APPLICATION NOTE

Thermo Scientific Cell Locker System Prevents Entry of Microorganisms for Protection of Sensitive Cell Cultures

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Abstract

The Thermo Scientific™ Cell Locker™ System in Thermo Scientific™ Heracell™ VIOS 160i and Thermo Scientific™ Forma™ Steri-Cycle i160 CO_2 Incubators offers enhanced stability, protection and flexibility for culturing sensitive cell types. Equipped with dual 0.2 µm membrane filters to allow airflow, the design is such that each Cell Locker Chamber, when closed, prevents entry of microorganisms, allowing segregation and quarantine of projects or cell types within a single CO_2 incubator. Independent tests were performed to test the sterility of the Cell Locker System and chamber design. A nebulizer was used to circulate *B. diminuta, S. aureus* and *M. orale* microorganisms inside the Cell Locker System, and agar plates captured any that settled. None were able to enter a closed chamber installed in the Cell Locker System. Next, individual Cell Locker Chambers were removed from the Cell Locker System, equipped with the optional transport door, then placed in a sealed cabinet and bombarded with *B. diminuta*. These tests showed that microorganisms could not gain entry to a closed Cell Locker Chamber outside the Cell Locker System. Combined, these results show that the Cell Locker System design provides secure culturing, isolates projects or cell types, and prevents cross-contamination of cultures.

Introduction

As cell biology innovations and applications accelerate, cell biologists are increasingly working with fragile, sensitive cell cultures in cutting edge fields including cancer, neurology, virology, immunology, cardiology, and gene therapy, as well as investigation into capabilities for stem cells, cartilage, muscle, and more. For such applications, microbial contamination is a constant danger to cultured cells and thus to downstream work. Understanding this risk has driven development of the Cell Locker System, a revolutionary approach to cell culture. The Cell Locker System, available in Heracell VIOS 160i and Forma Steri-Cycle i160 CO_2 Incubators, provides isolation for individual cultures, projects, cell types or user stocks in a single incubator, saving space in the laboratory while providing six segregated incubation chambers. These CO_2 incubators feature contamination control technologies including: Thermo Scientfic™ THRIVE™ active airflow, powering a HEPA air filtration system designed to provide ISO Class 5 cleanroom air quality in 5 minutes after every door opening; a covered, integrated humidity reservoir; and Thermo Scientific™ Steri-Run™ 180˚C automated sterilization cycle.

The Cell Locker System (see Figure 1) features six separate chambers, each one effectively serving as a quarantine chamber. With use of proper aseptic technique and sterile reagents and materials, the design of the Cell Locker System means that cultures incubated in a Cell Locker Chamber are protected from cross-contamination. This is especially important for sensitive, precious cells like primary cells, stem cells, neurons and immune cells which are more laboriously manipulated yet slower growing such that they are at greater risk. The Cell Locker System also provides enhanced environmental stability not possible in a traditional incubator, described in detail elsewhere.[1]

Cell Locker Chambers are composed of transparent polycarbonate, allowing for visual monitoring of culture vessels during transport. The two sides of the Cell Locker Chamber each feature a large 0.2 µm membrane filter (see Figure 3) which allows air exchange but prevents transmission of microorganisms. These replaceable filters are hydrophobic, enhancing gas transmission to and from the chamber, and oleophobic, repelling oils from handling and lipids from cells and body fluids. They are also resistant to organic solvents and have been tested for cytoxicity and biosafety. When closed, the Cell Locker Chamber structure – including the close-fitting door with silicone gasket and the dual 0.2 µm membrane filters – prevents transmission of even the smallest microorganisms. Although the smallest microorganisms, parvoviruses, are about 0.02 µm,[2] still they would not pass through these filter pores. This is due to the structure of the hydrophobic polyethersulfone (PES) filter. While some membrane filters are "straight-through" pores, the PES membrane is composed of a random three dimensional mesh which presents a labyrinth for particles. Physical laws of particle movement in air including sedimentation, interception, impaction, diffusion, and electrostatic attraction,[3] and the hydrophobic nature of the filter material, mean that when filtering air, the effective pore size is actually about ten times smaller, or 0.02 µm.[4] Even at this pore size, smaller particles are also caught due to collision with air molecules which slow their speed, and to electrostatic attraction, which combine to adhere these tiny particles to the mesh. As shown in Figure 2, different membrane filter pore sizes and construction have very different efficiencies. The 0.2 µm PES membrane used in the Cell Locker System filter is 100% efficient at capturing even the smallest particles.

