

Est. 2001

Kevin Murray

Sales Director

BMP

Better Manufacturing Practice

For Cell Therapies

BioSpherix

Est. 1982

cGMP

Good Manufacturing Practices

= QUALITY



GMP Xvivo Barrier Isolator System – a practical and economical alternative to cleanrooms for cGMP compliant production of human cells for clinical use.

CytoCentric Barrier Isolator

- **PROTECT CELLS (aseptic)**
- **PROTECT PEOPLE (virus, vector, prion)**
- **OPTIMUM CELL ENVIRONMENT (temperature, R.H., CO_2, O_2, etc.)**
- **P.A.T. READY (Process Analytic Technology, quality-by-design)**

↓ RISK = ↑ TRANSLATION

GMP XVIVO Barrier Isolator

Superior Process Control

1. Uninterruptible Conditions (Full time optimization)
2. Dynamic incubation options

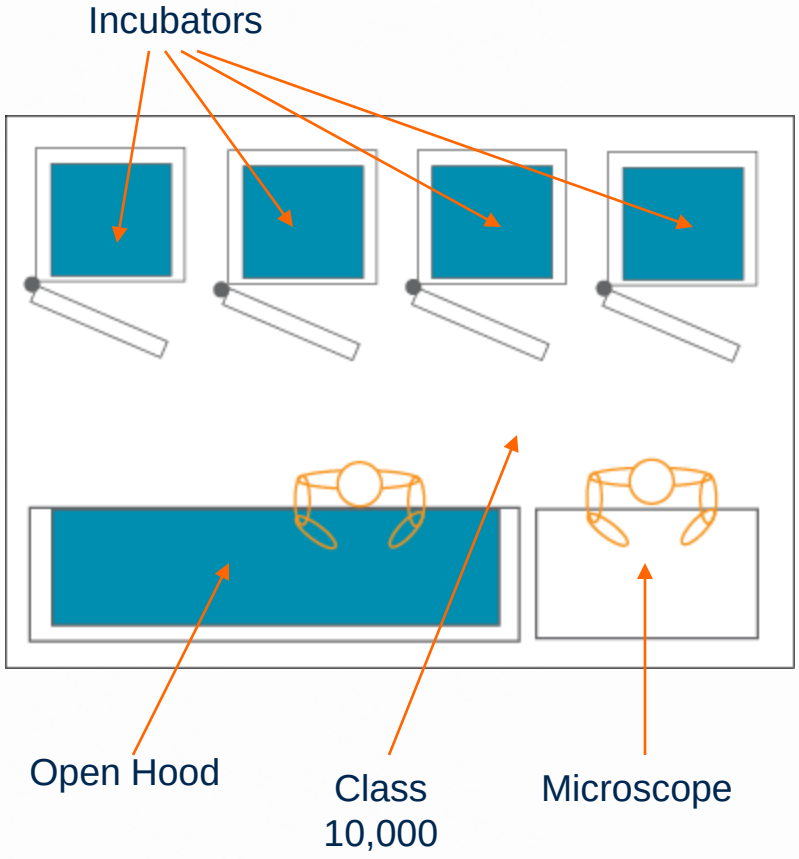
GMP XVIVO Barrier Isolator

1. Uninterruptible Conditions

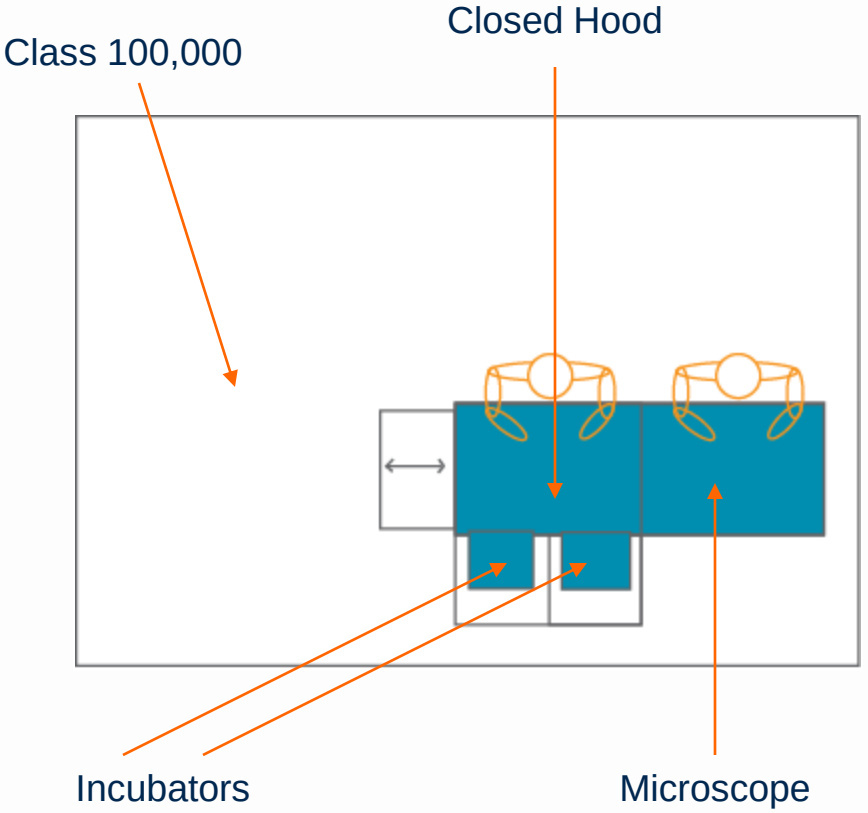
- No exposure to sub-optimal cell conditions
- Higher quality cell product
- More consistent results – for entire process
- Continuously monitor and control the cell production from start to finish
- No temperature, pH, O_2 or CO_2 shocks, resulting from carrying your cultures across the room from the incubator to the open hood

