

Effective Contamination Control with CO₂ Incubators

Ines Kristina Hartmann¹, James Jarvis²

¹Eppendorf AG, ²Eppendorf, Inc.

Executive Summary

CO₂ incubators provide an optimal cell growth environment by maintaining a humidified atmosphere with temperature and carbon dioxide control. These conditions not only promote cell growth, but also the growth of contaminants, like bacteria, yeast, molds and other fungi. The contamination-reducing features of an incubator's functional design and the effectiveness of its self-decontamination system must be considered in choosing an instrument. In this paper, we compare various strategies for preventing contamination in CO₂ incubators, from the functional design of the device to self-disinfection programs. We also give some useful tips to prevent contamination when using CO₂ incubators.



Introduction

Sources of contamination

Contamination is a major cause of frustration when culturing cells. There are many sources of contamination, either direct or indirect. Direct sources are contaminated reagents, media or seed culture. Media and reagents from reputable suppliers are rarely delivered contaminated nowadays. New cell lines can introduce contaminants into the lab, especially when they are given from lab to lab. They should be quarantined before culturing with the rest of the cells. Direct contamination can be prevented by stringent testing. Indirect sources of contamination include lab surfaces, equipment and personnel. Germs are spread predominantly by cross-contamination. This can be prevented by good aseptic techniques, regular cleaning, and disciplined adherence to scheduled maintenance programs for equipment. Good functional design of equipment and regular use of automatic self-decontamination programs can further help to minimize contamination.

How do contaminants get into a CO₂ incubator?

The CO₂ incubator may become an indirect source of contamination. Unlike a biological safety cabinet, an incubator cannot prevent the influx of airborne contaminants, as the door must be opened during routine use. The incubator chamber can also be contaminated by carelessness in aseptic techniques, and unnoticed splashes from cell culture vessels. Some CO₂ incubators use a HEPA filter to remove microorganisms from the air in the chamber, but if the filter is not changed regularly it can harbor and spread the germs. In addition, a HEPA filter is no protection against mycoplasma contamination as these microorganisms are only 0.1-0.3 μm in size and therefore are not filtered out.

Why do contaminants spread in CO₂ incubators?

Once a contaminant enters an incubator, the warm, humid environment promotes further propagation. This can pose a risk to cultures if contamination remains undetected and regular cleaning and maintenance schedules are not implemented.

How to prevent contamination in a CO₂ incubator?

Using proper aseptic technique along with a regular cleaning and disinfection schedule will help to minimize the spread of contaminants. These contamination-prevention habits will be easier, faster and more effective with well-designed CO₂ incubators that incorporate self-disinfection.

General tips on preventing contaminations with CO₂ incubators

- > Avoid fan-assisted air circulation – a recently published guideline for good cell culture practice recommends non-fan-assisted incubators, to reduce airborne contamination [1].
- > Make shelves and racks easy to remove for quick cleaning.
- > Choose an incubator equipped with an automatic self-disinfection program.
- > Do not place your incubator directly on the floor. A base with castors offers the possibility of flexible movement and improved access to the back side for cleaning; it also keeps the unit up off the floor, so that dust and dirt cannot enter when you open the door.
- > Use CO₂ and N₂ gases of ‘high grade’ quality (> 99.5 %), and in-line gas HEPA filters.
- > Minimize the frequency and duration of door openings by keeping the contents organized for fast, easy access, and use split inner doors if frequent openings are unavoidable.
- > Only touch the incubator with fresh and disinfected gloves.
- > Implement regular cleaning and maintenance schedules.

Product design & functional solutions

Comparison of different contamination control strategies

Design solutions and functional measures to prevent contamination in CO₂ incubators that are available today:

HEPA (High-Efficiency Particulate Air) filters can be installed in CO₂ incubators to filter particles from the air. This requires fan-assisted air circulation in the chamber in order to aspirate air through the filter. However, the addition of the fan and the duct results in a more complex interior chamber design. The advantage is actively filtered air. There are several disadvantages:

- > As a result of the complex interior, there are seams and corners in which contaminants can grow.
- > Splashes may stay undetected and become a potential breeding ground for contaminants.
- > More time has to be spent in dismantling the unit in preparation for cleaning and disinfection.
- > The forced air flow created in the chamber may result in a higher chance of germ entry. Contaminants that are too small to be filtered out get spread throughout the chamber (e.g. mycoplasma and viruses).
- > Unless the filter is replaced according to the maintenance schedule, it can become clogged and turn into a source of contamination.

