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Dispelling five myths about CO_2 incubator HEPA filtration systems

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Introduction

A high efficiency particulate air (HEPA) filter is a three-dimensional, pleated depth filter (Figure 1) designed to reduce particulate numbers in air. Because a HEPA filter is a three-dimensional mesh of fibers, it is resistant to air passing through, so force is required to push or pull the air through the mesh. Thus, a CO_2 incubator without a circulating fan to move air cannot offer in-chamber HEPA filtration. Thermo Scientific™ CO_2 incubators have offered in-chamber HEPA filtration systems since 1984.

Figure 1: A HEPA filter with a cutout showing the three-dimensional depth structure of the filter medium. This example also includes a molecular sieve medium to capture volatile organic compounds (VOCs).

Why we designed an in-chamber HEPA filtration system for our CO_2 incubators

When any incubator door is opened, the air inside mixes with the surrounding laboratory air. Normal indoor room air contains 30-1000 microorganisms per liter [1]. This is largely, but not entirely, due to the people working in the lab, because each of us carries an average of 10,000 microorganisms per centimeter of skin [2]. For this reason—in any incubator—cultured cells are at risk each time the incubator door is opened, and in-chamber HEPA filtration helps to capture these circulating microorganisms before they settle on the culture vessels, the shelves or in the water reservoir. But how well and how efficiently an in-chamber HEPA filtration system does this job entirely depends on the airflow design, the fan speed, the chamber size and more. If the air circulation is too slow, more microorganisms have time to settle within the incubator. If the air circulation is too fast, the air may desiccate the cultures. Poorly designed air circulation could allow dead spots where the air is not sufficiently filtered.

There is some misinformation circulating around CO_2 incubator HEPA filtration systems. In this e-book, we dispel these myths with data-based information.

A HEPA filtration system will capture particles of any size

Myth 1 **A HEPA filter will not capture anything smaller than 0.3 µm, so small viruses and mycoplasmas are still a risk to cultured cells.**

Particles larger than 0.4 µm in diameter, are captured by impaction: they are so large that they hit the wall of the filter and are caught. Large particles are also captured by interception, where particles collide with each other, which slows their speed and changes their direction, causing them to hit the filter wall. The smallest particles, less than 0.1 µm in size, are captured by diffusion; this is essentially Brownian motion where the particle is so tiny that it collides with air molecules that change the particle’s direction, causing it to hit the filter. As shown in Figure 2, particles of 0.2 µm in size fall between these processes which is why these are the least efficiently caught. For an H13 HEPA filter, the most penetrating particle size (MPPS) is usually 0.2 µm with an efficiency of 99.95%, and smaller and larger particles are captured with even greater efficiency, approaching 100% as shown in Figure 2. This means that those insidious mycoplasmas and small viruses are indeed captured by an in-chamber HEPA filtration system, which efficiently helps to protect your cultures.