Comparison

Conventional Cleanroom

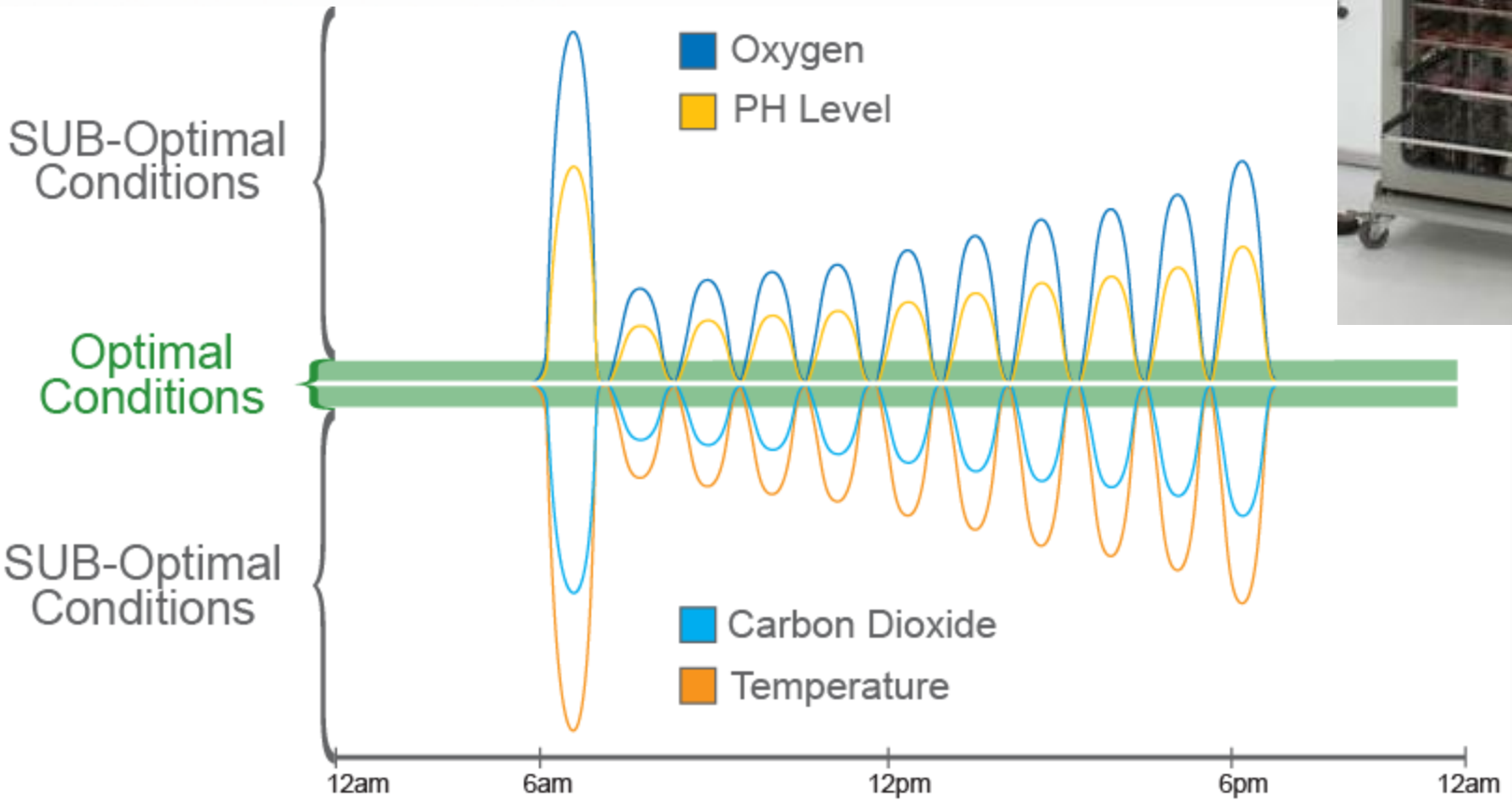


GMP XVIVO Barrier Isolator



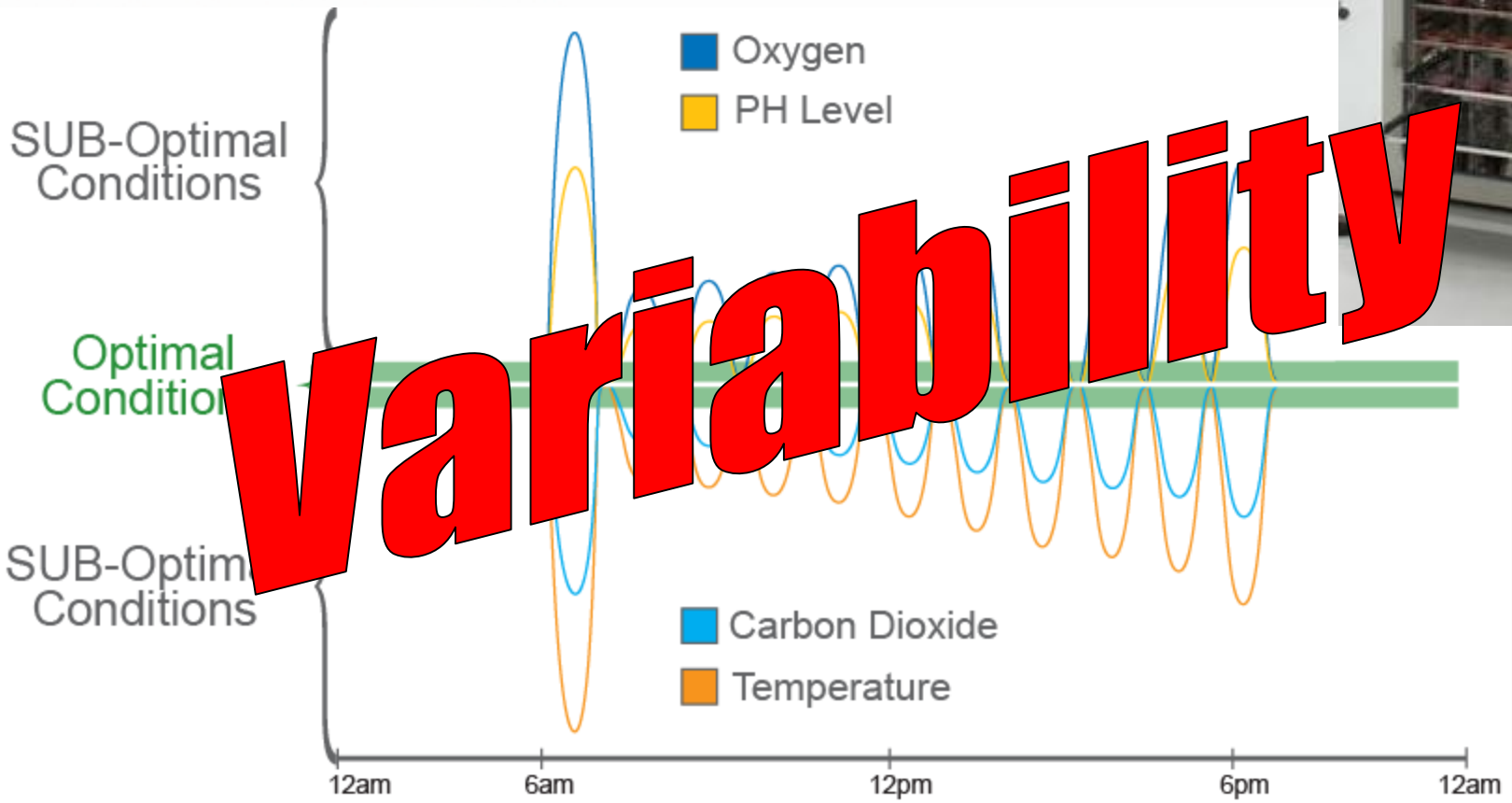
Cleanroom Incubator





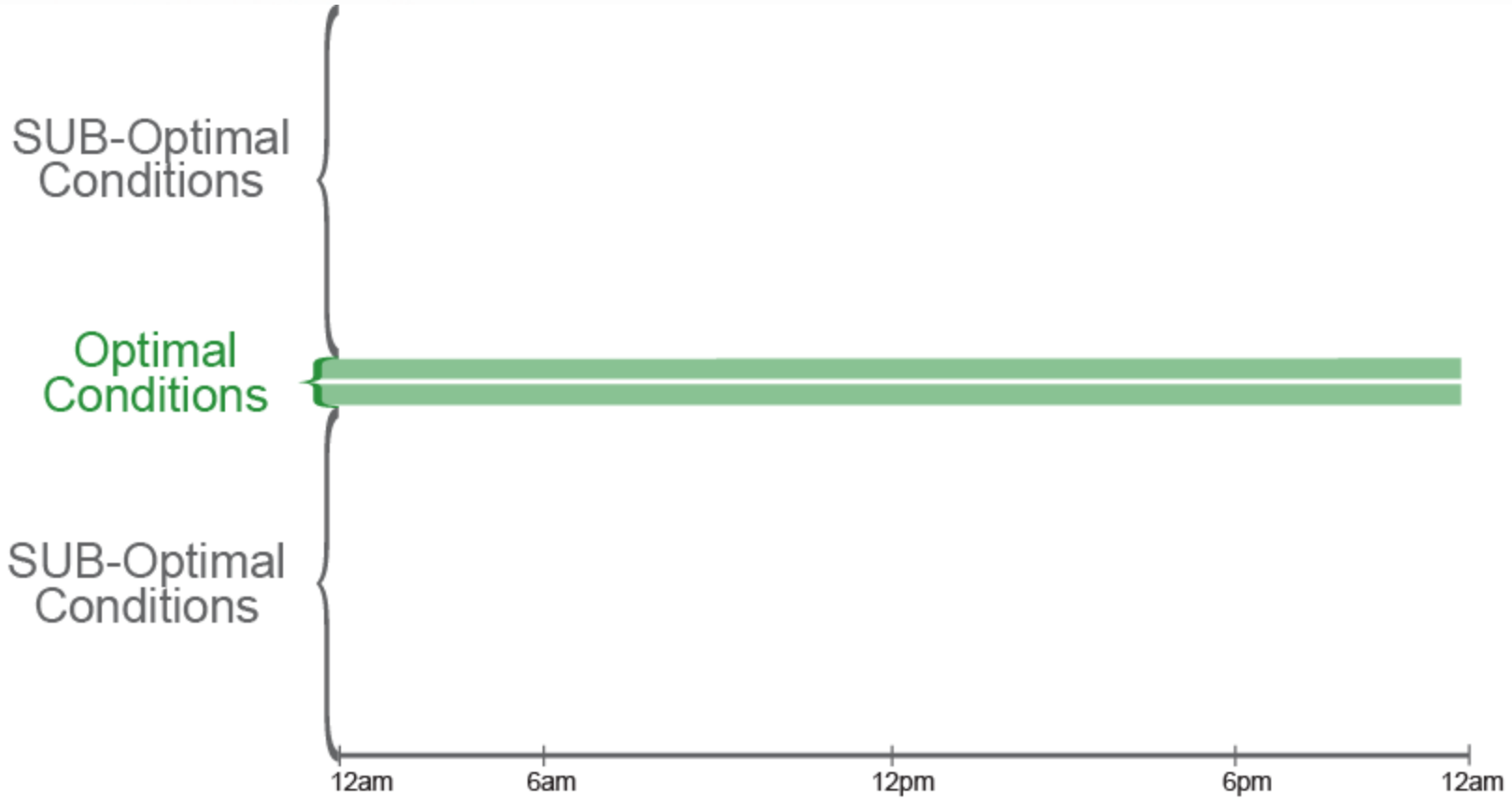
Cleanroom Incubator





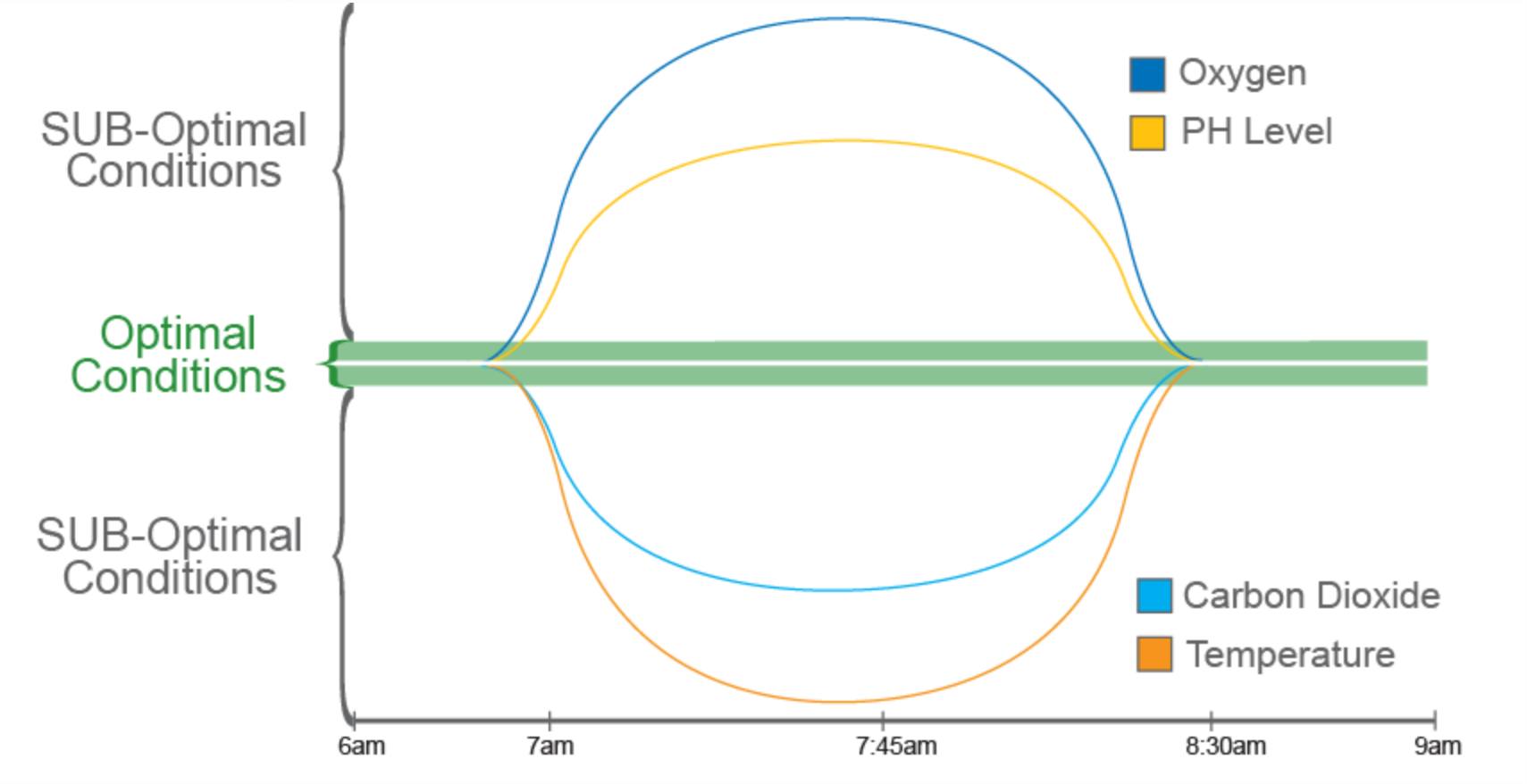
Variability

GMP Xvivo - *Incubator*

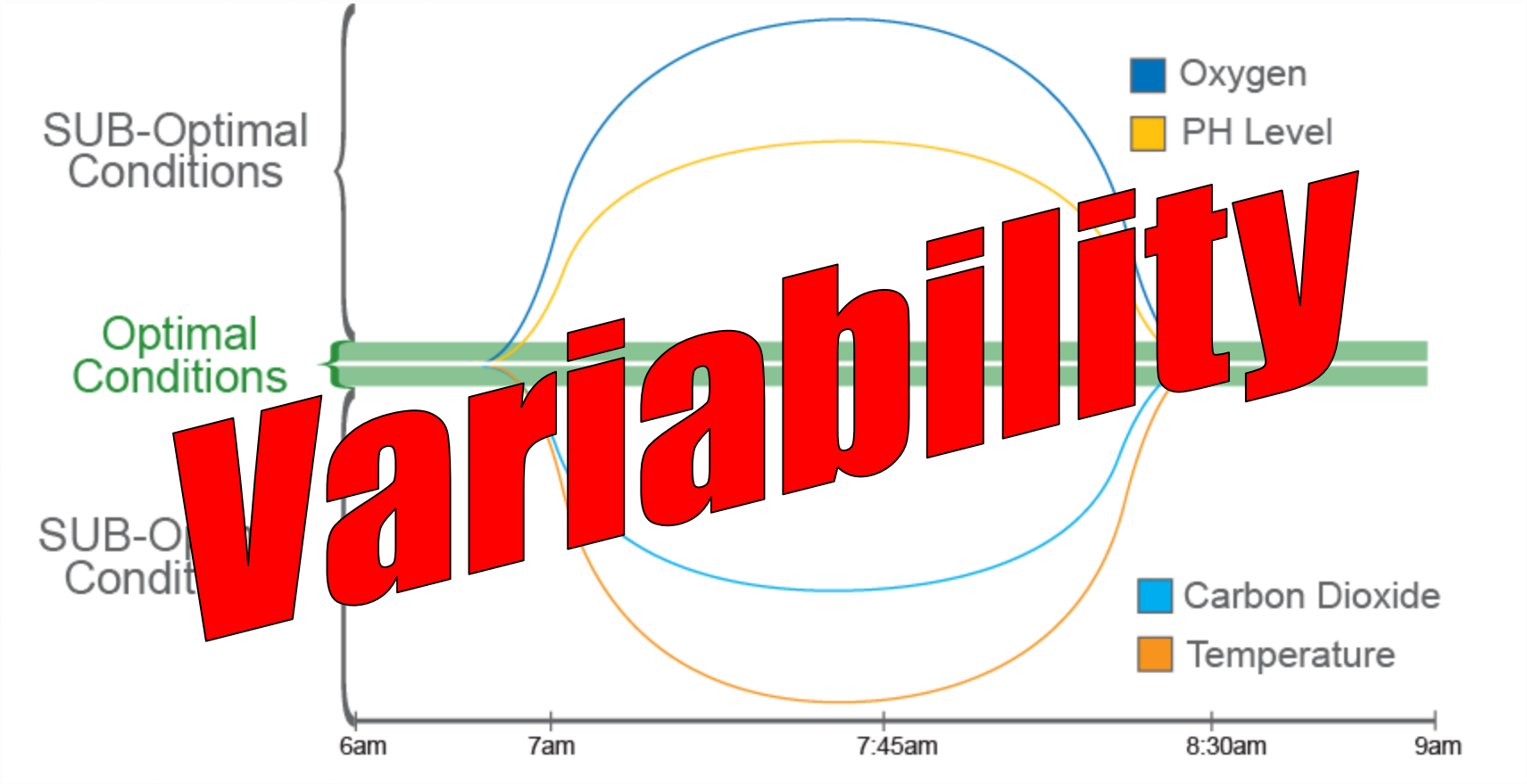


Incubator opens into optimized and aseptic workspace

Cleanroom Hood

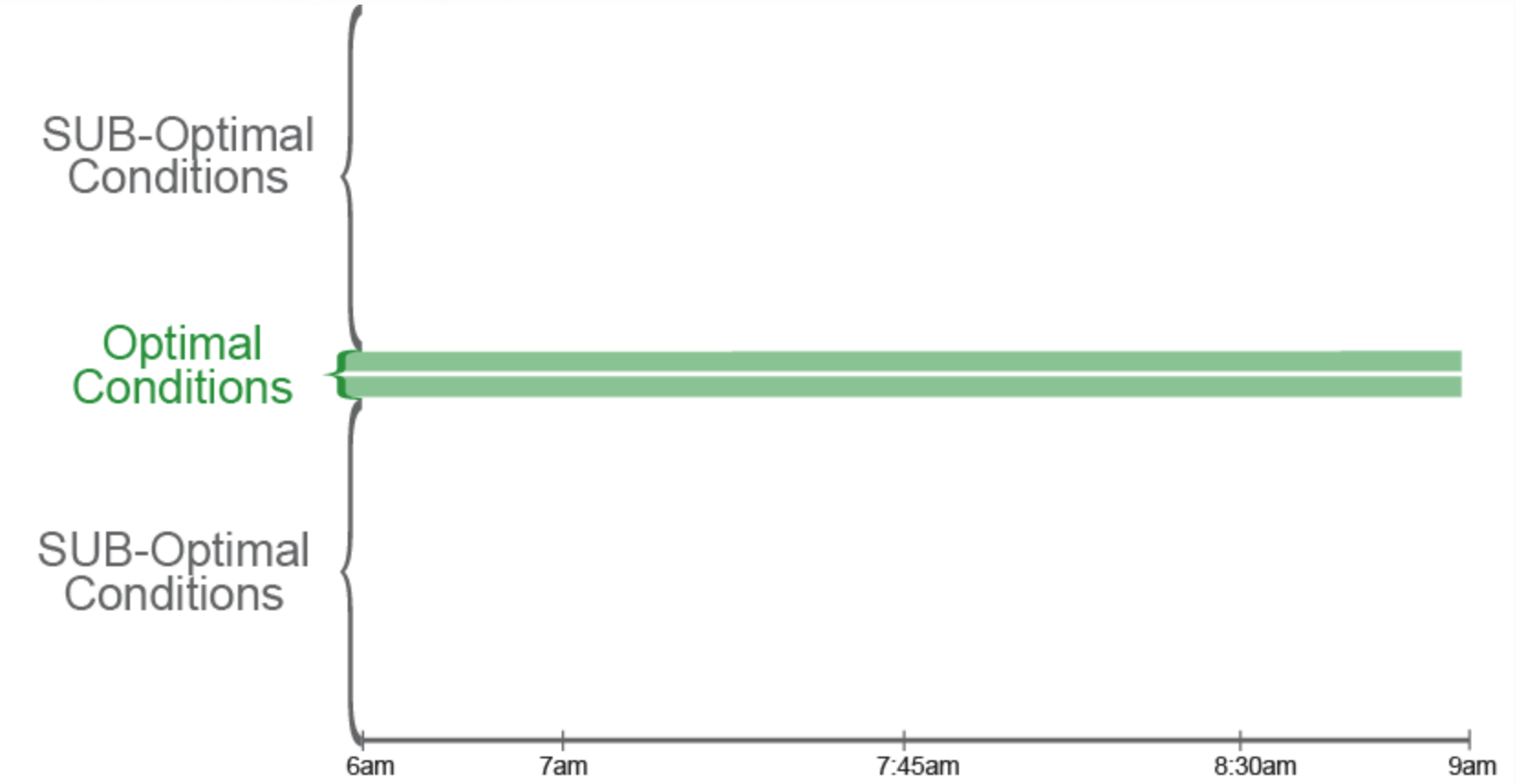


Cleanroom Hood



45 minute manipulation – 1 culture

GMP Xvivo – *Closed Hood*



Cell processing is done in optimized and aseptic workspace





Cleanroom Hood

BioSpherix Hood



Regulatory Guidance

- **"Systems that promote greater product and process understanding can provide a high assurance of quality on every batch and provide alternative, effective mechanisms to demonstrate validation."**

  (FDA's Guidance for Industry: PAT – A framework for Innovative Pharmaceutical Development, Manufacturing, and Quality Assurance; Sept. 2004)