Nevertheless, to ensure confidence in the protection provided, the complete Cell Locker System was tested using different approaches by two different, independent commercial test facilities.[5,6,7] The goal of these tests is to demonstrate that microorganisms cannot enter a closed Cell Locker Chamber when used in the Cell Locker System.

In these tests, a forced air nebulizer was used to circulate microorganisms into the airstream of the Cell Locker System. Petri plates containing appropriate culture agar were place on all available surfaces outside and inside the Cell Locker Chambers. If microorganisms cannot get into the Cell Locker Chambers, these plates remain sterile while plates outside the Cell Lockers will capture circulating microorganisms.

Figure 1: The Cell Locker System inside a Heracell VIOS CO_2 Incubator provides six segregated isolation chambers for critical cells or projects inside a single incubator. The inner transport door (black arrow) is optional and can be removed. The inner incubator door is segmented such that each Cell Locker Chamber has its own individual door, but the entire inner door can be opened, providing access for cleaning.

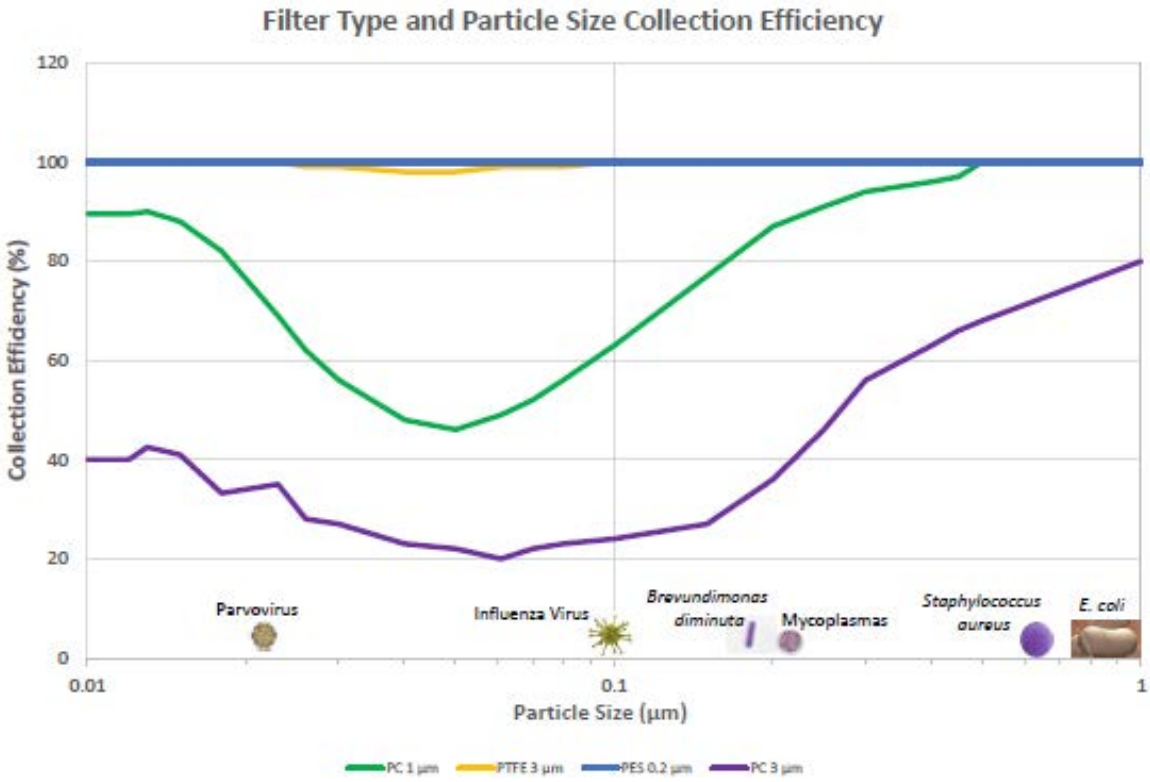

Figure 2: Efficiency of capturing varied particle sizes with different filter materials and pore size is shown. The polyethersulfone (PES) membrane used in the Cell Locker System filter, with a pore size of 0.2 µm, captures 100% of particles at all sizes. A polytetrafluoroethylene (PTFE) membrane with a 3 µm pore size can miss some particles in the 0.02-0.1 µm size. In contrast, a polycarbonate (PC) filter with smooth circular "straight-through" pores will miss many particles. Example microorganisms are positioned according to their relative size. The graph is based on Lindley 2016.

Cell Locker System includes optional transport door

Each Cell Locker Chamber is provided with an optional transport door and sliding work tray. Both can be used in the Cell Locker System. In this case, the smaller glass door in the incubator inner door is opened, then the transport door with the sliding work tray attached is pulled open (see Figure 1). No transport doors were used in the test of the System, because the transport door may be redundant for most labs. However, the transport door provides valuable flexibility for users who want to remove a Cell Locker Chamber from the Cell Locker System, for example to provide protected transport by taking