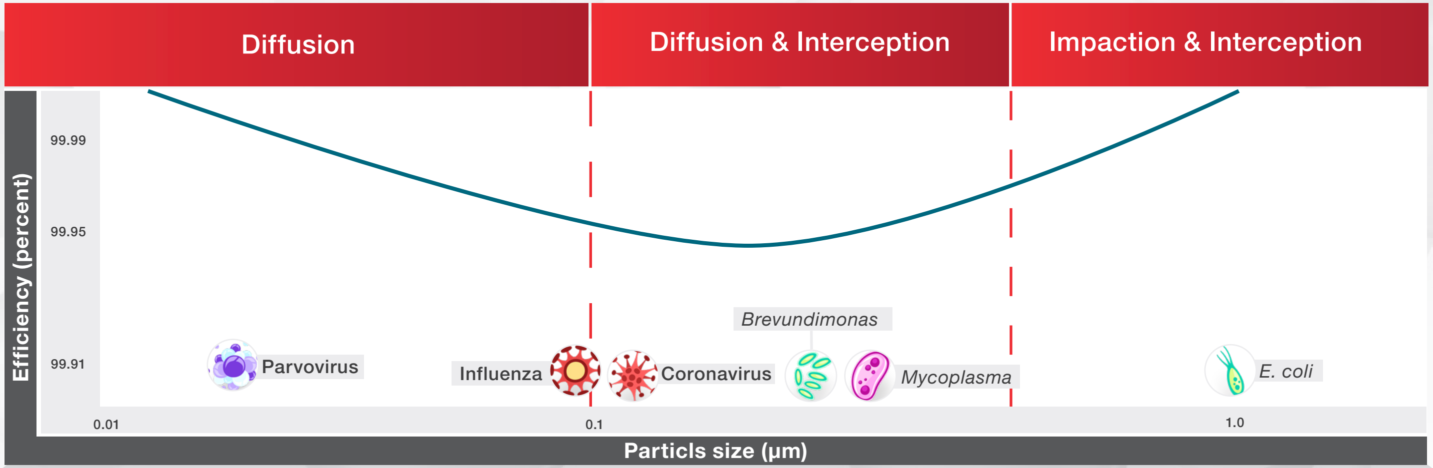
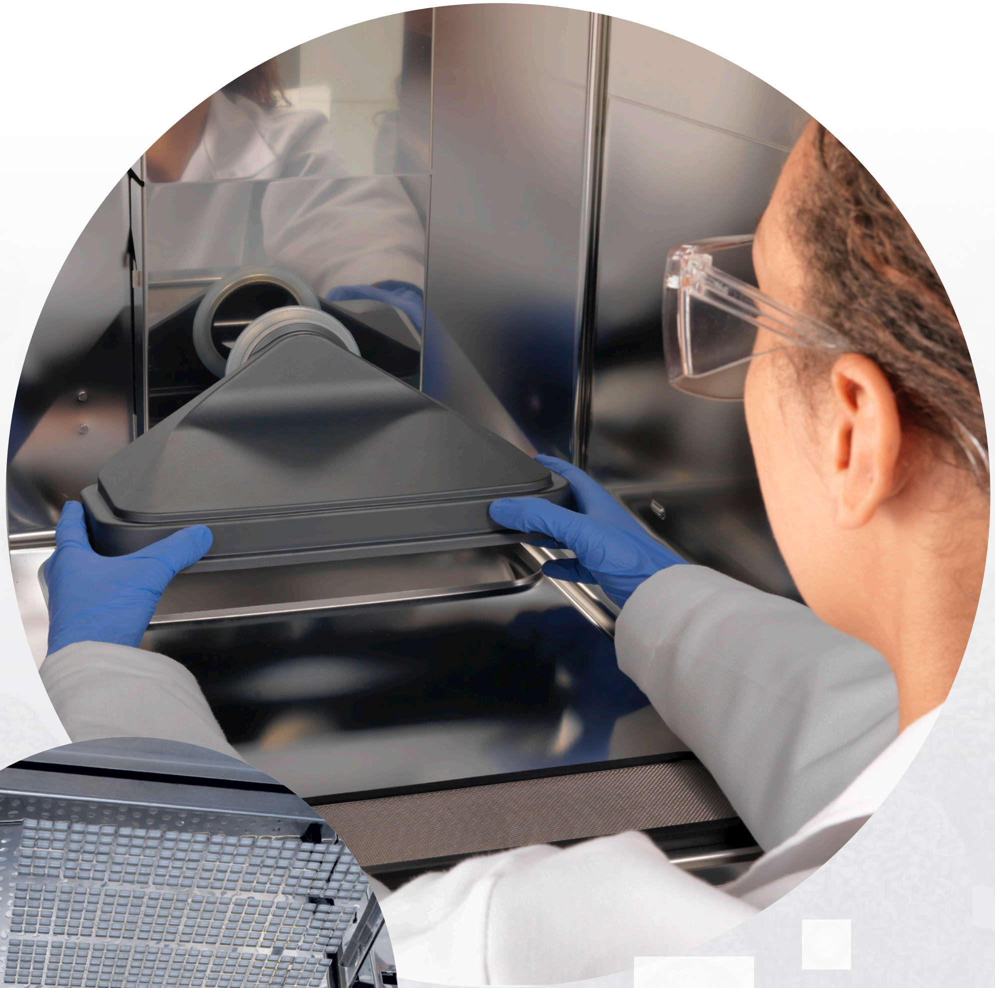


