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Thermo Scientific Cell Locker System Isolates Stem Cells and Other Delicate Cultures, Protecting from Contamination and Enhancing Environmental Stability in a Shared Incubator

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ABSTRACT

Delicate cultures including stem cells and primary cells are increasingly used in cell therapy research and development for applications in oncology, immunology, neurology and more. Commonly, these cells require daily manipulation, potentially exposing them to contamination. Such cells are also uniquely reactive to environmental variation. To address these challenges, we developed the Thermo Scientific™ Cell Locker™ System, a new CO_2 incubator design which includes six individual transparent Cell Locker chambers. Tests show the Cell Locker System provides enhanced stability, contamination control and flexibility for isolation of labile cell types or sensitive projects in a shared incubator. Each Cell Locker chamber features dual 0.2 µm membrane filters which allow air and humidity exchange but prevent transmission of microorganisms including mycoplasmas. Functional studies show that when one Cell Locker chamber is opened, remaining chambers in the Cell Locker System provide environmental stability for temperature, humidity, and CO_2/O_2 atmosphere, maintaining ideal conditions not possible in a standard incubator. Gas use and media evaporation were both reduced by 50%. Multiple clones of induced pluripotent stem cells from different donors were evaluated and found to be indistinguishable from control incubators where conditions and manipulations had previously been optimized, showing that the Cell Locker System design and materials facilitated optimal cell growth. The Cell Locker System represents a novel approach for culturing stem cells and other sensitive cultures used in cell therapy applications.

INTRODUCTION

Sensitive cells like stem cells, primary cells, neural cells and immune cells are much less resilient. Changing culture conditions affect them comparatively more than immortalized cell lines. Unlike established cell lines which have been growing in culture for decades, slow growing cell types or those which have only been passaged a few times have not had the opportunity to adapt (mutate) to artificial, two-dimensional *in vitro* culture conditions. These fragile cell types generally require more exacting and labor intensive effort to successfully culture.[1] Also, any microbial contamination is comparatively more costly if these cultures have to be discarded and work repeated.

Recognizing increasing use of such fragile cells types in basic and applied research for regenerative medicine, neuroscience, immunotherapy, cell therapy, oncology, autoimmune diseases and more, the Cell Locker System was designed to:

- better protect these cells.
- provide a much more stable environment.
- prevent cross contamination.
- conserve atmospheric gases.

MATERIALS AND METHODS

TEMPERATURE

Temperature was measured following DIN 12880 as physical limitations allowed. 3 validated PT100 temperature probes were placed in each of 6 chambers in the Cell Locker System (18 total). Each probe was 1.5 cm above the shelf, 1.5 cm from the front or back edge of the chamber, and equidistant from the sides. The incubator was set at maximum humidity and operated undisturbed for 12 hrs before the test. Each measurement was 10 sec and the test lasted 22 hrs. Uniformity equals the difference between the highest and lowest recorded temperatures. 4 different units were tested for 5 tests total.

HUMIDITY, CARBON DIOXIDE AND OXYGEN

Humidity was measured using a FHAD 462 relative humidity (RH) sensor (AHLBORN, Germany). CO_2 was measured using a GMM221 infrared sensor (Vaisala, Finland) used without the protective cover. O_2 was measured using a FCX_MC95-C zirconium sensor (Pewatron, Switzerland). Sensors were positioned in the geometric center of each chamber. Units equilibrated 12 hrs before testing. Figures 3, 4 and 5 are each a composite of multiple tests using three sensors in each of 3 Cell Locker chambers, and measured following a 30 sec door opening. RH tests were performed 4 times on each of 3 different units for 12 tests total. CO_2 tests were performed 4 times (3 chambers per test) on 4 different units for 16 total tests. 5 different oxygen concentrations were tested (results not shown). Tests were performed on 2 different units.

GAS CONSUMPTION

Gas consumption was measured using an electronic mass flow meter F-11B-10K-ABD (Bronkhorst, The Netherlands) positioned on the gas inlet port of the incubator. The gas consumption /day or /hr is calculated by measuring the gas flow (L/min) and multiplying by the time that the gas valve is open. This total is then divided by the test duration.

MICROBIAL TESTING

A nebulizer was used to circulate active cultures of *Staphyloccus aureus, Brevunidmonas diminuta* or *Mycoplasma orale* within the Cell Locker System, captured on open sterile agar dishes. The incubator was sterilized and all Cell Locker System interiors were sprayed with 70% ethanol and allowed to air dry before each test. The incubator was at 37 ˚C, with no CO_2. No HEPA filter was used in the System, because the filter would collect the microorganisms. Tests were performed three times.