a closed Cell Locker Chamber directly to the biological safety cabinet, to a different incubator, or even a different laboratory (see Figure 3). A closed Cell Locker Chamber equipped with the transport door will protect cultures from exposure to microorganisms circulating in the air of the laboratory, the hallway, or farther afield. This innovation means that for the first time, cultures can be continuously protected from circulating airborne contamination.

Because of this option, we wanted to test the containment of individual Cell Locker Chambers equipped with the transport door. In these tests, four Cell Locker Chambers with the transport door were positioned inside a sealed cabinet and bombarded with microorganisms at a higher airspeed than in the incubator chamber. Again, if microorganisms are prevented from entering, then individual Cell Locker Chambers can be used anywhere to protect cultures from airborne contamination. This setup also allowed testing of different gasket designs to determine if any performed better at preventing entry of microorganisms.

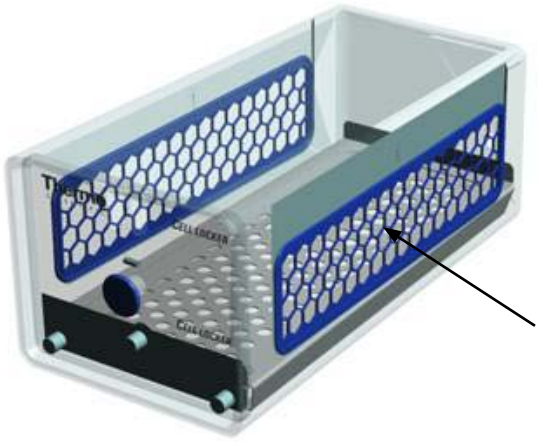

Figure 3: An individual Cell Locker Chamber can be used with the optional transport door and sliding work tray, to safely transport cultures to a BSC, a different incubator, or even to a different laboratory. The sides of the Cell Locker Chamber feature dual 0.2 µm membrane filters (black arrow) to allow air circulation, but prevent transmission of microorganisms. The transport door at the front and the stainless steel (or optional 100% copper) work tray are optional and need not be used in the Cell Locker System, and are easily removable if not desired.

Test Materials and Methods

Each test was performed three times.
Three microorganisms were used:

- *Brevundimonas diminuta* is a bacterium slightly larger than 0.2 µm,[4] used in a standard test to validate membrane filter pore size.[4,8] We wanted to use this microorganism in practical tests of the Cell Locker System to validate the design for limiting circulation of even very small microorganisms.
- *Mycoplasma orale* (DSM 25590) is a common cell culture contaminant. Mycoplasmas are very small, 0.15-0.3 µm [9] and due to their ubiquitous nature, we also wanted to use one species to test the Cell Locker System.
- *Staphylococcus aureus* (ATCC 6538) is commonly found on human respiratory tissues and skin, is another common cell culture contaminant, and about 0.8 µm in size.