Figure 2: A HEPA filter captures particles of all sizes, but with different efficiencies. The efficiency depends on the filter depth and construction, the airflow velocity, and the different physical processes involved, including impaction, interception and diffusion. Example microorganisms captured by these processes are arranged by size. Particles of around 0.2 µm are captured with the lowest efficiency of 99.95%. Smaller and larger particles are captured with greater efficiency, approaching 100%.

Microorganisms cannot escape a HEPA filter

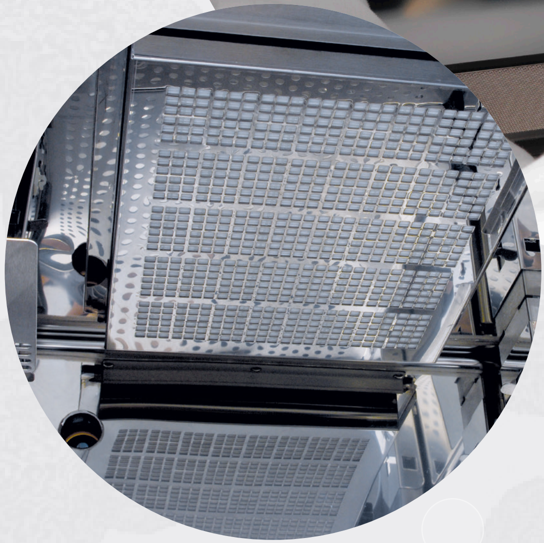
Myth 2 Microorganisms can escape and grow again.

As long as there are no holes or tears in the HEPA filter medium, microorganisms cannot escape a HEPA filter. The filter medium is hydrophobic, so over time, microorganisms will desiccate and die. The amount of time this takes will be different for varied species. Experiments have proven this; the only way microorganisms were recovered and viable was when the filter was cut into pieces and pulverized on a vortex mixer while immersed in nutrient growth medium [3]. When handled with care, an in-chamber HEPA filter is shown to help protect cultures for one year. A used HEPA filter should be disposed with the laboratory biological waste.



Particles are quickly captured from incoming air

In Thermo Scientific CO_2 incubators, the airflow is designed to filter the entire chamber air volume every 60 seconds, resulting in ISO Class 5 air cleanliness in 5 minutes after the door has been closed, following a thirty second door opening. ISO 14644-1 has classified air cleanliness on a log base 10 system for accepted numbers of particles of prescribed sizes [4].



An example of how a Thermo Scientific™ Vios™ iDx CO_2 Incubator cleans the air is shown in Figure 3. As the door remains closed, the air passing continuously through the HEPA filter becomes ever cleaner. Shorter door openings or cleaner laboratory air likely result in ISO Class 5 conditions even faster.

Be aware that different CO_2 incubator air filtration designs will not meet this performance. Some do not reach ISO Class 5 at all. Some CO_2 incubators place a HEPA filter in an external box where air is removed from the chamber through tubing. This system is driven by a pump, not a circulating fan. The pump driven system could leave dead spots in the incubator chamber where air is not circulated. Manufacturers of CO_2 incubators with different HEPA filtration systems may not provide performance data, but this is required to understand how these different designs – especially with filtration occurring outside the incubator chamber – affect the air quality inside the incubator where cultures reside.

