Smart Notes

What is a certified cleanroom-compatible CO_2 incubator design, and why is this an important consideration for any cell therapy or gene therapy process?

A cleanroom-certified CO_2 incubator is one that has been tested and proven to adhere to air quality limits within Grade A cleanroom standards according to ISO 14644-1 [1]. Such tests are performed according to ISO 14644-14 by an experienced and independent third-party test organization.

Particulates are closely monitored in cleanrooms according to ISO 14644-14 and the leading international pharmacopeias from the United States [2], the European Union [3], and Japan [4]. While the vast majority of cleanroom particulates come from the staff working there, an estimated 15% are due to equipment [5].

Since particulates were the reason for 22% of U.S. Food and Drug Administration (FDA) recalls of sterile injectables from 2008 to 2012 [6] and the second leading cause of recalls from 2009 to 2019 [7], particulates are a primary concern for biological drug manufacturers. This does not include microbial contamination, which is the leading cause for sterility assurance recalls. Particulates present in a sterile injectable can represent a variety of dangers for a patient, from an unintended immune response to a pulmonary embolism.

Particulates sourced from equipment can be due to materials of construction, including painted surfaces, plastics, insulation and packaging materials, glass, metal parts with sharp edges, or crevices that could harbor microorganisms. It is difficult to estimate the number of particulates that could come from a given instrument; the only way to know for sure is by testing. And, for unbiased data, that testing should be performed by an independent third party, not by the manufacturer.

Thermo Scientific™ Heracell™ Vios™ CR CO_2 Incubator

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How is a cleanroom-compatible CO_2 incubator design tested and certified?

A CO_2 incubator should be tested in an ISO Class 4 cleanroom that has been recently validated to ensure it meets this rating. The particle monitor should be recently calibrated and certified as well. At least seven points on and around the CO_2 incubator should be measured for particle release, with special attention to ventilation openings and doors. Each measuring point should be measured for 20 minutes at the working incubator temperature of 37°C. After this measurement, the point with the highest particle counts should be further monitored for 100 minutes. Since higher temperatures will generate more particulate emissions, a high-temperature sterilization cycle should be monitored in the same way [8]. Results from one cleanroom-certified CO_2 incubator design are shown in Figure 1.

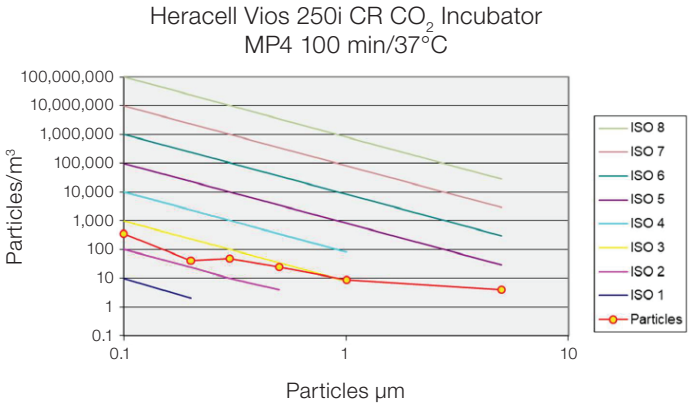

Figure 1. Particle counts from a Heracell Vios 250i CR CO_2 Incubator at measuring point 4 (MP4), measured with the incubator set to 37°C with no CO_2 gas or humidity provided, demonstrate that the incubator operates within ISO Class 5 limits.

Table 1. Types of extrinsic particles and their relative prevalence in recalls of injectable drugs by the U.S. FDA [7].

1	Glass
2	Active pharmaceutical ingredients
3	Stainless steel
4	Hair
5	Silicone, plastics
6	Fiber
7	Rubber

Why should we be concerned about particulates in a cleanroom?

In an injectable pharmaceutical, particulates pose a risk to the patient receiving the injection. Particulates include microorganisms that can cause disease, an inflammatory immune reaction, or tissue damage [7]. However, even nonviable particulates are dangerous. Depending on the particulates' size and composition, they can cause blood vessel damage, a blood clot, a pulmonary embolism, or tissue damage in lungs or liver [5].

What types of particulates in a cleanroom may be due to equipment?

Extrinsic particulates are those that are foreign and not part of the drug or the drug process [5]. Table 1 lists the types of extrinsic particles found in a cleanroom and their relative frequency. Cleanroom HEPA filtration systems capture particulates and maintain air quality within a given ISO classification.

As a central piece of equipment in a cell therapy or cellular genetic engineering process, a cleanroom-compatible CO_2 incubator provides an important benefit in the control of contamination and particulates. This is accomplished by capturing emitted particulates through an onboard HEPA filtration system. Additional features that contribute to cleanroom compatibility include ease of use, control, and cleaning, the electropolished interior and shelving, in-chamber HEPA filtration to further protect precious cultures, compatibility with the dry, noncondensing STERIS VHP™ process, and IP54-compliant electronics.

Summary

A certified cleanroom-compatible CO_2 incubator supports regulatory compliance with a design that controls particulate emissions and ultimately manages potential disruption to the cleanroom environment, limiting risks to the safety, purity, and quality of the end product.

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References

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