the electronics – is drawn into four filtered vents (B: wide blue arrows).

Figure 2. Overview of the process used to test CO_2 incubator suitability for use in a cleanroom, in accordance with ISO 14644-14: 2016. A measuring point with the highest particle emissions detected was located at the upper right side, above the top door hinge. This point was used for the final ISO class compatibility determination.

Prepare cleanroom	Prepare incubator	Initial incubator tests	Test highest emission point
1	2	3	4
• Ensure particle counter has current calibration certificate • "Rinse" room with air for 1 hour • Test cleanroom air cleanliness • 3 min. at 6 measuring points • Determine ISO class	• Clean and disinfect incubator surfaces with 70% IPA • Visually confirm no residue • "Rinse" with air for 1 hour • Test at 37°C with no humidity or gas added	• Initiate short scan to find locations with highest particle emissions • Measure the points with highest emissions (6 points, 20 min. each) • Point located at the upper right side had highest emissions	• Scan point located at upper right side, above the top door hinge for 100 minutes total
Initial scan during Steri-Run	**Test highest emission point during Steri-Run**	**Analyse results**	**Determine compatibility with ISO class**
5	6	7	8
• Initiate Steri-Run cycle • Scan for highest particle counts • Determine location with highest emissions	• Measure at highest emissions point for 12 hours cycle duration (Upper right side, door hinge) • Particle counts increased at higher temperature • Repeat two times	• Calculate statistical values for all particle readings • Calculate the z-values • Refer to ISO 14644-1:2015	• Determine the ISO Class 5 suitability for all configurations tested

Visual inspection and requirements for GMP compatibility

The incubators were visually inspected as per requirements in ISO 14644-14:2016, with the objective being to identify any damage or incorrect assembly as well as to assess surface cleanliness and imperfections in design, all of which have the potential to impact the quantitative emissions tests. Both models made a very good overall impression (Figure 3). The units were further evaluated according to ISO 14644-14:2016 for incorporation of cleanroom compliant design principles, including the selection of appropriate materials and surface finishes as well as cleanability. The overall assessment of these CO_2 incubators resulted in granting ISO Class 5 and Grade A/B compatibility certification at 0.5 µm and 5.0 µm (5,6).

The outer and inner surfaces are made of brushed (exterior) and electropolished (interior) stainless steel and were very easy to clean, leaving no residue. Initial cleaning of any laboratory or clinical device is critical, since any particles remaining from manufacturing, packaging, transportation or initial assembly could impact the process. For each of these test models, any residues were easily removed, leaving a clean, smooth surface.

Before the particulate emissions testing commenced, any aspects that could influence the assessment results were taken under consideration, including variability between different sizes, different mode of operation, or use of clean room-specific accessories. Therefore, four separate tests were conducted in order to achieve an ISO Class 5 and Grade A/B compatibility certification at 0.5 µm and 5.0 µm:

- Heracell Vios 160i CR unit in the representative mode of operation at 37 °C
- Heracell Vios 160i CR unit for the full duration of the 180 °C sterilization cycle
- Heracell Vios 250i CR unit in the representative mode of operation at 37 °C
- Heracell Vios 250i CR unit for the full duration of the 180 °C sterilization cycle

For a cell culture/cell therapy application, and the certification goal, the particle sizes for consideration are identified by the EU-GMP guideline (10) and “Guidance for Industry – Sterile Drug Products Produced by Aseptic Process” (11) as 0.5 µm and 5.0 µm.

Representative mode of operation

As it is not possible to test every possible condition generated by CO_2 incubators, a representative mode of operation was defined as 37 °C, without humidity or gas. Since the chamber atmosphere has no impact to the particle emission of the device, no added humidity or gas was included as part of the particulate testing. However, since higher temperatures can cause increased particle emissions, these CO_2 incubators were also tested during the complete Steri-Run 180 °C automated sterilization cycle.