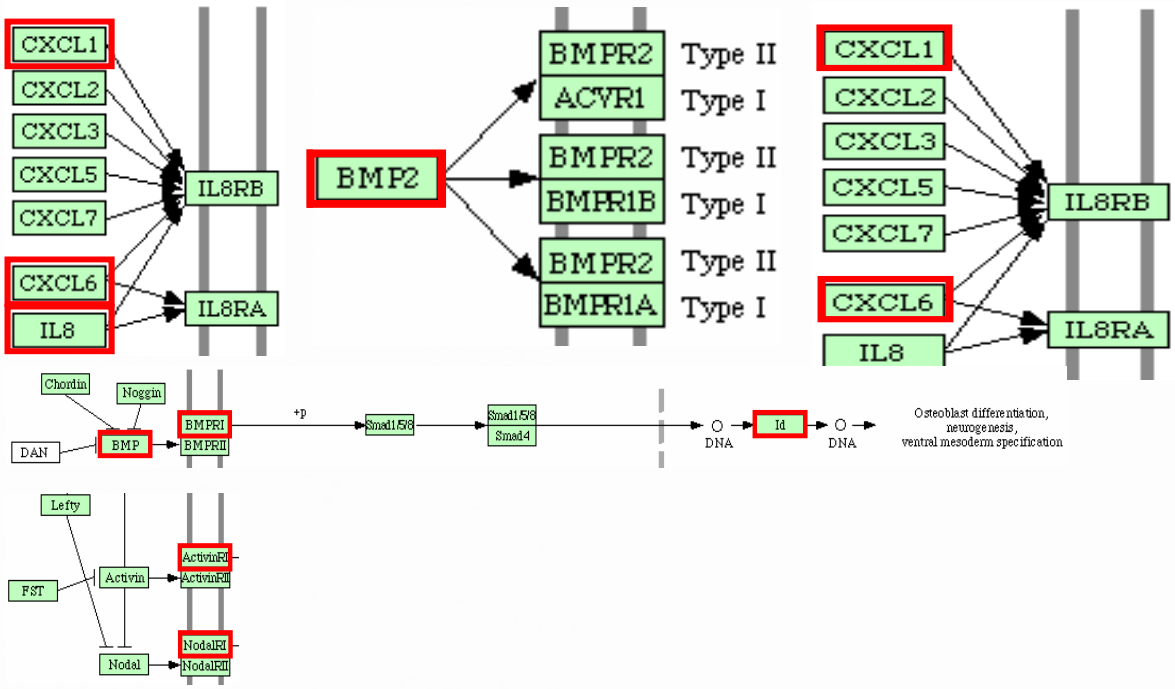

PC Control : Process Chamber Control
Nick
11/02/2010 10:28:04
Communication Ok
No Alarms
Back
Process Chamber 1 Control
Particle Sensor ON
Light ON
Infusion EMO SetPoint
175 Pa.
OK
Pump ON
Chart
Alarms Setup
Dilution Factor
CRAC ON
Vacuum EMO SetPoint
-200 Pa.
OK
Process
Set Point
OutPut Up
OutPut Down
Mode
Control
O2
19.8 %
20.0 %
8.9 %
0.0 %
Auto
ON
Tuning
Calibration
CO2
5.0 %
5.0 %
0.8 %
0.0 %
Auto
ON
Tuning
Calibration
RH
23 %
23.0 %
N/A %
0.0 %
N/A
ON
Tuning
Calibration
Chamber Pressure
86 Pa.
80 Pa.
N/A %
0.0 %
Auto
ON
Tuning
Calibration
S.S. Pressure
1190 Pa.
N/A Pa.
N/A %
N/A %
N/A
Tuning
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VOC
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N/A %
0.0 %
N/A
ON
Tuning
Calibration
CLO2
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N/A %
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Tuning
Calibration
Particles
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N/A /m3
N/A %
N/A %
N/A
Tuning
Calibration
P.C. TEMP
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26.0 'C
9.6 %
N/A %
Auto
ON
Tuning
Calibration
Floor 1 Temp
36.4 'C
37.0 'C
6.5 %
N/A %
Auto
ON
Tuning
Calibration


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Chamber Pressure
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CLO2
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Tuning
Calibration
Particles
0/m3
N/A /m3
N/A %
N/A %
N/A
Tuning
Calibration
P.C. TEMP
25.5 'C
26.0 'C
9.6 %
N/A %
Auto
ON
Tuning
Calibration
Floor 1 Temp
36.4 'C
37.0 'C
6.5 %
N/A %
Auto
ON
Tuning
Calibration
Quality by Design

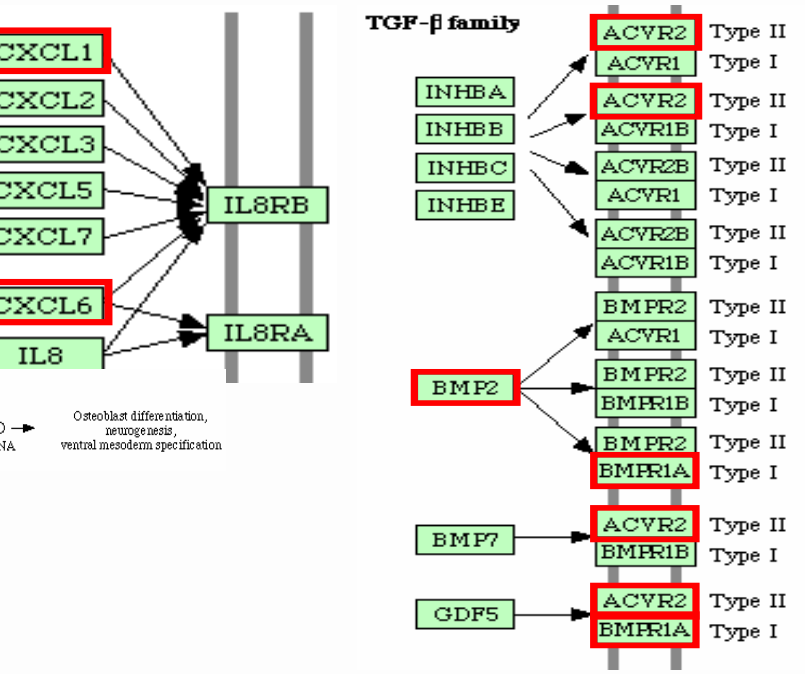
Oxygen and Stem Cells – hMSC(6)

Down-regulation in 2% UI and 2% UI + Workstation of genes associated with TGF-β, BMP2, IL8RA and IL8RB signaling. (KEGG pathways).

2% UI



2% UI + Workstation

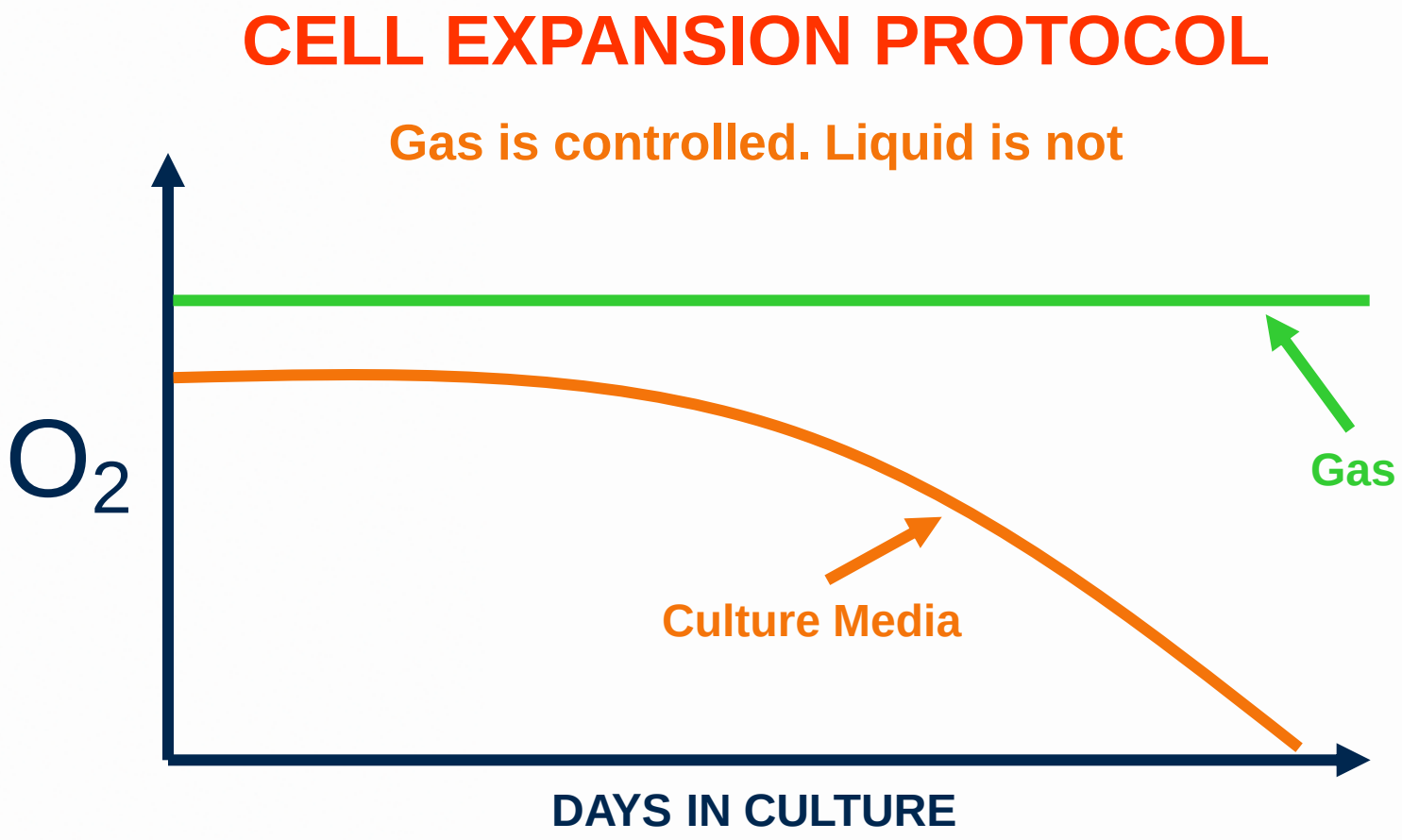


Minor environmental changes in O2 induce long-lasting transcriptional alterations (Nick Forsyth – Keele University ISSCR 2010)

2. Dynamic incubation options

- Cell cultures are dynamic in nature
- Incubation conditions need to adjust to keep up with these dynamic cultures
- This capability is unique to the Xvivo Barrier Isolator