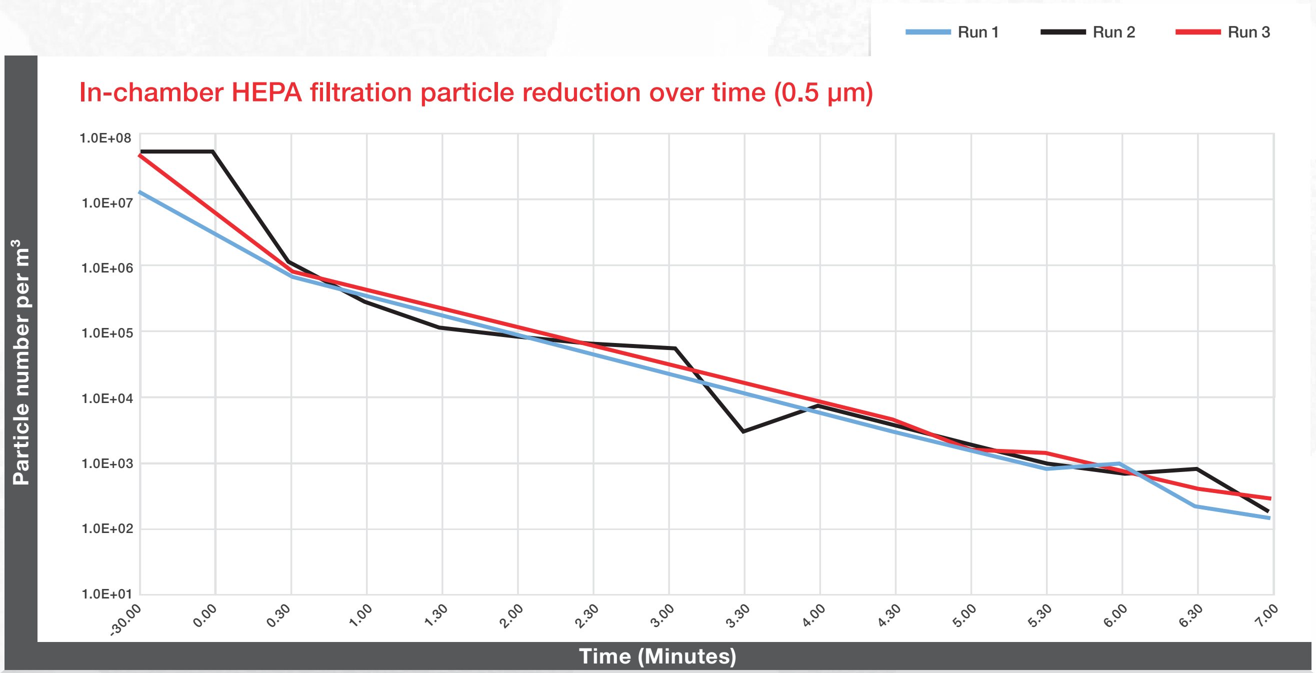


Figure 3: In-chamber HEPA filtration provides ISO Class 5 conditions in 5 minutes after a door opening. Representative test results showing that in the Vios iDx 255 CO_2 incubator, the in-chamber HEPA filtration combined with the Thermo Scientific™ THRIVE™ Active Airflow generates ISO Class 5 conditions in 5 minutes following a 30 second incubator door opening (both inner and outer doors) and the air continues to get cleaner over time, reaching lower than ISO Class 4 conditions in seven minutes.

A CO_2 incubator HEPA filter costs little and lasts one year

Myth 3 A CO_2 incubator HEPA filter is expensive and hard to maintain.

We sometimes hear Myth 3 as a reason for cell biologists to avoid using a CO_2 incubator with in-chamber HEPA filtration, but it simply is not true. You may hear rumors of prices as high as $1,000 USD each, but in truth our HEPA filters cost about one-tenth this amount. You may also hear that they must be replaced often, but our in-chamber HEPA filters are designed to last for one year. They do need to be removed from the incubator if preparing to run a high-temperature sterilization cycle, because the filter sealant will melt. If desired, the HEPA filter could be stored in a plastic bag or surgical wrap during the sterilization cycle then re-installed following the cycle. And our in-chamber HEPA filters can be easily replaced with no tools needed. Before installation, a new HEPA filter should be carefully inspected to ensure there are no tears or holes in the pleated filter medium.

A HEPA filter is a three-dimensional depth filter

Myth 4 There can be different types of HEPA filters in a CO_2 incubator.