Particle monitoring and collection device

To measure particulate emissions, a light scattering airborne particle counter (LSAPC) with a sampling probe that collected at least 28,3 L/min of air was employed. Lasair® III 110 is a laser diode-based discrete particle counter with 1.0 cubic feet per minute (CFM) flow rate (Particle Measuring Systems, Colorado, USA). The device was calibrated prior to performing the tests (5,6).

Evaluation of the test environment

The tests were conducted in a 12 m^2 cleanroom equipped with powerful Fan-Filter-Modules including pre-filter F7 and H14 HEPA filters positioned at the rear of the room, resulting in a horizontal displacement flow. The airflow was characterized prior to installing the CO_2 incubators to ensure the test requirements were met, as follows:

In the cleanroom, particles were measured at six different sampling locations for three minutes each with a collection volume of 28.3 L per test.

The airflow velocity was measured, confirming a range of 0.38-0.43 m/s, meeting the ISO 14644-14:2016 requirement for the test environment of 0.3-0.5 m/s.

The cleanroom met ISO Class 4 conditions according to ISO 14644-1:2015. (7) Therefore, the test environment met the requirement to be at least one ISO class cleaner than the environment where the CO_2 incubators are intended to be used (5,6).

Figure 3. Heracell Vios 160i CR and Heracell Vios 250i CR incubators photographed during the initial visual inspection and cleaning.

Preparatory identification of High Particle Concentration (HPC) locations

The purpose of this initial test was to identify the locations on the CO_2 incubators which emit the highest concentration of particles. These sites of highest emission are included in the final suitability measurement. All areas of the CO_2 incubators, but especially edges, seals and openings, were carefully assessed in this first pass.

Following the approximate identification, a precise determination of particle emission hot spots was carried out using a systematic scan during normal operation at 37 °C. Based on this initial scan, six high emission points were identified for each unit (see measuring points examples in Figure 4, Figure 5 and Figure 6). Next, twenty separate measurements of one minute each were taken, for a total 20 minutes at each point (5,6).

Determination of the highest emission point

For normal operation at 37 °C, only one measuring point, that with the highest particle emission, was tested for the extended 100 minutes specified by ISO 14644-14:2016. Consistently for both models tested, the highest measurement point was determined to be at the upper right side, above the top door hinge (see Figure 6). Therefore, consecutive independent 100 single readings, 1 min. each, were taken at that measurement location for total 100 minutes.

In the next step of the validation process the CO_2 incubators were rigorously tested for the total duration of the Steri-Run 180 °C sterilization cycle. As with the 37 °C tests, the highest particle concentration (see Figure 6) was found to be at the top right corner of the door, next to the hinge (5,6).

Data processing

The evaluation was carried out at both 0,5 µm and larger and at 5,0 µm and larger. Accordingly, the ISO class determination was performed as per ISO 14644-1:2015 (7).

The statistical values calculated for each size range of particles were as follows: arithmetic mean, maximum, minimum, standard deviation, standard error and upper confidence limit and, most importantly, determinant z-value. The calculation of the z-value was carried out using the below equation:

$$z = (G\text{-}UCL) / SE$$

where:

G = limit; UCL = upper confidence limit 95%; SE = standard error

The goal was to achieve the calculated value of “z” larger than 1.645, which meant that the class limit G (here, G is the maximum number of particles permitted within ISO Class 5) could not be exceeded with the confidence level of 95 %. Per ISO 14644-14:2016, these CO_2 incubators could then be considered compatible with an ISO Class 5 environment at the particle size ranges of 0,5 µm and larger and 5,0 µm and larger (5,6).

Figure 4. MP 2 near the right corner of the display on Heracell Vios 250i CR unit.

Figure 5. MP 6, on the back of Heracell Vios 160i CR, close to the HEPA filter.

Figure 6. Heracell Vios 160i CR at the highest emission point MP 3, located on the upper right side, above the top door hinge.

