Cleaning and disinfection of a Heracell Vios CR or Forma Steri-Cycle CR CO_2 incubator in a GMP environment

Introduction

In a cell culture cleanroom or associated environment, microbial contamination or particulate detritus represents a risk for a cell-based therapeutic. Microorganisms were discovered in 8.45% of biopharmaceutical cultures in 2010 [1] and *Mycoplasma* species in 15–35% of all cell cultures in 2015 [2]. A 2016 analysis of nearly 30,000 batches of autologous patient cells showed that these blood cells can carry their own microbes, appearing in 0.06% of cases [3]. Viral contamination of cultures used in production of biological therapies remains a concern [4]. But it is not just microorganisms that are potentially dangerous; nonviable particulates were responsible for 22% of FDA recalls during the period 2008–2012 [5] and represented the second leading cause of recalls in 2009–2019 [6]. Because of these risks, scientists, process engineers, and facility staff emphasize cleaning and disinfection of production laboratories and cleanrooms, and the equipment and supplies therein. Clearly, disinfection practices must include proper use of chemical cleaners and disinfectants, since overuse or improper application can damage the facility and equipment. Due to the diversity of chemical cleaners and disinfectants available and the variability of application practices, this application note provides data-based recommendations for cleaning and disinfection practices specific to carbon dioxide (CO_2) incubators used in cell and gene therapy cleanrooms—and especially laboratories and associated environments operating under good manufacturing practice (GMP) guidelines. Specific procedures are presented for cleaning and disinfecting the Thermo Scientific™ Heracell™ Vios™ CR and Thermo Scientific™ Forma™ Steri-Cycle™ CR CO_2 incubators – CTS™ Series, which are certified as cleanroom-compatible [7].

Cleaner and disinfectant options—benefits and risks

There are many different types of surface cleaners, with different compositions and concentrations. Some chemicals are appropriate for use at low concentrations but are damaging at higher concentrations, while other chemicals can cause corrosion over time. Several chemical cleaners emit dangerous fumes that can have mild, moderate, or severe toxicity which could affect laboratory staff, but also could affect cultured cells, and this toxicity can be cumulative [8,9]. The level of risk varies with the chemical and concentration, but in every case, it is important to check the safety data sheet and manufacturer's recommendations for use.

A chemical cleaner or disinfectant may be appropriate for use on many surfaces and still be a poor choice for laboratory equipment. Even equipment with similar materials of construction may have exposure to different conditions that affect resistance to chemical disinfectants. For example, a disinfectant may be appropriate for cleaning a stainless steel biological safety cabinet (BSC) but not for a water bath, which is usually heated and always immersed.

Corrosion due to chemicals is exacerbated in a CO_2 incubator for culturing cells derived from tissues because of the increased heat and humidity, but especially because of the CO_2 gas itself. CO_2 gas readily dissolves in any liquid. This property is beneficial when used with growth media, to help balance culture pH. But when combined with the high humidity provided by the incubator, the CO_2 gas generates weak carbonic acid throughout the incubator chamber. This weak acid plus the warm temperature and high humidity can have synergistic corrosive effects when mixed with strong disinfectants.

The type of water used to provide humidity can also contribute to in-chamber corrosion. In all cases, only sterilized distilled water should be used for a humidity source in the CO_2 incubator; never use highly purified water such as deionized or ultrapure (Type 1) water [10] because water with very low ionic content is aggressive. Since water seeks equilibrium, it will actively pull ions from anything it touches, including from stainless steel, copper, and glass. If the inner glass door of a CO_2 incubator is starting to appear cloudy, this is an early sign that the water supply is helping to cause deterioration of the glass and soon may affect the metal chamber as well. Sterilized distilled water can be prepackaged as water for irrigation (WFI) or cell culture–grade water, but in all cases it should have a conductivity of 1–20 µS/cm (resistivity of 50 K-1 MΩ-cm) [10].

General recommendations for cleaners and disinfectants

In the CO_2 incubator, any spills or dirt should be cleaned up immediately—especially if the spill is growth medium—because these will provide nutrients for unwanted microorganisms. For cleaning detergents, we recommend using only mild soap with minimum dwell or exposure time. Remove the detergent using clear distilled water, 70% ethanol (EtOH), or 70% isopropanol (IPA).

> Follow manufacturers' directions for all disinfectants, then remove residues with 70% EtOH or 70% IPA.