Conventional Incubator

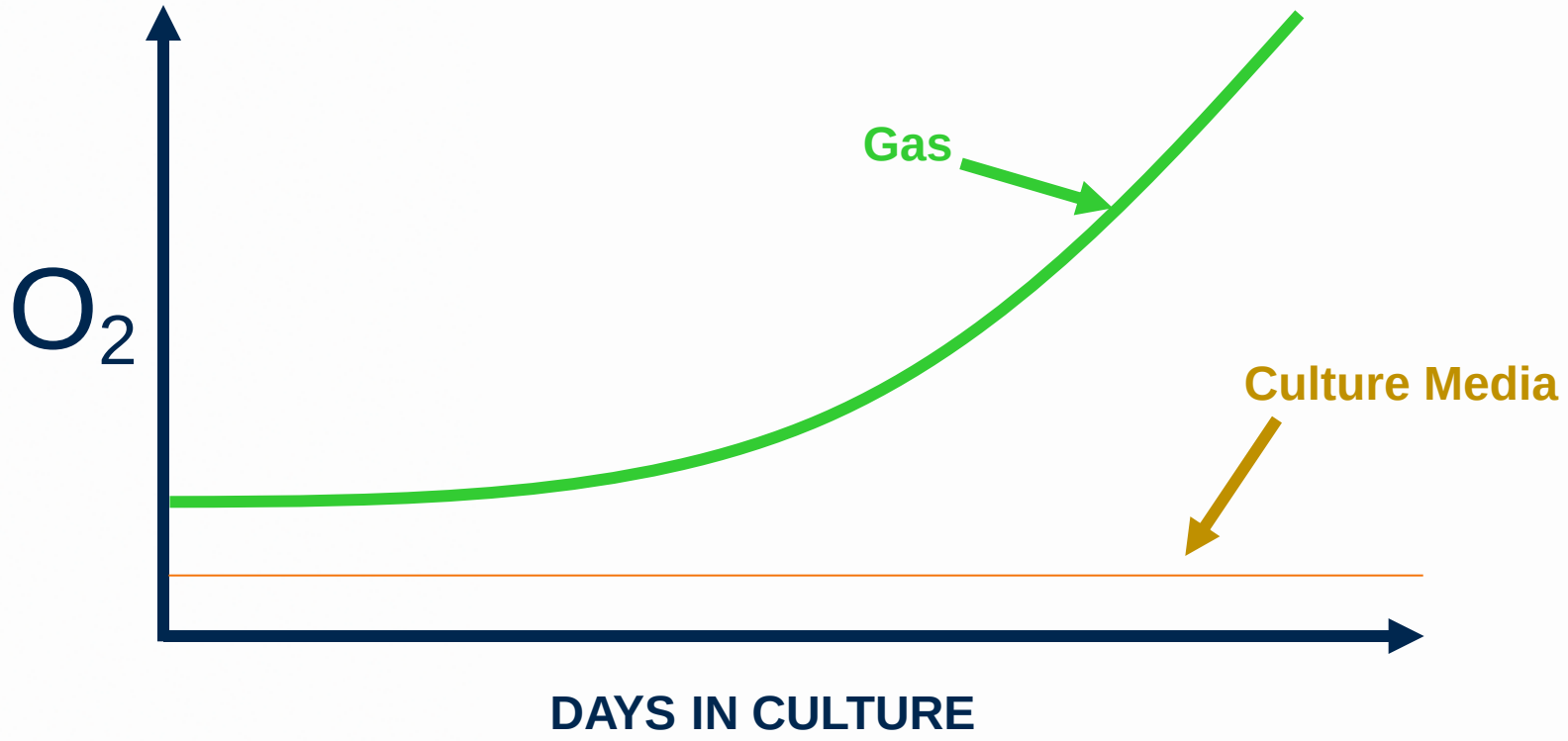


GMP XVIVO Barrier Isolator

XVIVO DYNAMIC OXYGEN

CELL EXPANSION PROTOCOL

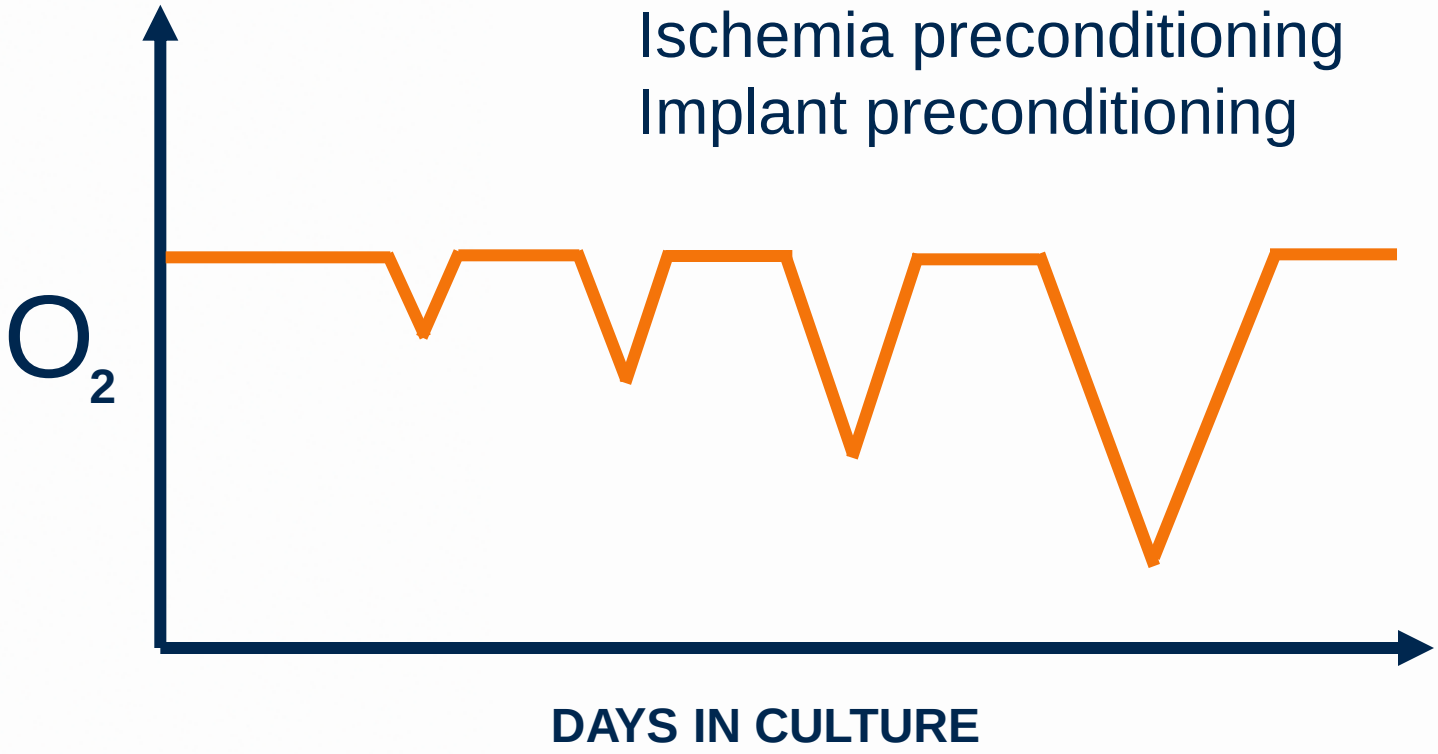
Gas is controlled. Liquid is controlled.



GMP XVIVO Barrier Isolator

XVIVO DYNAMIC OXYGEN

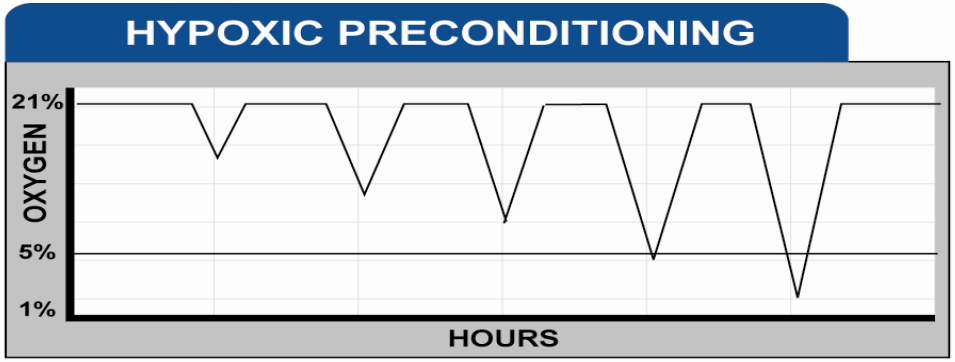
Hypoxia preconditioning
Ischemia preconditioning
Implant preconditioning

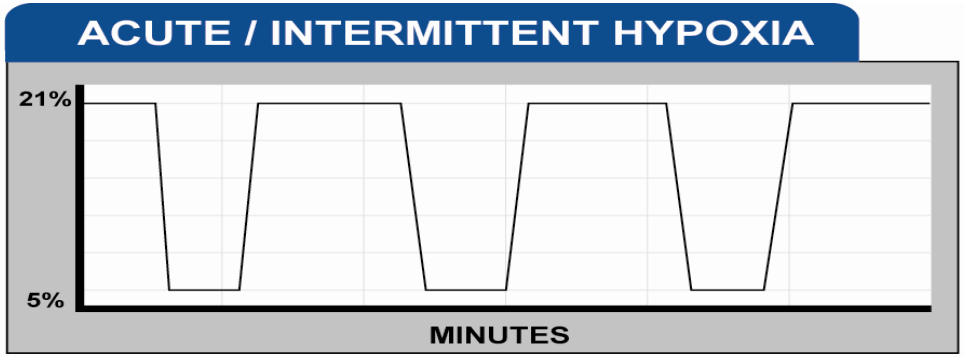


Benefits of Preconditioning Cells

- Cells cultured and processed in room air conditions adjust to a 21% O2 environment
- Injecting these cells into a low O2 environment (<5%) causes cell shock and death
- Preconditioning cells prior to injection into this low O2 environment will enhance engraftment and viability