All Cell Locker Chamber and System/incubator interiors were wiped or sprayed with 70% ethanol and allowed to air dry before each test. The Heracell VIOS CO_2 incubator (part of the Cell Locker System) was sterilized using the Steri-Run automated sterilization cycle [10] and set at 37˚C, with no CO_2 gas connected. No HEPA filter was used in the Heracell VIOS incubator, because the HEPA filter would collect the circulating microorganisms.

Tests of the Cell Locker System using circulating microorganisms

Microorganisms were circulated throughout the Cell Locker System via a nebulizer and the Thermo Scientific THRIVE active airflow system without the HEPA filter installed, and the optional transport door was not used in any Cell Locker Chamber. Two liters of sterile distilled water were used in the covered integrated humidity reservoir in the tests for *M. orale* and *S. aureus*, with no water used during the *B. diminuta* tests. One positive and one negative control plate for the bacteria were used. The negative control was unopened and sterile; the positive control contained a spread of the bacteria tested. These plates were not opened during the test.

The nebulizer was driven by a compressed gas canister, with the tubing running out through the incubator access port. For the *B. diminuta* tests, the nebulizer was positioned on the middle shelf between two Cell Locker Chambers, and for the *M. orale* and *S. aureus* tests, the nebulizer was between the two Cell Locker Chambers on the top shelf (see Figure 4A).

Sterile Petri plates containing the proper agar were positioned inside and on top of the Cell Locker Chambers (see Figure 4B). The plates on top should capture circulating bacteria even if none can enter the Cell Locker Chambers. Just before commencing each test, the plates inside and on top of the Cell Locker Chambers were uncovered. Then the incubator door was closed and the nebulizer switched on. After 1 hour, the nebulizer was switched off. Each Cell Locker Chamber door in the incubator segmented inner glass door was opened one at a time, and lids were aseptically placed on each dish. When all of the dishes in the Cell Locker Chambers were covered, then the large incubator inner door was opened and the plates on top of each Cell Locker Chamber were covered. For the *B. diminuta* tests, each Cell Locker Chamber had four plates inside and one on top. For the *S. aureus* and *M. orale* tests, four plates were inside and eight plates were on top of each Cell Locker Chamber. After the proper incubation period, the colonies on each plate were counted and recorded. Each separate colony represents one bacterium. Any plates with more than 300 colonies were scored as too numerous to count (TNTC).

Figure 4A: Nebulizer placement in the Cell Locker System. The tubing was led through the access port in the upper left back wall of the incubator.

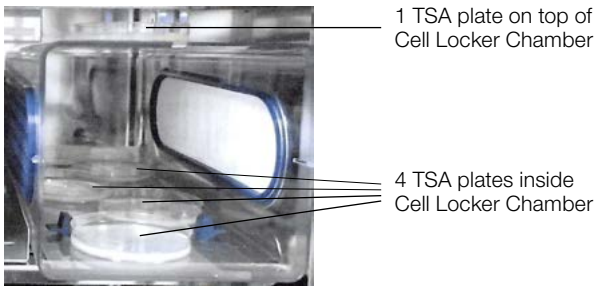

Figure 4B: Positioning of plates inside Cell Locker Chambers in the Cell Locker System. Four plates were positioned inside each Cell Locker Chamber for each test. One to eight plates were positioned on top of each Cell Locker Chamber (see individual tests for details).

Brevundimonas diminuta

A new vial of *Brevundimonas diminuta* was obtained from the National Collection of Industrial and Marine Bacteria (United Kingdom). An actively growing suspension was prepared and the concentration determined by serial dilution onto tryptone soya agar (TSA) plates, incubated at 30 ±2˚C for 48 hours and counted. The suspension was diluted for a final concentration of 7.10 x 10^3 colony forming units/milliliter (CFU/ml) and used as a spray suspension in the nebulizer. The total circulated ranged from 1.50 x 10^5 to 1.69 x 10^5 CFU (21-23 g), determined by weighing the nebulizer before and after each test.