STEM CELL CULTURE

Induced pluripotent stem (IPS) cell clones from 4 donors were thawed and cultured in 60 mm dishes and mTeSR™1 media (STEMCELL Technologies, Vancouver BC, Canada) and incubated at 37˚C, 5% CO_2. Cells were incubated in two groups, half in a Thermo Scientific™ Forma™ 3130 CO_2 incubator, half in a Cell Locker System. Cells were incubated 9-28 days, observed daily and passaged/frozen as required. After cryopreservation, cells were thawed, replated and re-evaluated.

EVAPORATION RATE

Evaporation was determined using 0.52 cm Petri dishes containing 15 g of distilled water, 3 dishes per Cell Locker chamber, 18 total. The System was set to 37 ˚C and CO_2 set to 0. After 24 hrs 16 min, the dishes were weighed again using a precision balance 572 (Kern, Germany) with an accuracy of +/- 0.01 g. No incubator or Cell Locker chamber door was opened during the test.

RESULTS

Figure 1. Cell Locker System Isolates Cultures

Cell Locker System consists of six individual protected chambers inside a Thermo Scientific™ Heracell™ 160i or a Thermo Scientific™ Forma™ i160 Steri-Cycle CO_2 incubator. 0.2 µm filter membranes permit air exchange but protect from cross-contamination and maintain ideal conditions.

Figure 2. Temperature in Closed Cell Locker Chambers Remains Stable When One is Opened

A stable temperature simulating body temperature native to cultured cells is critical to all cultured cells, but especially for sensitive cell types. A temperature of only 1˚C above 37˚C is detrimental and even immortalized cells will be degraded with temperatures of 2˚C above normal.[2]

When Cell Locker chamber 4 is opened for 30 seconds, the temperature drops from 37˚C to 35 ˚C. Following the door closing, the temperature rapidly recovers.

Meanwhile, the temperature in chambers 1, 2, 3, 5 and 6 remains steady at 37 ˚C. Results are consistent for any opened Cell Locker chamber.

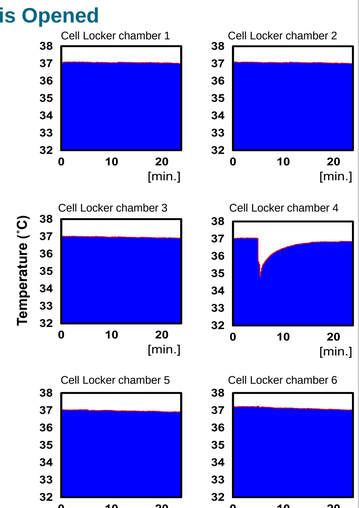


Figure 3. Relative Humidity in Closed Cell Locker Chambers Remains Constant When One is Opened

High relative humidity is critical for sensitive cells because it prevents evaporation of water from growth media. Only water evaporates; the remaining salts, minerals and other nutrients become more concentrated as a result, and can reach concentrations which are toxic for many cell types.

The Cell Locker System speeds humidity recovery, providing improved maintenance of precisely balanced growth media.

When Cell Locker chamber 4 is opened for 30 seconds, the humidity level drops to ambient – here, approximately 30%. But the remaining unopened chambers hold at the incubator set humidity of 93.8% +/- 3%. Results are consistent for any opened Cell Locker chamber.

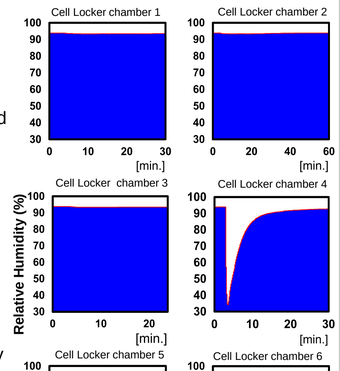


Figure 4. Carbon Dioxide Concentration in Closed Cell Locker Chambers Remains Constant When One is Opened

Carbon dioxide works with the buffer in growth media to maintain stable culture pH, mimicking the vertebrate bloodstream. All cells in the body produce CO_2 as a by-product of metabolism, and in the blood, bicarbonate ions are a native buffer. In the lungs, waste CO_2 is approximately 5%, similar to the concentration commonly used in culture, in concert with 2 mg/ml sodium bicarbonate. When pH drifts too far from physiological 7.4, cells may develop morphological stress indicators such as granules in the cytoplasm, membrane detaching and other changes.[2,3]

When Cell Locker chamber 4 is opened for 30 seconds, the CO_2 level drops rapidly, similar to any CO_2 incubator. The remaining chambers remain at stable CO_2. Results are consistent for any opened Cell Locker chamber.