Reference to ISO 14644-1:2015 classification system

A reference to the ISO 14644-1:2015 classification system was made. ISO 14644-1:2015 Table 1 was used to identify the respective ISO classification (7).

Particle size ranges 0,5 µm and larger and 5,0 µm and larger were used in determining cleanroom suitability.

Table 1. ISO classes of air cleanliness by particle concentration according to ISO 14644-1:2015.

DIN EN ISO 14644-1:2015 ISO Class number (N)	Maximum allowable concentrations (particles/m³) for particles equal to and greater than the considered sizes, shown below					
	≥0,1 µm	≥0,2 µm	≥0,3 µm	≥0,5 µm	≥1,0 µm	≥5,0 µm
1	10					
2	100	24	10			
3	1,000	237	102	35		
4	10,000	2,370	1,020	352	83	
5	100,000	23,700	10,200	3,520	832	
6	1,000,000	237,000	102,000	35,200	8,320	293
7				35,2000	83,200	2,930
8				3,520,000	832,000	29,300

Reference to EU GMP Annex 1: Manufacture of Sterile Medicinal Products classification system

A reference to the EU GMP Annex 1: Manufacture of Sterile Medicinal Products classification system was made. The table listing maximum permitted airborne particle concentrations for each grade is shown (10).

While the requirements are very similar to ISO 14644-1:2015, there are some differences. Annex 1 only lists two particle size ranges; greater than or equal to 0,5 µm and greater than or equal to 5,0 µm. In addition, while the current ISO 14644-1:2015 does not list limits for 5,0 µm and larger, Annex 1 lists 20 particles for Grade A and 29 particles for Grade B.

Table 2. Clean room and clean air device classification according to EU GMP Annex 1.

	Maximum permitted number of particles per m³ equal to or greater than the tabulated size			
	At rest		In operation	
Grade	**0,5 µm**	**5,0 µm**	**0,5 µm**	**5,0 µm**
A	3,520	20	3,520	20
B	3,520	29	352,000	2,900
C	352,000	2,900	3,520,000	29,000
D	3,520,000	29,000	Not defined	Not defined

Results:

Documentation and statements regarding cleanroom suitability

In this technical note only results from the measurement classification testing are presented, as the critical determinant for cleanroom suitability. The step by step procedure and data for every performed test is available upon request (5,6).

Results for Heracell Vios 160i and 250i CR single units at 37°C

The Heracell Vios 160i CR and 250i CR models (100 min, 37°C) have achieved ISO Class 5 certification according to ISO 14644-14:2016 during normal use (see below for sterilization tests). An example evaluation of the measurements for Heracell Vios 160i CR is presented in Figure 7.

Results for Heracell Vios 160i and 250i CR models during the Steri-Run sterilization cycle

The measurements performed during the full duration of the 180 °C Steri-Run sterilization cycle (12 hours cycle, tested twice for each model) demonstrated that even during this intense heat, these CO_2 incubators can be classified ISO Class 5. The evaluation of the measurements for Heracell Vios 160i CR during the Steri-Run cycle is presented in Figure 8. Results for the Heracell Vios 250i CR incubators are similar and are not shown. The measurements for both sizes of particles (0.5 µm and 5.0 µm), were within ISO Class 5 and Grade A/B for both models.

Conclusions:

Multiple rigorous validation procedures were conducted to evaluate airborne particle concentration from the innovative Heracell Vios CR and Forma Steri-Cycle CR CO_2 incubators. ISO 14644-14:2016 is the globally accepted standard for assessment of equipment to be placed in the stringent cleanroom environment, and in these tests, all methodology and data documentation strictly followed ISO 14644-14:2016.

The results prove that these innovative CO_2 incubator designs are suitable to operate in ISO Class 5 cleanrooms. The advanced technology incorporated into this design significantly reduces particle emission.

Heracell Vios 160i and 250i CR CO_2 incubators underwent comprehensive testing by measurement of airborne particle concentration. Both models achieved ISO Class 5 and Grade A/B compatibility according to ISO 14644-14:2016 for both particle size ranges 0,5 µm and larger and 5,0 µm and larger.