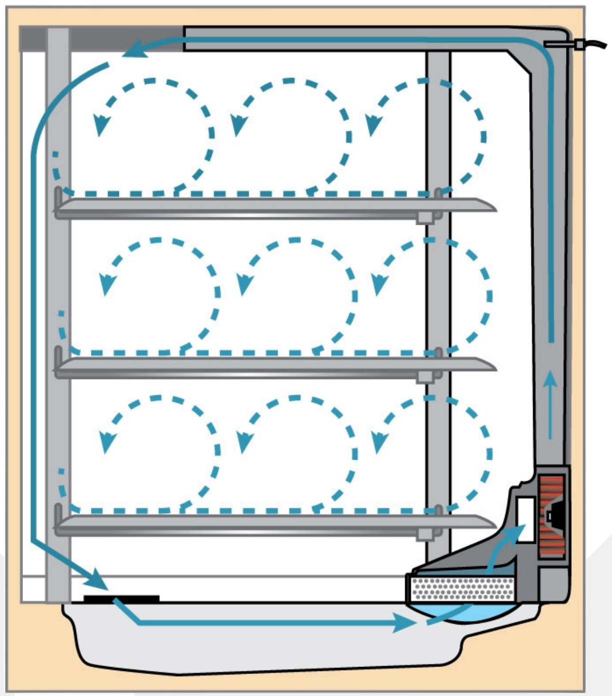
Some manufacturers have stated that their CO_2 incubators have multiple HEPA filters. This is incorrect and may be due to confusion regarding what is or is not a HEPA filter. As stated above, a HEPA filter is a three-dimensional depth filter. If the filter in question is a two-dimensional membrane filter, this is not a HEPA filter. Figure 4 shows a side-by-side comparison. A round, two-dimensional membrane (or disc) filter is commonly used for filtering impurities from CO_2 and other gases used in the CO_2 incubator. A membrane filter is not appropriate for use in forced air applications and thus by definition is not a high efficiency particulate air (HEPA) filter.

Figure 4: Side by side comparison. A three-dimensional, pleated depth HEPA filter (top) next to a two-dimensional membrane disk air filter (below). Zoomed in view shows the three-dimensional mesh structure of the HEPA filter and indicates why a circulating fan is required to force air through the fibers

An in-chamber HEPA filtration system is non-unidirectional

Myth 5	**HEPA filtration in a CO_2 incubator works like a design in a BSC or in a clean bench.**

Many biologists and process engineers are familiar with a unidirectional HEPA filtration system in a biological safety cabinet (BSC) or clean bench. Non-unidirectional HEPA filtration systems are slightly different. As described in ISO 14644-3 [5], "The recovery performance of a non-unidirectional cleanroom is affected by air distribution characteristics such as ventilation effectiveness, thermal conditions, and obstructions." In the CO_2 incubator, the HEPA filtration system is a non-unidirectional system, since air filtration must circulate around shelves and culture vessels. Also, when the incubator door is opened, dirty air enters and mixes with the already cleaned air. When the door closes, the HEPA filtration system will continuously filter the air, increasing the chamber air cleanliness. As a result, the chamber air is progressively diluted to an ever-cleaner environment as shown in Figure 3.



Conclusions

A well-designed HEPA filtration system in a CO_2 incubator should circulate the entire chamber air volume through the HEPA filter approximately every sixty seconds to clear particulates of all sizes from the air and to limit settling inside the incubator. This system protects sensitive cell cultures by removing airborne microorganisms that could represent a contamination risk. A HEPA filtration system is most effective in combination with a circulating fan. Thermo Scientific HEPA filters are inexpensive, and are easy to maintain and to install. Due to the constraints posed by incubator parts and loaded culture vessels, this is a non-unidirectional airflow system, cleaning the air by dilution, not by separating clean air from dirty air. Such a design should be proven effective according to ISO 14644-3:2019 as well as easy for users to maintain.

References

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4. International Standard ISO 14644-1. (2022) Cleanrooms and associated controlled environments – Part 1: Classification of air cleanliness. International Organization for Standardization (ISO).
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