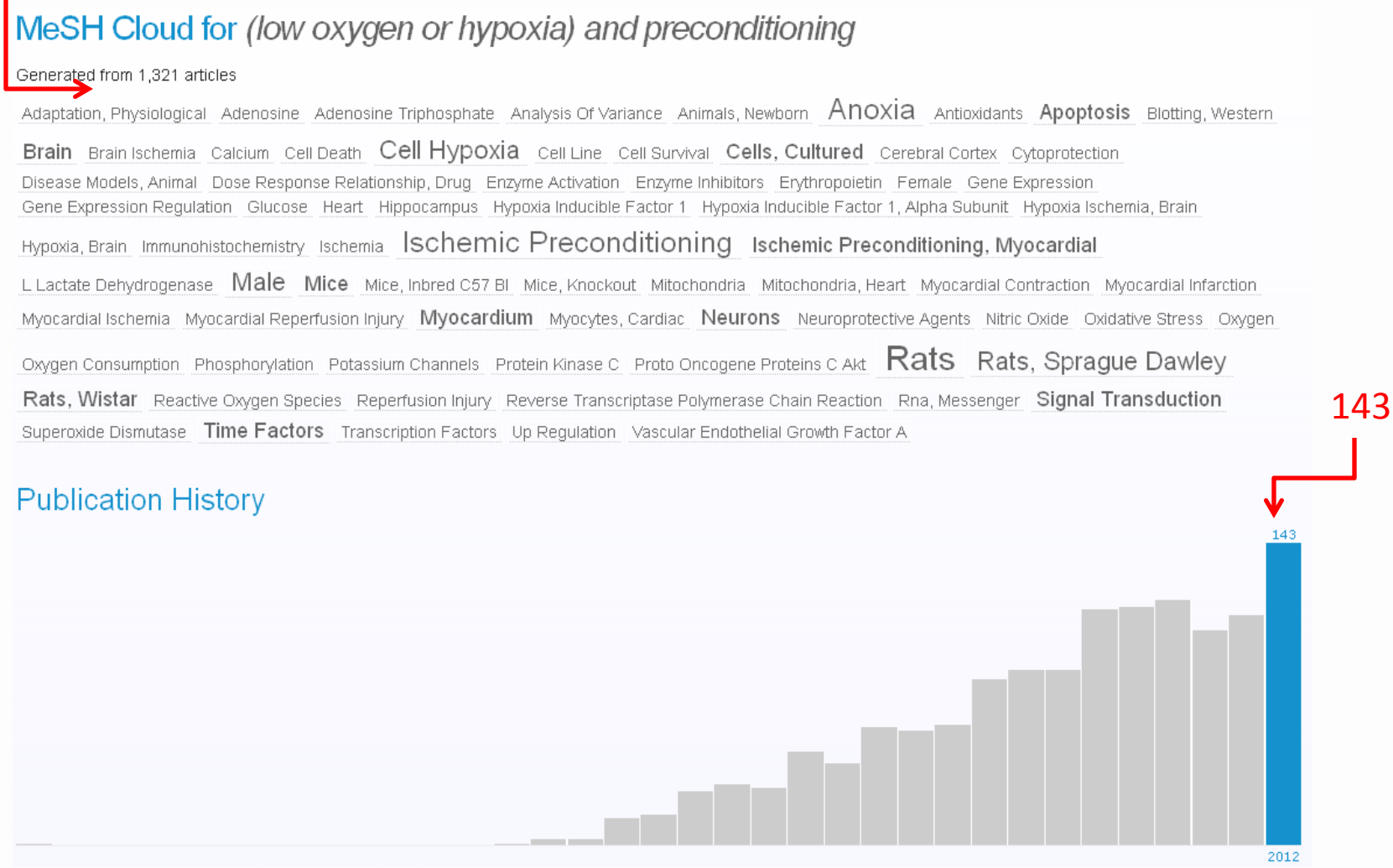
Benefits of Preconditioning Cells





Benefits of Preconditioning Cells

1321 Articles



Benefits of Preconditioning Cells

PubMed Results
Items 1 – 12 of 21 (Display the 21 citations in PubMed)

1. **Preconditioning mesenchymal stem cells with caspase inhibition and hyperoxia prior to hypoxia exposure decreases apoptosis and increases cell survival.**
Saini U, Gumina RJ, Wolfe B, Kuppusamy ML, Kuppusamy P, Boudoulas KD.
J Cell Biochem. 2013 Jun 21.

2. **Hypoxic preconditioning with cobalt of bone marrow mesenchymal stem cells improves cell migration and enhances therapy for treatment of ischemic acute kidney injury.**
Yu X, Lu C, Liu H, Rao S, Cai J, Liu S, Kriegel AJ, Greene AS, Liang M, Ding X.
PLoS One. 2013 May 9;8(5):

Effects of in vitro low oxygen tension preconditioning of adipose stromal cells on their in vivo chondrogenic potential: application in cartilage tissue repair.
Portron S, Merceron C, Gauthier O, Lesoeur J, Sourice S, Masson M, Fellah BH, Geffroy O, Lallemand E, Weiss P, Guicheux J, Vinatier C.
PLoS One. 2013 Apr 30;8(4)

4. **Noninvasive imaging of myocyte apoptosis following application of a stem cell-engineered delivery platform to acutely infarcted myocardium.**
Godier-Furnémont AF, Tekabe Y, Kollaros M, Eng G, Morales A, Vunjak-Novakovic G, Johnson LL.
J Nucl Med. 2013 Jun;54(6):977-83.

5. **Mesenchymal stem cells: promising for myocardial regeneration?**
E, Harmsen MC.
Curr Stem Cell Res Ther. 2013 Jul;8(4):270-7.

6. **Hypoxia-preconditioned mesenchymal stromal cells improve cardiac function in a swine model of chronic myocardial ischaemia.**
Jaussaud J, Biais M, Calderon J, Chevaleyre J, Duchez P, Ivanovic Z, Couffinhal T, Barandon L.
Eur J Cardiothorac Surg. 2013 May;43(5):1050-7

Benefits of Preconditioning Cells

7. **Preconditioning of stem cells by oxytocin to improve their therapeutic potential.**
Noiseux N, Borie M, Desnoyers A, Menaouar A, Stevens LM, Mansour S, Danalache BA, Roy DC, Jankowski M, Gutkowska J.
Endocrinology. 2012 Nov;153(11):5361-72.

8. **Apelin 13: a novel approach to enhance efficacy of hypoxic preconditioned mesenchymal stem cells for cell therapy of diabetes.**
Mottaghi S, Larijani B, Sharifi AM.
Med Hypotheses. 2012 Dec;79(6):717-8

9. **Hypoxic preconditioning enhances the therapeutic potential of the secretome from cultured human mesenchymal stem cells in experimental traumatic brain injury.**
Chang CP, Chio CC, Cheong CU, Chao CM, Cheng BC, Lin MT.
Clin Sci (Lond). 2013 Feb;124(3)

10. **Stem cell-based delivery of Hypoxamir-210 to the infarcted heart: implications on stem cell survival and preservation of infarcted heart function.**
Kim HW, Jiang S, Ashraf M, Haider KH.
J Mol Med (Berl). 2012 Sep;90(9):997-1010

11. **The role of SDF-1-CXCR4/CXCR7 axis in the therapeutic effects of hypoxia-preconditioned mesenchymal stem cells for renal ischemia/reperfusion injury.**
Liu H, Liu S, Li Y, Wang X, Xue W, Ge G, Luo X.
PLoS One. 2012;7(4)

12. **Concomitant activation of miR-107/PDCD10 and Hypoxamir-210/Casp8ap2 and their role in cytoprotection during ischemic preconditioning of stem cells.**
Kim HW, Mallick F, Durrani S, Ashraf M, Jiang S, Haider KH.
Antioxid Redox Signal. 2012 Oct 15;17(8):1053-65

Benefits of Preconditioning Cells

Curr Stem Cell Res Ther. 2013 Jul;8(4):270-7.

Mesenchymal stem cells: promising for myocardial regeneration?

Przybyt E, Harmsen MC.

University of Groningen, University Medical Center Groningen, Department of Pathology and Medical Biology, Cardiovascular Regenerative Medicine Research Group, Hanzeplein 1 (EA11), 9713 GZ Groningen, The Netherlands.

Abstract

The pandemic of cardiovascular disease is continuously expanding as the result of changing life styles and diets throughout the Old and New World. Immediate intervention therapy saves the lives of many patients after acute myocardial infarction (MI). However, for many this comes at the price of adverse cardiac remodeling and heart failure. Currently, no conventional therapy can prevent the negative aftermath of MI and alternative treatments are warranted. Therefore, cardiac stem cell therapy has been put forward over the past decade, albeit with modest successes. Mesenchymal Stem Cells (MSC) are promising because these are genuine cellular factories of a host of secreted therapeutic factors. MSC are obtained from bone marrow or adipose tissue (ADSC). However, the heart itself also contains mesenchymal- like stem cells, though more difficult to acquire than ADSC. Interestingly, mesenchymal cells such as fibroblasts can be directly or indirectly reprogrammed to all myocardial cell types that require replacement after MI. To date, the paracrine and juxtacrine mechanisms of ADSC and other MSC on vessel formation are best understood. The preconditioning of, otherwise naive, stem cells is gaining more interest: previously presumed deleterious stimuli such as hypoxia and inflammation, i.e. causes of myocardial damage, have the opposite effect on mesenchymal stem cells. ***MSC gain a higher therapeutic capacity under hypoxia*** and inflammatory conditions. In this review, mesenchymal stem cells and their working mechanisms are put into the perspective of clinical cardiac stem cell therapy.

Benefits of Preconditioning Cells

J Cell Biochem 2013 Jun 21. doi: 10.1002/jcb.24609. [Epub ahead of print]

Preconditioning mesenchymal stem cells with caspase inhibition and hyperoxia prior to hypoxia exposure decreases apoptosis and increases cell survival.

Saini U, Gumina RJ, Wolfe B, Kuppusamy ML, Kuppusamy P, Boudoulas KD.
Department of Medicine, Division of Cardiovascular Medicine, and The Dorothy M. Davis Heart and Lung Research Institute, The Ohio State University, Columbus, Ohio.

Abstract

Myocardial infarction is a leading cause of mortality and morbidity worldwide. Occlusion of a coronary artery produces ischemia and myocardial necrosis that leads to left ventricular (LV) remodeling, dysfunction and heart failure. Stem cell therapy may decrease infarct size and improve LV function; the hypoxic environment, however, following a myocardial infarction may result in apoptosis, which in turn decreases survival of transplanted stem cells. Therefore, the effects of preconditioning mesenchymal stem cells (MSC) with hyperoxia (100% oxygen), Z-VAD-FMK pan-caspase inhibitor (CI), or both in a hypoxic environment in order to mimic conditions seen in cardiac tissue post-myocardial infarction were studied in vitro. MSCs preconditioned with hyperoxia or CI significantly decreased apoptosis as suggested by TUNEL assay and Annexin V analysis using fluorescence assisted cell sorting. These effects were more profound when both, hyperoxia and CI, were used. Additionally, gene and protein expression of caspases 1, 3, 6, 7 and 9 were down-regulated significantly in MSCs preconditioned with hyperoxia, CI, or both, while the survival markers Akt1, NFκB, and Bcl-2 were significantly increased in preconditioned MSCs. **These changes ultimately resulted in a significant increase in MSC proliferation in hypoxic environment as determined by BrdU assays compared to MSCs without preconditioning. These effects may prove to be of great clinical significance when transplanting stem cells into the hypoxic myocardium of post-myocardial infarction patients in order to attenuate LV remodeling and improve LV function**.