Staphylococcus aureus

An actively growing culture of the bacteria was used to prepare a 70 ml solution with 6 x 10^3 CFU/ml. *S. aureus* bacteria were cultured on Baird Parker agar plates and incubated at 37˚C for 24-48 hours and counted. During the 1 hour nebulization period, about 16 ml of solution was used, for a total circulation of 9.6 x 10^4 CFU.

Mycoplasma orale

An actively growing culture of the bacteria was used to prepare a 70 ml solution with 5.8 x 10^3 CFU/ml. These bacteria were cultured on agar according to the recipe from the European Pharmacopeia 2.6.7 [11] and incubated at 37˚C under humid conditions for up to 14 days. During the 1 hour nebulization period, about 16 ml of solution was used, for a total circulation of 9.28 x 10^4 CFU.

Tests of the Cell Locker Chambers with transport cover using sprayed microorganisms

Four individual Cell Locker Chambers were equipped with the optional transport door and sliding work tray, and positioned closed in a Class III Microbiological Safety Cabinet (MSCIII) as shown in Figure 5. One of the Cell Locker Chambers had the transport door propped open approximately one centimeter. The cabinet air circulation was switched off before the test. Each Cell Locker Chamber contained four open TSA plates inside, and one open plate on top.

A 30 ml actively growing suspension of *B. diminuta* prepared as explained above with a concentration of 7.10 x 10^5 CFU/ml was used in a spray nebulizer. The nebulizer operated for one hour, and a small fan positioned under the nebulizer helped to circulate the bacteria in the cabinet. A total of 4.2 g was sprayed with a pressure of 26 pounds per square inch (PSI), for a total circulation of 2.98 x 10^6 CFU. After the nebulizer was switched off, the cabinet fan was switched on to vent any remaining airborne bacteria. Then the TSA plates were covered and incubated at 30˚C for 48 hours and counted.

Figure 5: Four Cell Locker Chambers with the optional transport door, and three different door gaskets, were positioned inside an MSC III cabinet. A nebulizer sprays the bacterial suspension and a small circulating fan helps to disperse the bacteria. Each Cell Locker Chamber contains four opened TSA plates inside and one opened TSA plate on top.

Results and discussion:

For each test, all positive control plates showed growth of the test bacteria, and no negative control plates showed any growth (results not shown).

Cell Locker System Tests

These tests were performed on the Cell Locker System inside the Heracell VIOS 160i CO_2 incubator, with three different very small microorganisms, *B. diminuta*, *M. orale*, and *S. aureus*. Each microorganism was tested three times on different days, and no Cell Locker Chamber showed any contamination. In fact, all of the opened agar plates inside all Cell Locker Chambers remained sterile during the 1 hour exposure to the circulating airborne bacteria. In contrast, all of the opened agar plates exposed on the exterior top of each Cell Locker Chamber showed significant growth of the test bacteria. See Tables 1, 2 and 3. Together, these results demonstrate that no microorganisms were able to gain access to the interior of the closed Cell Locker Chambers, even these tiny microorganisms. Thus, when properly handled, the design of the Cell Locker System inside the Heracell VIOS 160i CO_2 incubator prevents any airborne contamination of cultures inside closed Cell Locker Chambers. The Cell Locker System will isolate projects and cell types, preventing cross-contamination from circulating airborne microorganisms. It is important to note, however, that just as with any vessel or chamber, airborne contaminants can enter when the Cell Locker Chamber door is opened.

Table 1: Results are shown using *B. diminuta* to test containment of the Cell Locker System in the Heracell VIOS CO_2 Incubator. None of the plates inside the Cell Locker Chambers collected any bacteria. In contrast, every plate on top of the Cell Locker Chambers was contaminated.