UV light is another measure intended to eliminate air and waterborne contaminants that may have entered the chamber. The UV lamp is usually isolated from the cell culture chamber by a plenum cover over the humidity pan, and the light is directed at both the circulated, humidified air and the water in the pan. The lamp automatically switches on for a specified period after each door opening. Establishing directional airflow requires an additional fan and a duct at the back of the incubator. Although UV treatment of the air and water in incubators has been found effective [2], relative humidity above 70 % was found to adversely impact the effectiveness of UV [3]. In addition, the UV lamp must be replaced periodically to maintain its effectiveness.

Fan-less chamber design: Unlike the forced airflow in a fan-assisted incubator, the fan-less incubator circulates air gently, by convection. The potential risks of turbulent airflow – drying of samples, vibration, and further spread of contaminants – are fully eliminated. And the chamber design has no complex interior structures in which germs can hide. By its plain design, with no seams and hidden corners, contaminants rarely have the chance to grow without being detected. If any spillages occur, they can be disinfected immediately, as all surfaces of the chamber are easily accessible.

Table 1: Pros and Cons of different contamination control strategies in CO₂ incubators

Measure	Pro	Con
HEPA filter (using fan)	<ul style="list-style-type: none"> > Active filtering of particles from the air reduces airborne spread of contaminants that are bigger than 0.2 µm 	<p>Chamber design is complex with duct, seams, possible welding joints and concealed corners:</p> <ul style="list-style-type: none"> > Splashes may stay undetected > Contaminants may spread undetected > Time-consuming preparation for cleaning > Regular investment in new filter / UV lamp <p>Fan-assisted air circulation:</p> <ul style="list-style-type: none"> > In-chamber fan may create turbulences > May increase chance of germ entry
UV light (using fan)	<ul style="list-style-type: none"> > Direct exposure kills air + waterborne contaminants 	<p>UV:</p> <ul style="list-style-type: none"> > Penetration limited to direct exposure > Influence of humidity on effectiveness [3]
Plain chamber design / Fan-less design	<p>Fan-less design</p> <ul style="list-style-type: none"> > No potential risk of turbulences > Reduce airborne spread of contaminants <p>Plain chamber design:</p> <ul style="list-style-type: none"> > Low chance of contamination formation > Chamber surfaces are easily accessible > Rapid preparation for cleaning > No extra investment in spare parts 	<ul style="list-style-type: none"> > No active measures to reduce airborne contaminants

Conclusion: All strategies have pros and cons (Table 1). On one hand, HEPA filters and UV light can actively reduce the airborne germ load. On the other, both measures require fan-assisted circulation of chamber air. This airflow may increase the chance of airborne contamination, and it requires a complex interior chamber that is more time-consuming to dismantle and clean, more likely to harbor hidden contamination, and leaves less room for cultures. The chamber air is not actively cleaned if the HEPA filter or UV light is omitted, but as result of the fan-less design all parts of the chamber are visible and easily accessible. A plain design means also less chance of hidden contamination.

The Eppendorf solution: The deep-drawn chamber of Eppendorf CO₂ incubators is made from a single sheet of stainless steel, with no seams (Figure 1). The Eppendorf direct-heating technology results in natural and gentle convection circulation of the chamber atmosphere and does not require a fan or complex internal parts. There are no hidden corners to contaminate, and spills can be seen and wipe-disinfected. The racking and shelves can be removed in less than two minutes for quick cleaning and disinfection.



Figure 1: Eppendorf seamless easy-to-clean incubator chamber with rounded corners prevents contamination formation and allows quick cleaning procedures.