The validation procedure also included rigorous testing performed during the entire duration of the 180 °C Steri-Run sterilization cycle. Both model sizes can be classified for the particle size rages 0,5 µm and larger and 5,0 µm and larger to ISO Class 5 and Grade A/B according to ISO 14644-14:2016.

In addition to the rigorous testing for particle emissions, Heracell Vios CR and Forma Steri-Cycle CR CO_2 incubators received GMP compliance certification based on the stainless-steel exterior, sealed casing, electropolished stainless steel interior, and IP54 electronics. All these design features contribute to easy cleaning, compatibility with commonly used cleanroom chemical disinfectants, and were evaluated as part of this independent testing (12).

Heracell Vios and Forma Steri-Cycle CR CO_2 incubators represent the first commercially available GMP and ISO Class 5 cleanroom certified models. This design will present a huge advantage to many pharma and biotech companies, driven by GMP regulatory goals and dedicated to manufacturing of safer and exquisite quality cell therapy products.

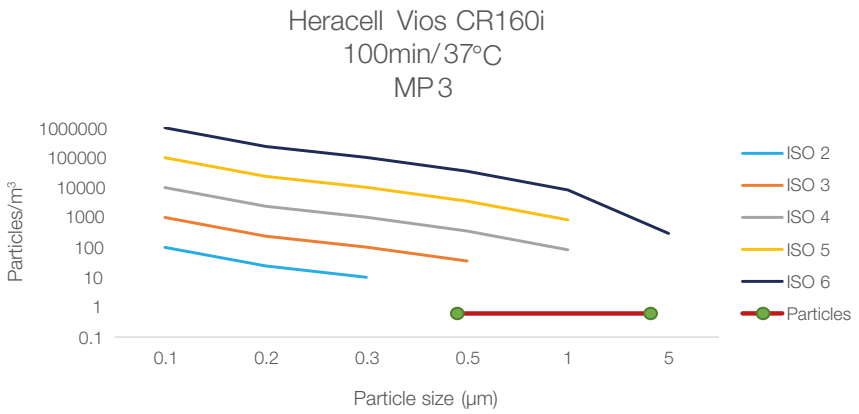

Figure 7. Summary of 0.5 µm and 5.0 µm particle per cubic meter of air, recovered from Heracell Vios 160i CR unit, at the hot spot MP 3, 100 minutes, 37 °C.

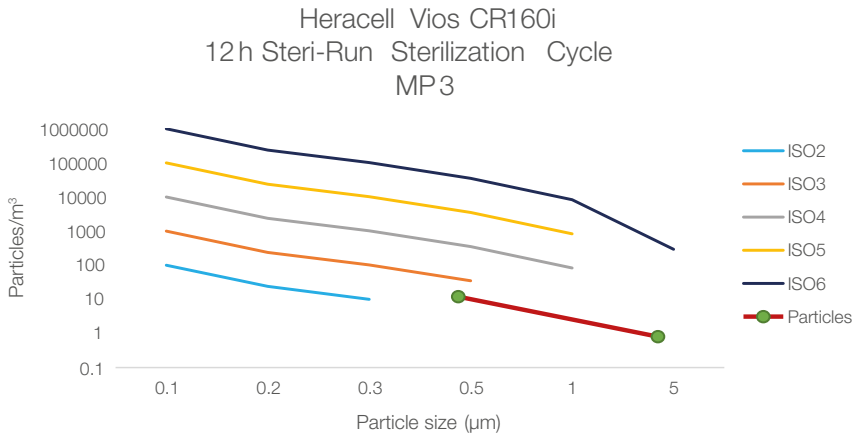

Figure 8. Summary of 0.5 µm and 5.0 µm particles per cubic meter of air recovered from Heracell Vios 160i CR unit, at the hot spot MP 3, during 180 °C Steri-Run sterilization cycle.

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