Cell Locker System Test Results, *B. diminuta*						
7.1 x 10^3 CFU/mL suspension	**Four agar plates opened inside each Cell Locker Chamber**			**Positive control agar plate opened on top of each Cell Locker Chamber**		
Cell Locker Chamber position	**Test 1 1.63 x 10^5 CFU**	**Test 2 1.69 x 10^5 CFU**	**Test 3 1.50 x 10^5 CFU**	**Test 1**	**Test 2**	**Test 3**
Top Left	0+0+0+0	0+0+0+0	0+0+0+0	75	51	108
Top Right	0+0+0+0	0+0+0+0	0+0+0+0	71	59	95
Middle Left	0+0+0+0	0+0+0+0	0+0+0+0	75	135	125
Middle Right	0+0+0+0	0+0+0+0	0+0+0+0	60	142	130
Bottom Left	0+0+0+0	0+0+0+0	0+0+0+0	35	49	24
Bottom Right	0+0+0+0	0+0+0+0	0+0+0+0	47	37	26

Table 2: Results are shown using *S. aureus* to test containment of the Cell Locker System in the Heracell VIOS CO_2 Incubator. None of the plates inside the Cell Locker Chambers collected any bacteria. In contrast, every plate on top of the Cell Locker Chambers was contaminated.

Cell Locker System Test Results, *S. aureus*						
9.6 x 10^4 CFU/mL circulated	**Four agar plates opened inside each Cell Locker Chamber**			**Positive control agar plate opened on top of each Cell Locker Chamber**		
Cell Locker Chamber position	**Test 1**	**Test 2**	**Test 3**	**Test 1**	**Test 2**	**Test 3**
Top Left	0+0+0+0	0+0+0+0	0+0+0+0	TNTC x 8	TNTC x 8	TNTC x 8
Top Right	0+0+0+0	0+0+0+0	0+0+0+0	TNTC x 8	TNTC x 8	TNTC x 8
Middle Left	0+0+0+0	0+0+0+0	0+0+0+0	TNTC x 8	TNTC x 8	TNTC x 8
Middle Right	0+0+0+0	0+0+0+0	0+0+0+0	TNTC x 8	TNTC x 8	TNTC x 8
Bottom Left	0+0+0+0	0+0+0+0	0+0+0+0	TNTC x 8	TNTC x 8	TNTC x 8
Bottom Right	0+0+0+0	0+0+0+0	0+0+0+0	TNTC x 8	TNTC x 8	TNTC x 8

Table 3: Results are shown using *M. orale* to test containment of the Cell Locker System in the Heracell VIOS CO_2 Incubator. None of the plates inside the Cell Locker Chambers collected any bacteria. In contrast, every plate on top of the Cell Locker Chambers was contaminated.

Cell Locker System Test Results, *M. orale*						
9.28 x 10^4 CFU/mL circulated	**Four agar plates opened inside each Cell Locker Chamber**			**Positive control agar plate opened on top of each Cell Locker Chamber**		
Cell Locker Chamber position	**Test 1**	**Test 2**	**Test 3**	**Test 1**	**Test 2**	**Test 3**
Top Left	0+0+0+0	0+0+0+0	0+0+0+0	TNTC x 8	TNTC x 8	TNTC x 8
Top Right	0+0+0+0	0+0+0+0	0+0+0+0	TNTC x 8	TNTC x 8	TNTC x 8
Middle Left	0+0+0+0	0+0+0+0	0+0+0+0	TNTC x 8	TNTC x 8	TNTC x 8
Middle Right	0+0+0+0	0+0+0+0	0+0+0+0	TNTC x 8	TNTC x 8	TNTC x 8
Bottom Left	0+0+0+0	0+0+0+0	0+0+0+0	TNTC x 8	TNTC x 8	TNTC x 8
Bottom Right	0+0+0+0	0+0+0+0	0+0+0+0	TNTC x 8	TNTC x 8	TNTC x 8

Tests on individual Cell Locker Chambers with the transport door and different gaskets

Individual Cell Locker Chambers can be used with the optional transport door (shown in Figure 3) to remove cultures from the Cell Locker System and transport them in a protected fashion to a biological safety cabinet, a different incubator, or even a different laboratory. Tests were performed to challenge the containment of the transport door. In addition, different gasket designs were tested to confirm the level of gasket integrity required in sealing the transport door. These tests also challenge the membrane filter gasket and sealing, in a different way, being outside the Heracell VIOS CO_2 Incubator. We tested three different door gaskets, and a control with the Cell Locker transport door slightly ajar.