Copper chamber: Most incubators are available with a copper, copper alloy, or copper-plated chamber, instead of stainless steel. The antimicrobial properties of copper surfaces have been demonstrated repeatedly [4]. Alloys are most effective when containing at least 60 % copper [5].

The Eppendorf solution: The CellXpert® C170i is optionally available with a copper plated chamber, shelves and humidity pan (Figure 2).



Figure 2: Eppendorf copper chamber. Chamber, shelves and water tray are plated with 100 % copper.

Cleaning and maintenance schedules

None of the contamination control strategies described above can replace a regular, thorough cleaning and disinfection schedule. Implement a regular cleaning schedule for your incubator, at a frequency that depends on

several factors, including the number of users and their skills in aseptic technique. Please follow the instructions in your operating manual, as cleaning instructions may vary between manufacturers.

Daily: Inspect the incubator contents! Remove and disinfect any spills immediately with 70 % ethanol or isopropanol. Wipe with the disinfectant, rather than spraying it, as this enables complete wetting of the surface, and prevents the formation of aerosols that may harm you and your cells.

Weekly: Empty the water tray, clean it and wipe-disinfect it using 70 % alcohol. Refill the tray with water. Most manufacturers recommend using sterile distilled water.

Monthly: Once a month, up to every 6–8 weeks, empty the incubator fully. Using a lint-free cloth, clean the chamber interior with soapy water and rinse with fresh water, followed by wiping the surfaces with 70 % alcohol or an equivalent non-corrosive disinfectant. If you have an incubator with many hidden corners, fissures, ducts, or seams, you should pay special attention to these areas, as germs can hide there. Clean and disinfect the removed shelves and racking in the same way. Clean the exterior of

the incubator, especially the surfaces you touch, like the doors. Take care to keep the solutions from coming into contact with any mains electrical outlets or assemblies. If your incubator is equipped with an automatic disinfection program, first reinstall all parts that can withstand the disinfection program; check if the sensors can stay inside and keep the HEPA filter out, if the unit is equipped with one. Then run the disinfection program overnight, following the manufacturer's instructions.

Every 6 months: Replace the HEPA filter, if your unit is equipped with one.

Annually: Service should be done at least once a year by an authorized service engineer. Suppliers offer flexible service performance plans and contracts, according to your needs, from basic checks of sensors and functional parts up to replacement of worn parts. (For Eppendorf Service packages please visit: www.eppendorf.com/incubator-service)

Built-in automated self-decontamination functions

Incubators with integrated disinfection systems offer additional protection from contamination. Today, incubators are available with various built-in automatic self-disinfection systems, from moist- or dry-heat to hydrogen peroxide (H₂O₂) nebulization.

H₂O₂ nebulization is quicker than heat decontamination, but requires handling of a toxic reagent and periodic repurchase of the reagent specified by the manufacturer.

Moist-heat disinfection requires a long and tedious procedure, including draining of the water and refilling the reservoir. In addition, it leaves condensed water in the chamber at the end of the cycle, which increases the risk of recontamination. Condensed water has to be removed by a final wipe-disinfection of the chamber.

Dry-heat disinfection can be run overnight, and has the shortest preparation time. It also has the lowest chance of recontamination, as the chamber can be used directly afterward. Note that HEPA filters cannot stay in the incubator during high-temperature disinfection.

Table 2: Pros and Cons of different methods of self-disinfection programs in CO₂ incubators

Method	Pro	Con
H ₂ O ₂ nebulization	<ul style="list-style-type: none"> > Quick (approx. 3 hours*) *excluded preparation time 	<ul style="list-style-type: none"> > Handling of toxic reagent > Regular cost of reagent > Tedious preparation before and after the procedure > High chance of recontamination due to manual repositioning of interior
High temperature (moist heat)		<ul style="list-style-type: none"> > Tedious preparation time > High chance of recontamination due to condensed water > Slow (overnight)
High temperature (dry heat)	<ul style="list-style-type: none"> > Short preparation time > Lowest chance of recontamination as chamber can be used right away after cycle 	<ul style="list-style-type: none"> > Slow (overnight)