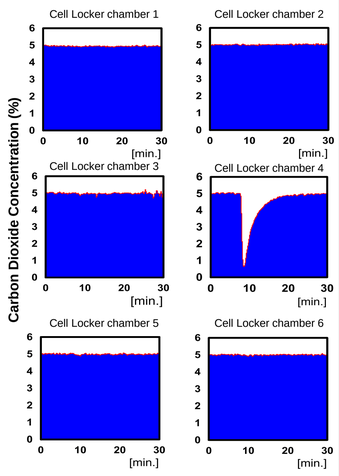


Figure 5. For Hypoxic Culturing, the Cell Locker System Maintains Nearly Stable Oxygen

Many primary cells, stem cells, neurons and other cell types grow faster with fewer stress markers, less differentiation, and longer life when under reduced oxygen, mimicking hypoxic conditions *in vivo*.[4] In the body, oxygen concentration varies from 1-4% in the brain and bone marrow to 12-14% in the bloodstream and lungs. Because oxygen affects nearly every cellular process, differences in oxygen can results in varied cell responses.

At 1% oxygen, when a standard hypoxic incubator door is opened for 30 seconds, the entire chamber rises to 15-16% oxygen, approaching atmospheric oxygen of 21%. But in the Cell Locker System, when one chamber is opened, the unopened chambers only increase about 2%. So cells remain in nearly stable oxygen for more uniform results. Results are consistent for any opened Cell Locker chamber.

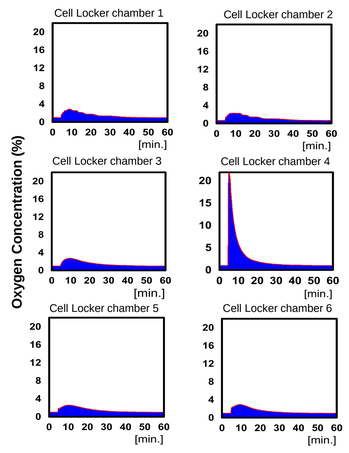


Table 1. The Cell Locker System Reduces CO_2 and N_2 Gas Consumption by Over 50%

	N_2 Gas Consumption Per Day	
Activities Per Day	Standard CO_2 Incubator	Cell Locker System
No Door Opening at 3% O_2	70 L	120 L
One x 30 sec. door opening at 3% O_2	236 L	80 L
Three x 30 sec. door openings at 3% O_2	778 L	360 L
Three x 30 sec. door openings at 5% O_2	548 L	224 L
Three x 30 sec. door openings at 1% O_2	1325 L	840 L
Six x 30 second door openings at 3% O_2	1486 L	600 L

Nitrogen gas is used to reduce oxygen concentration. If the doors are never opened, the Cell Locker System will use slightly more nitrogen gas over time, due to additional gaskets which slowly release gas. But in a typical user scenario with 30-second door openings throughout the day, the Cell Locker System uses only about half as much gas.

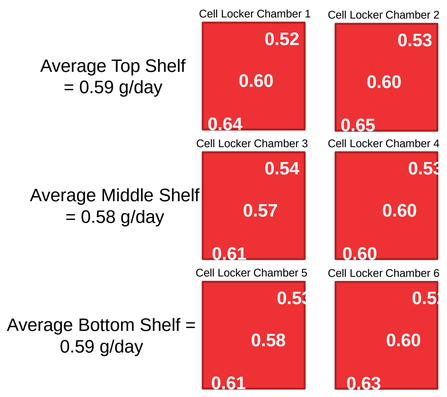
This means that for a typical stem cell research lab, a bottle of nitrogen gas will last twice as long. The effect is most impactful in a hypoxic system. CO_2 gas is also reduced by a similar percentage (results not shown). In the U.S., one canister of nitrogen gas holds 8500 L. At 3% O_2 and 6 door openings/day in the Heracell VIOS incubator, one canister will last 5.7 days. With the Cell Locker System, that canister will last 14.1 days.