For all disinfectants, follow the manufacturers' recommendations for use and dwell time, and in all cases, we recommend following with 70% EtOH or 70% IPA to remove the disinfectant residues. Table 1 lists recommended disinfectants. Gloves and eye protection should always be worn when handling chemical disinfectants. As with all processes in a cleanroom setting, and in accordance with ISO 14644-13 [11], all cleaning and disinfection practices must be validated. For example, use a wipe test or swab test following the procedure to document effectiveness of the process.

Table 1. Recommendations for chemicals used in manual wipe disinfection of CO_2 incubators. These disinfectants have proven compatibility with stainless steel, glass, and copper when used according to manufacturer's recommendations. 70% EtOH or 70% IPA are excellent choices for use following any other chemical, to remove residues that could cause corrosion over time. Disinfectant chemicals not listed are not recommended due to potential damage to incubator materials. For 100% copper interiors, a chemical disinfectant is not needed due to copper's natural properties. However, 70% EtOH or 70% IPA may be used without risk to the copper surface.

Type	Concentration	Example brand
Ethanol	70%	Any (common)
Isopropanol	70%	Any (common)
Quaternary ammonium	10% or less (2% or less is best)	Conflikt™, Lysol™ No Rinse, Fermacidal D2™
Hydrogen peroxide	1–3%	Any (common)
Hydrogen peroxide/peracetic acid/ acetic acid	1%/0.8%/<10%	Spor-Klenz™ Ready-to-Use (RTU) Sterilant (Steris Life Sciences)

Automated, on-demand systems for sterilization inside a CO_2 incubator

For any automated sterilization cycle, it is critical that the design be proven effective according to the standards in the U.S. and European pharmacopeias [12,13]. Briefly, this includes proof of elimination of at least one million heat-resistant spores of the bacterium *Bacillus subtilis* (also known as *Bacillus atrophaeus*), the approved biological indicator organism for dry-heat sterilization and for vaporized hydrogen peroxide (VHP) sterilization. In addition, the air should be continuously circulated using an in-chamber fan or blower. Look for independent, third-party testing documenting the effectiveness of a sterilization cycle [14].

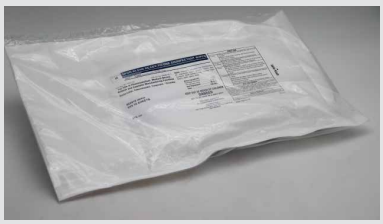
Specific to VHP systems, it is important to note that this chemical is toxic in high concentrations and that the U.S. Occupational Health and Safety Administration has set a

permissible exposure limit (PEL) of 1 part per million (ppm) per 8-hour workday [15]. For this reason, it is critical that any VHP process be proven effective and the chemical be proven neutralized at the completion of the process. Only a dry, noncondensing VHP system is recommended, where the humidity is carefully controlled. This is because if the VHP condenses into liquid, it can corrode equipment and materials over time. There are many suppliers offering VHP, and this term is applied to processes that include chemicals in addition to hydrogen peroxide, including peracetic acid, phenol, and acetic acid. We have seen several different VHP processes cause corrosion and cannot recommend them. Thermo Scientific™ CO_2 incubators have been tested with multiple cycles of Vaprox™ sterilant (Steris Life Sciences), a dry, noncondensing hydrogen peroxide application, and found to be compatible [16]. No VHP is compatible with 100% copper interiors since it will combine with the pure copper and oxidize, reducing the hydrogen peroxide concentration. Based on these considerations, our recommendation is to use the Thermo Scientific™ Steri-Run™ 180°C automated, high-temperature sterilization system for incubators with 100% pure copper interiors [14].

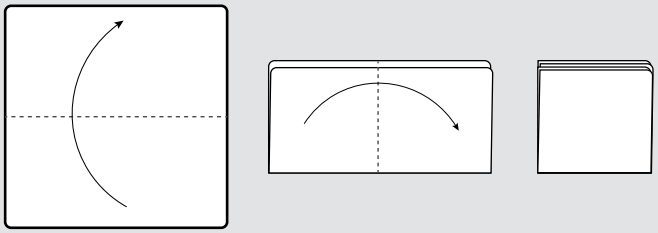
Procedure for applying disinfectant using a prepackaged cloth wipe

This procedure is similar for any cleanroom wiping procedure.

1. Remove one disposable cloth wipe, impregnated with disinfectant, from a resealable package. In the example below, a Spor-Klenz™ RTU disposable cloth wipe from Steris Life Sciences is shown. The cloth should be uniformly damp but not wet.



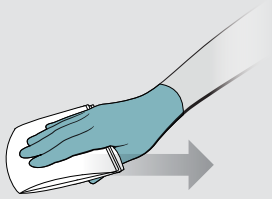
2. In midair, fold the cloth in half, then fold in half again so it is folded in quarters.