Conclusion: All measures have pros and cons (Table 2). Whereas H₂O₂ nebulization is fast, high heat decontamination can be scheduled overnight. Dry-heat requires very little preparation time, and has none of the labor and costs associated with reagents, apparatus, and the refilling of water reservoirs. It also has the lowest chance of recontamination after a disinfection cycle, as the chamber can be used right away once the water pan is refilled. With H₂O₂ nebulization, the liquid remaining after the cycle must be wiped away, the apparatus removed, and the shelves properly repositioned. With moist-heat, the water reservoir must be emptied and disinfected (as recommended by the manufacturers), so the chance of recontaminating the chamber is highest among the three methods.

The Eppendorf solution: The CellXpert CO₂ Incubators offer dry-heat, high-temperature disinfection. The optional automated high-temperature disinfection program provides an additional layer of protection against contamination. Using it is exceptionally easy. By just pressing one button, the disinfection cycle can be started; it heats all parts of the inner chamber up to 140-180 °C (depending on the model) and holds it for 2 hours. The whole process, about 14 hours in duration, can be conveniently completed overnight. The Eppendorf CO₂ sensor is heat resistant, and can stay inside during the cycle.

Conclusion

In summary, Eppendorf CO₂ Incubators make it easy to prevent contamination with their functional, easy-to-clean chamber design and effective dry-heat decontamination.

CellXpert® C170i Ordering Information

Device Options			Order no.				
Door Handle	Humidity Monitoring/ Water Level Monitoring	Copper	230 V, 50/60 Hz	230 V, 50/60 Hz	230 V, 50/60 Hz	230 V, 50/60 Hz	100–120 V, 50/60 Hz
			European	UK/HKG	Australia	China	USA/Japan
Right			6731 000.011*	6731 000.012*	6731 000.013*	6731 000.014*	6731 010.015*
Right		Yes	6731 000.511	6731 000.512	6731 000.513	6731 000.514	6731 010.515
Right	Humidity monitoring		6731 000.111*	6731 000.112*	6731 000.113*	6731 000.114*	6731 010.115*
Right	Water level monitoring		6731 000.211*	6731 000.212*	6731 000.213*	6731 000.214*	6731 010.215*
Right	Both		6731 000.311*	6731 000.312*	6731 000.313*	6731 000.314*	6731 010.315*
Left			6731 000.021*	6731 000.022*	6731 000.023*	6731 000.024*	6731 010.025*
Left		Yes	6731 000.521	6731 000.522	6731 000.523	6731 000.524	6731 010.525
Left	Humidity monitoring		6731 000.121	6731 000.122	6731 000.123	6731 000.124	6731 010.125
Left	Water level monitoring		6731 000.221	6731 000.222	6731 000.223	6731 000.224	6731 010.225
Left	Both		6731 000.321	6731 000.322	6731 000.323	6731 000.324	6731 010.325

*Stock article; all others are built-to-order

CellXpert® C170 Ordering Information

Device Options		Order no.				
Door Handle		230 V, 50/60 Hz	230 V, 50/60 Hz	230 V, 50/60 Hz	230 V, 50/60 Hz	100–120 V, 50/60 Hz
		European	UK/HKG	Australia	China	USA/Japan
Right		6734 000.011	6734 000.012	6734 000.013	6734 000.014	6734 010.015

All variants and accessories (with humidity monitoring, copper chamber etc.) are available on our website: www.eppendorf.com

Learn more about our incubators using the interactive product model here: www.eppendorf.com/CellXpert

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About Eppendorf

Eppendorf is a leading life science company that develops and sells instruments, consumables, and services for liquid-, sample-, and cell handling in laboratories worldwide. Its product range includes pipettes and automated pipetting systems, dispensers, centrifuges, mixers, spectrometers, and DNA amplification equipment as well as ultra-low temperature freezers, fermentors, bioreactors, CO₂ incubators, shakers, and cell manipulation systems. Associated consumables like pipette tips, test tubes, microtiter plates, and disposable bioreactors complement the instruments for highest quality workflow solutions.

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