In the results (see Table 4), plate 1 is closest to the Cell Locker transport door; plate 4 is farthest to the back. Plates 1 and 2 tend to capture the most bacteria, with fewer bacteria captured on plates 3 and 4. The test with the partially opened transport door worked perfectly, showing that many bacteria were able to enter this approximately 1 cm opening. Note that the Cell Locker Chamber with gasket B – which is slightly loose, not making contact with the side walls in all areas – had the lowest challenge from the bacteria, because it was positioned farthest from the nebulizer. Yet this is the Cell Locker Chamber which was contaminated. Both gasket A and gasket C prevented any bacterial entry to the chambers. The Cell Locker Chambers with gaskets A and C, with zero bacteria entering, were the closest to the nebulizer and thus should experience a slightly greater challenge than the chambers which were farther away, including those chambers with gasket B closed, and with gasket B with the transport door slightly open. Gaskets A and C represent the basis for production gaskets used in the Cell Locker System. This test proved again that no bacteria could enter the Cell Locker Chamber unless the transport door gasket was loose or the door was open, and that no bacteria could enter through or around the membrane filter. With this design, an individual Cell Locker Chamber can be used with the optional transport door to protect sensitive cultures from airborne contamination when removed from the Cell Locker System.

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Table 4: Shown are results testing four Cell Locker Chambers with the transport door and three different gaskets, and one Cell Locker Chamber with the transport door slightly open. Gasket B fit more loosely than gaskets A and C. Chambers with transport doors and gaskets A and C remained sterile. Gasket B was leaky, allowing bacteria to enter, and a chamber with the transport door slightly open also allowed entry of bacteria.

Test Results for Cell Locker Chambers With Transport Door, *B. diminuta*						
2.98×10^7 CFU	**Individual Cell Locker Chambers with Transport Door Facing Nebulizer in Aerosol Cabinet**					
Cell Locker gasket type	**Test 1**		**Test 2**		**Test 3**	
	Inside	**Outside**	**Inside**	**Outside**	**Inside**	**Outside**
Gasket A	0+0+0+0=0	124	0+0+0+0=0	277	0+0+0+0=0	217
Gasket B	6+1+0+0=7	34	21+3+0+0=24	TNTC	4+7+5+0=16	218
Open Door	27+103+19+8=157	53	317+210+74+142=734	TNTC	114+409+205+125=853	79
Gasket C	0+0+0+0=0	79	0+0+0+0	TNTC	TNTC x 8	149

Conclusions

Tests reported here are performed independently by two commercial test facilities,[5,6,7] and demonstrate that when properly installed, the Cell Locker System and individual Cell Locker Chamber design will prevent bacteria from entering. Using a Cell Locker Chamber with the transport door also protects cultures from airborne contaminants when transporting cultures to a biological safety cabinet, different incubator, or even a different lab.

These tests demonstrate the integrity of the Cell Locker System, as well as the design of the individual chamber, transport door, and gaskets for isolating cultures and preventing airborne cross-contamination. As Louis Pasteur proved,[12] and as Petri dishes demonstrate in labs every day around the world, a vessel does not need to be airtight to restrict bacteria. If microbes carried in air have to navigate a circuitous path, they will be caught. Due to the complex labyrinth mesh and other properties of the PES membrane, and to the physical processes involved in filtering air, the results also mean that the PES membrane filter with the Cell Locker Chamber design will restrict smaller particles including viruses also. Cell biologists who incorporate the Cell Locker System into their workflow will enjoy enhanced protection for sensitive cultures.

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