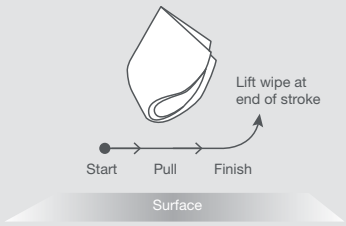
3. Hold the folded cloth between thumb and four fingers so that the fold is toward the surface to be cleaned and the edges are facing the palm of the hand.

4. If there are any spills or areas with more dirt, isolate and clean these first so as not to spread the problem further. Once those areas are cleaned, you can now proceed with a more regimented cleaning.

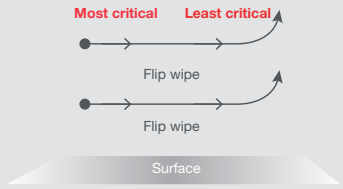
5. Starting at the furthest spot and at the top of the area to be cleaned, wipe in a straight line toward you.



6. Now refold the cloth to expose a new unused side, and repeat the straight-line wipe, overlapping no more than 10–20% of the previous straight-line wipe. At the end of each stroke, lift the wipe completely and cleanly from the surface.



7. Repeat until the cloth has been used eight times, and no side has been used more than once. Then discard the used cloth and take a new disposable cloth wipe.

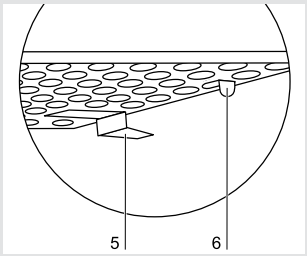


8. Continue until all surfaces have been cleaned and disinfected.

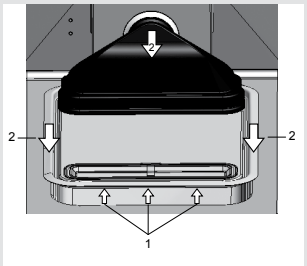
Procedure for cleaning and disinfecting a Heracell Vios CR or Forma Steri-Cycle CR CO_2 incubator

1. Remove any cultures to a different incubator.
2. Turn off the gas supply and switch off the power to the CO_2 incubator.
3. With a receiving container ready, connect the quick-connect tubing and empty the water reservoir.

4. Carefully remove the shelves, shelf supports, and shelf rails. To remove the shelf, the front of the shelf must be gently lifted over the tip protection stop (6) and disengaged from the shelf support guide (5) while sliding forward off the shelf support.



5. Remove the water reservoir prefilter and slide out the water reservoir cover, including the airbox containing the HEPA filter, by lifting the water reservoir cover at (1) as shown.



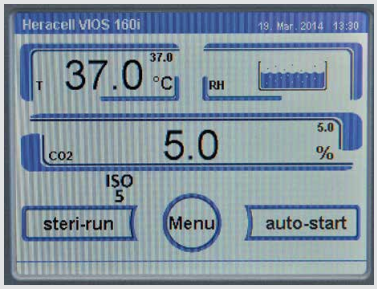
Slide the water reservoir cover as at (2), pulling it out of the incubator chamber. Then remove the airbox and HEPA filter from the water reservoir cover.

There is no need to remove the HEPA filter from the airbox for this cleaning/disinfection procedure. However, if a Steri-Run sterilization cycle will be operated following the disinfection, the HEPA filter should be removed at this time and stored or discarded. If the HEPA filter will be retained and further used following the Steri-Run cycle, we recommend storing the HEPA filter in a plastic bag or surgical wrap during the sterilization cycle.

6. Remove the air duct from the incubator ceiling by unlocking the keyholes from the pins in the ceiling and removing the back duct from the back wall.
7. Starting at the back of the ceiling, wipe with disinfectant toward the front.
8. Next, wipe the back wall from top to bottom, then the left side and right side, wiping each from ceiling to floor and starting at the back, moving toward the front. Finally, wipe the water reservoir and its sides from back to front and left to right.
9. If a disinfectant other than 70% EtOH or 70% IPA was used, repeat steps 7–8 using 70% EtOH or 70% IPA.
10. Using the disinfectant cloth, wipe the inside of the plenum pieces starting with the back piece, inside first, then outside. Repeat with 70% EtOH or 70% IPA and replace in working position.
11. Repeat step 10 for the airbox exterior and interior (if the HEPA filter was removed), the water reservoir cover, and the prefilter.
12. Repeat step 10 for the shelf brackets and rails, then for the shelves.
13. Repeat step 10 for the door gasket and interior glass door. Wipe the door top, side, and bottom edges with the door open.
14. Repeat step 10 for the exterior of the glass door and close. Repeat step 13 for the interior of the outer door and door gasket, and also wipe the door edges. Close the outer door.
15. Wipe the exterior with disinfectant, including the top and all sides. Also wipe the incubator feet and casters. If necessary, engage the castors and roll the incubator stack out for access to each side and the rear. Repeat with 70% EtOH or 70% IPA. A cleanroom mop may be used to apply disinfectant to the areas between incubators in a stack, including the stacking adaptor. As with hand wiping, the mop should be applied back to front in a straight-line motion. Ensure that all areas are wiped.