Table 2. Cell Locker System Design Prevents Entry of Microorganisms to Closed Cell Locker Chambers

Cell Locker Chamber	Microorganism Concentration					
	Staphylococcus aureus		*Brevunidmomas diminuta*		*Mycoplasma orale*	
	ATCC 6538		ATCC 19144		DSM 25590	
	9.6 x 10^4 CFU		1.6 x 10^5 CFU		9.3 x 10^4 CFU	
Location	Inside	Outside Top	Inside	Outside Top	Inside	Outside Top
Top Left	0	TNTC	0	78	0	TNTC
Top Right	0	TNTC	0	75	0	TNTC
Middle Left	0	TNTC	0	112	0	TNTC
Middle Right	0	TNTC	0	111	0	TNTC
Bottom Left	0	TNTC	0	36	0	TNTC
Bottom Right	0	TNTC	0	37	0	TNTC

Results are summaries of 3 tests for each microorganism. Open sterile agar plates were placed inside or on top of each closed Cell Locker chamber. Active cultures were circulated inside a closed Cell Locker System using a nebulizer. Concentrations and results from plate counts are averages over the 3 tests. CFU = colony forming unit. TNTC = too numerous to count (>300). For *S. aureus* and *M. orale,* 8 plates and for *B. diminuta*, 1 plate were placed on top of each chamber. Each chamber contained 4 plates.

Figure 6. Evaporation is Reduced by 50% in the Cell Locker System



When water evaporates, salts, nutrients and other additives in growth media can reach concentrations which are toxic to cultured cells. Limiting evaporation is especially critical in small volume assays such as those in microwell plates, organ-on-a-chip cultures, and microfluidic systems.

Three plates with 15 g water were placed in each chamber and after 24 hrs, weighed. Heracell VIOS CO_2 incubators show an average of 1.3 g/day evaporation in similar tests. But the Cell Locker System provides even more stable high humidity, reducing evaporation over a 24 hour period by >50%, with an evaporation rate average of 0.58 g/day.

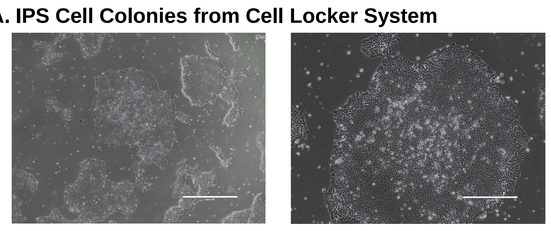
Table 3. IPS Cells had the Same Quality Following Culture in the Cell Locker System Compared to an Optimized Incubator

IPS Cells	Cell Locker System	Forma 3130 Incubator
Colonies Observed	> 50	>50
Spots of differentiation	None	None

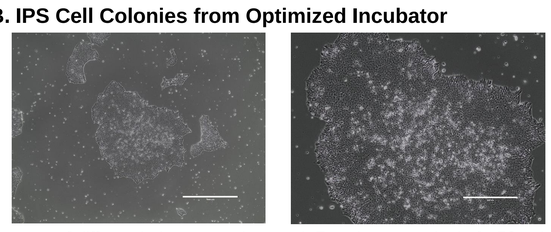
Results are a summary of IPS cells derived from 4 different donors, split into two populations and grown in parallel either in a Cell Locker System or in a Thermo Scientific Forma 3130 water jacketed incubator. Procedures were developed in the 3130. All parallel cultures grew at the same rate, and all passages were performed on the same day. Following cryopreservation and thawing, cells were re-plated and assessed for quality.

Figure 7. Typical IPS Cell Colonies Show the Same Quality Following Cryopreservation and Regrowth in the Cell Locker System and in an Optimized Incubator

A. IPS Cell Colonies from Cell Locker System



B. IPS Cell Colonies from Optimized Incubator



No spots of differentiation were observed. Results are typical of >50 colonies observed for each culture type.

CONCLUSIONS

The Thermo Scientific Cell Locker System represents a novel approach to providing improved culturing conditions for sensitive cell types such as primary cells, stem cells, and more. This patented design maintains desired conditions so that cells spend more time in an environment that mimics physiological conditions. Under such conditions, cultured cells will provide responses that better model those in the intact organism, for better predictions of human responses for drug and disease modeling for studies in regenerative medicine, cell therapy, oncology, neuroscience, immunology and more.

Compared to a Thermo Scientific CO_2 incubator which itself has outstanding conditions, the Cell Locker System remains more stable, recovers faster, shows 50% less evaporation and uses 50% less gas even under hypoxic conditions. Microorganisms are prevented from entering closed Cell Locker chambers. Stem cells showed the same ideal characteristics in the Cell Locker System as in an optimized traditional incubator.

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