thermo scientific

16. When complete, perform a test to validate the success of the cleaning and disinfection process.
17. Switch on the incubator power.
18. If desired, initiate a Steri-Run sterilization at this time.



19. Following the Steri-Run cycle, an Auto-Start cycle may be operated if desired.
20. When ready for culturing, aseptically fill the water reservoir with sterilized distilled water, turn on the gas supply, and ensure that conditions have equilibrated before returning cell cultures to the incubator chamber.

Conclusions

Clearly, proper cleaning and disinfection of laboratory equipment used in a GMP cleanroom environment is critical to the success of any cell therapy or gene therapy production. Choosing the proper disinfectant is important to the long-term quality maintenance of the equipment. Use of the wrong disinfectant or an improper application of a disinfectant can cause corrosion, which poses a risk to the equipment and to the therapeutic product. The products and procedures recommended here have been carefully selected and tested for compatibility with Heracell Vios CR and Forma Steri-Cycle CR CO_2 incubators.

References

1. Armstrong SE, Mariano JA, Lundin DJ (2010) The scope of mycoplasma contamination in the biopharmaceutical industry. *Biologicals* 38: 211.
2. Wilder CN, Reid Y (2015) Mycoplasma quality control of cell substrates and biopharmaceuticals. *American Pharmaceutical Review.*
3. Mizutani M, Samejima H, Terunuma H, Kino-oka M (2016) Experience of contamination during autologous cell manufacturing in cell processing facility under the Japanese Medical Practitioners Act and the Medical Care Act. *Regenerative Therapy* 5:25.
4. Barone PW et al. (2020) Viral contamination in biologic manufacture and implications for emerging therapies. *Nature Biotechnology* 38:563.
5. Tawde SA (2014) Particulate matter in injectables: Main cause for recalls. *Journal of Pharmacovigilance* 3(1).
6. Eglovitch JS (2019) FDA: Despite improvement, particulate-related injectables recalls remain a concern. *Pink Sheet, Informa Pharma Intelligence.*
7. Wronksi K, Bates MK, Low L (2021) Heracell Vios CR and Forma Steri-Cycle CR-CTS series CO_2 incubators achieve proven compatibility with an ISO Class 5 Grade A/B cleanroom. Thermo Fisher Scientific in press.
8. Shen S, Yuan L, Zeng S (2009) An effort to test the embryotoxicity of benzene, toluene, xylene, and formaldehyde to murine embryonic stem cells using airborne exposure technique. *Inhalation Toxicology* 21(12):973.
9. McDermott C, Allshire A, van Pelt FNAM, Heffron JJA (2007) Sub-chronic toxicity of low concentrations of industrial volatile organic pollutants in vitro. *Toxicology and Applied Pharmacology* 219:85.
10. Thermo Scientific (2016) Smart Note: How can using the wrong type of water to provide in-chamber humidity cause corrosion in my CO_2 incubator? Thermo Fisher Scientific SNCO2WATER2016.
11. International Organization for Standardization (2017) Cleanrooms and associated environments – Part 13: Cleaning of surfaces to achieve defined levels of cleanliness in terms of particle and chemical classifications. ISO 14644-13:2017(E).
12. The United States Pharmacopeial Convention (2013) *The United States Pharmacopeia and National Formulary.*
13. European Directorate for the Quality of Medicines & HealthCare, Council of Europe (2020) *European Pharmacopoeia* 10th ed. Strasbourg France.
14. Thermo Scientific Steri-Run sterilization cycle proves total sterilization (2015) Thermo Fisher Scientific ANCO2STERIRUN 0215.
15. The National Institute for Occupational Safety and Health, Centers for Disease Control and Prevention. Hydrogen peroxide. **https://www.cdc.gov/niosh/npg/npgd0335.html**. Accessed 29 March 2021
16. Thermo Scientific CO_2 incubators are compatible with multiple cycles of vaporized hydrogen peroxide (2021 in preparation).

Find out more at **thermofisher.com/cleanroomco2**

ThermoFisher SCIENTIFIC