Benefits of Preconditioning Cells

Eur J Cardiothorac Surg. 2012 Oct 25. [Epub ahead of print]

Hypoxia-preconditioned mesenchymal stromal cells improve cardiac function in a swine model of chronic myocardial ischaemia.

Jaussaud J, Biais M, Calderon J, Chevaleyre J, Duchez P, Ivanovic Z, Couffinhal T, Barandon L.
Adaptation cardiovasculaire à lischémie, INSERM, Pessac, France.

CONCLUSION:

MSC engraftment with hypoxic preconditioning significantly improves capillary density and cell survival, resulting in improvement in global, regional and diastolic left ventricular functions. This highlights the therapeutic potential of transplanting hypoxic-preconditioned MSC in the setting of chronic ischaemic heart failure.

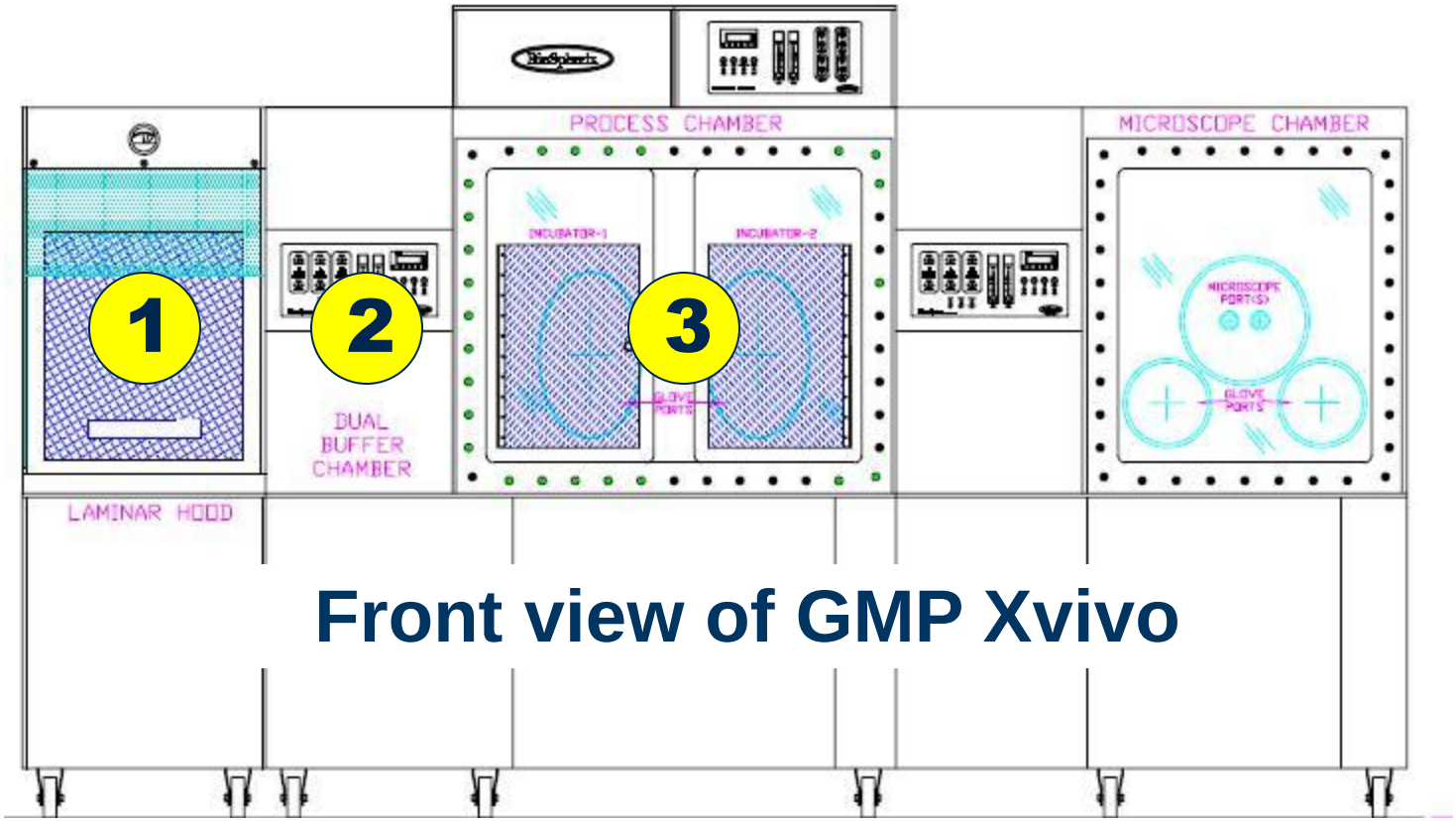


GMP Xvivo Barrier Isolator - Features

1- **Laminar Flow Hood:** A staging area for bringing all items in and out of the system. All items are wiped down before they enter the buffer chamber / air-lock (2).

2- **Buffer Chambers (air-lock):** All outside air is removed via log reduction and dilution factoring. Dual chambers separate product and waste. Outside air never enters processing or incubation areas (3).

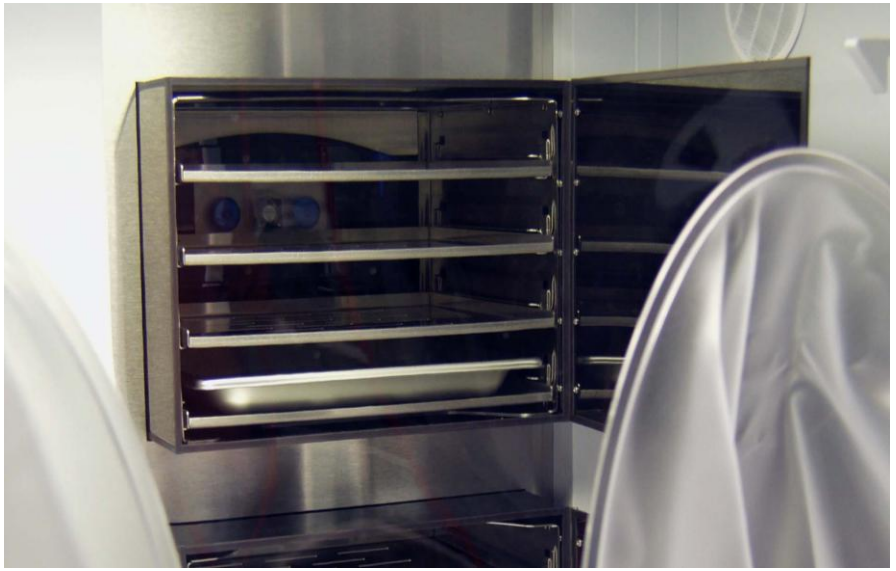
3- **Processing Chamber:** Class 100 – ISO 5 inside entire system. Absolute hepa filtration with approx. 70 air changes per hour. Total control of pressure, particles, VOCs, O2, CO2, temp. and reduced humidity are just some of the options.



Front view of GMP Xvivo

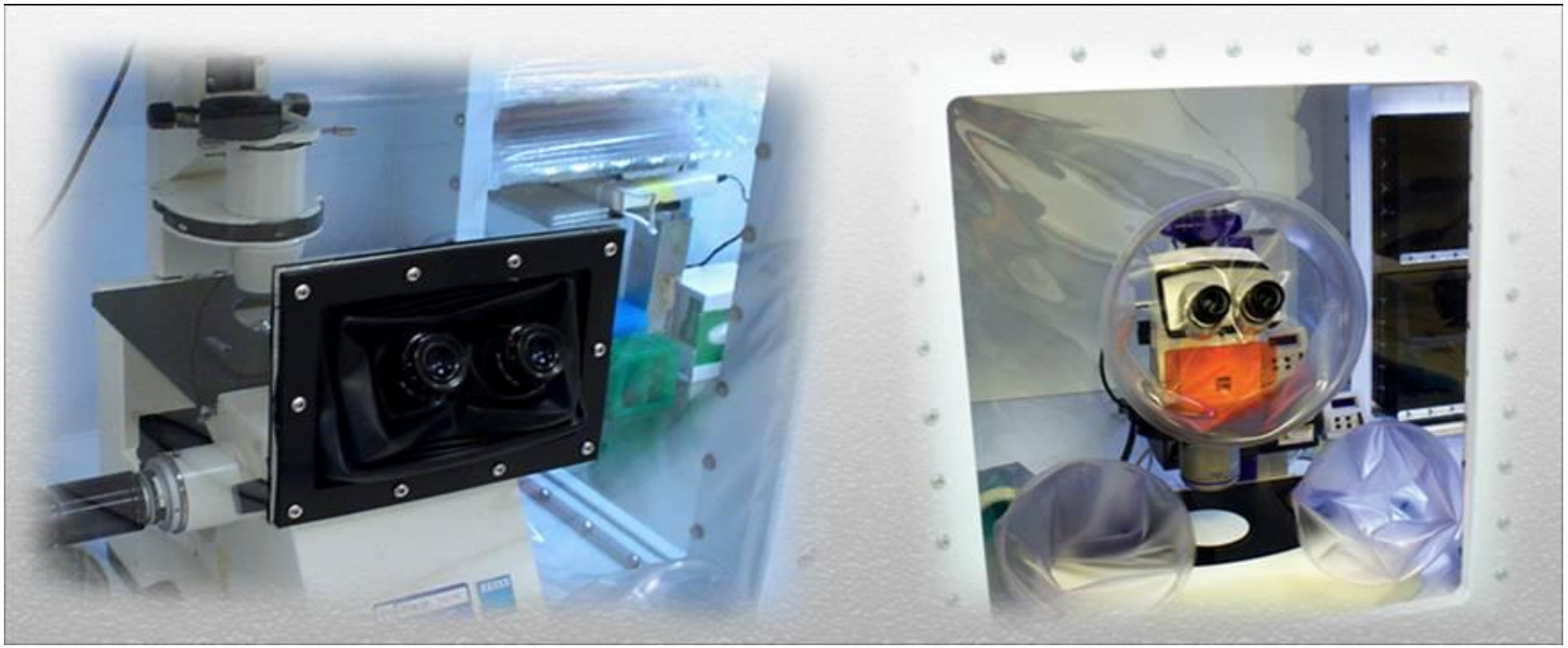







SANYO

Incorporate ANY third party equipment:



- Microscopes
- Centrifuges
- Cell Sorters
- Automated Robotics
- Flow Cytometers
- Bio-reactors

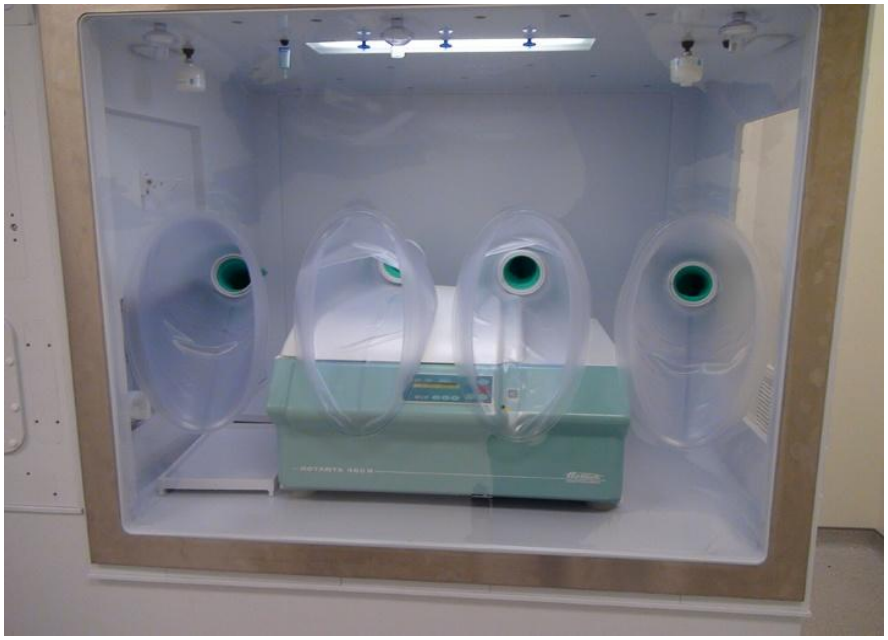




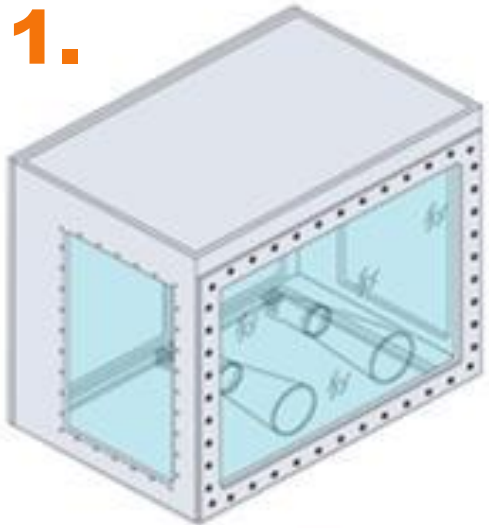


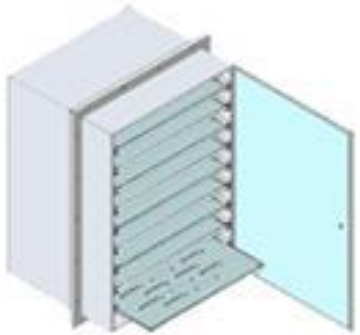


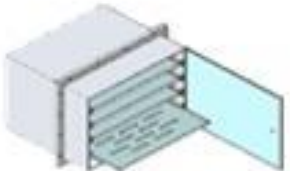


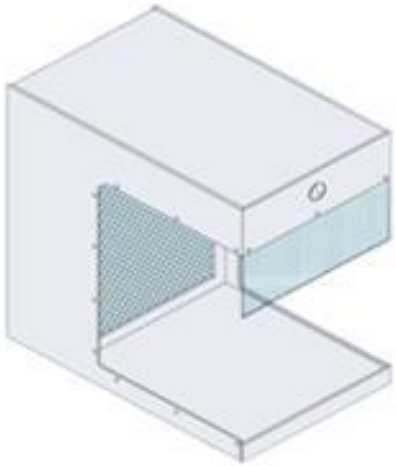


1.





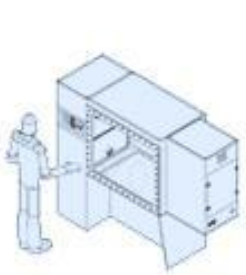


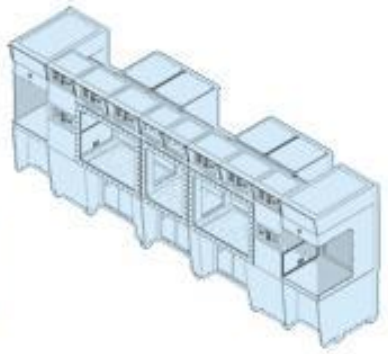


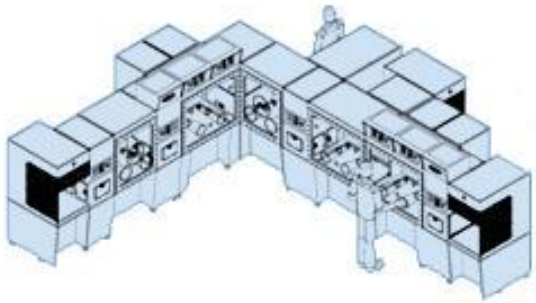
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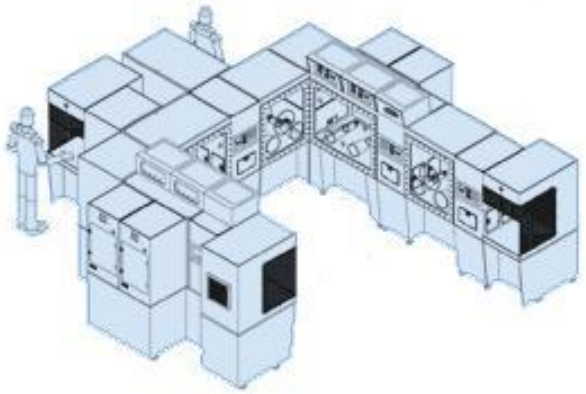


3.







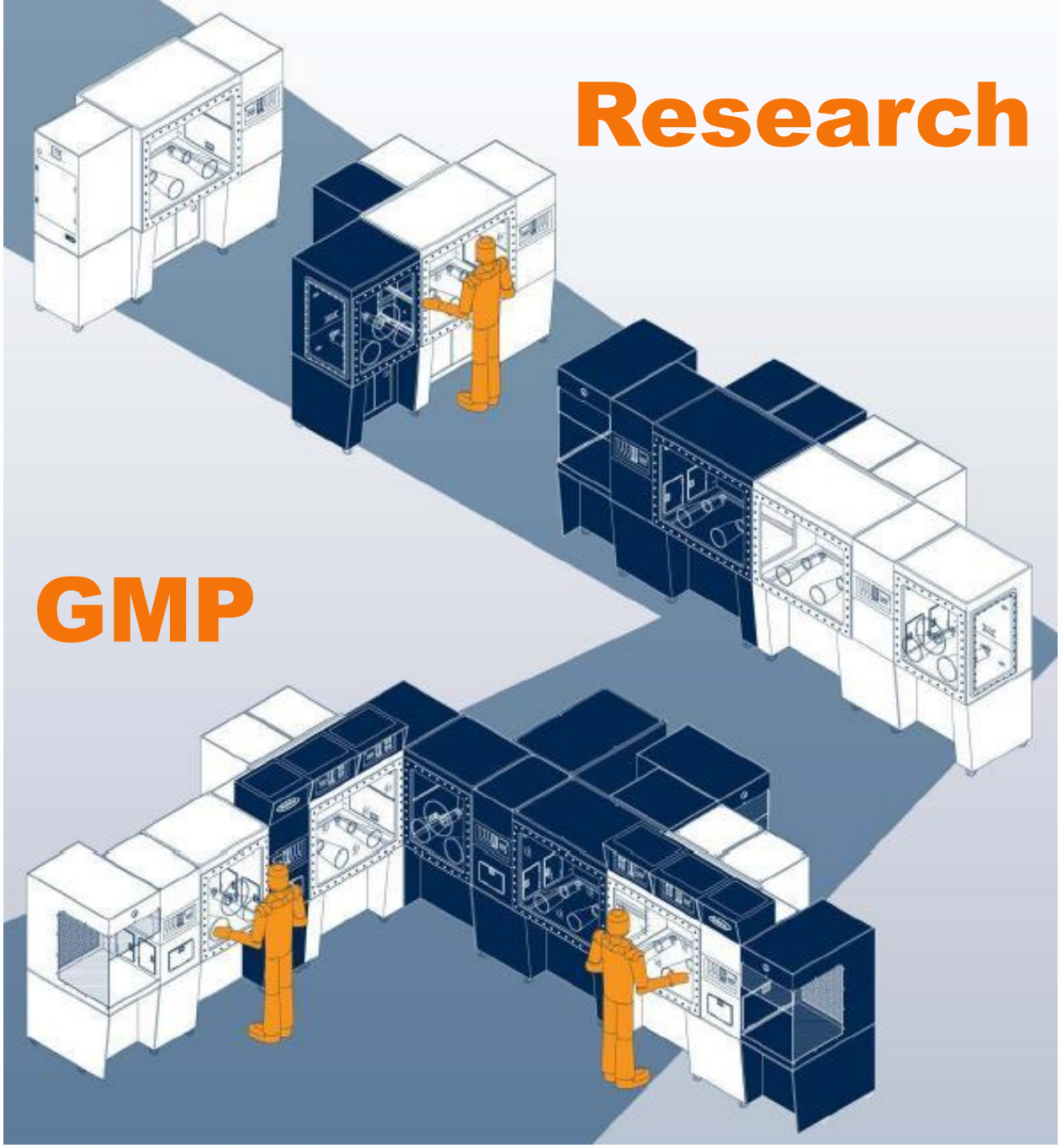


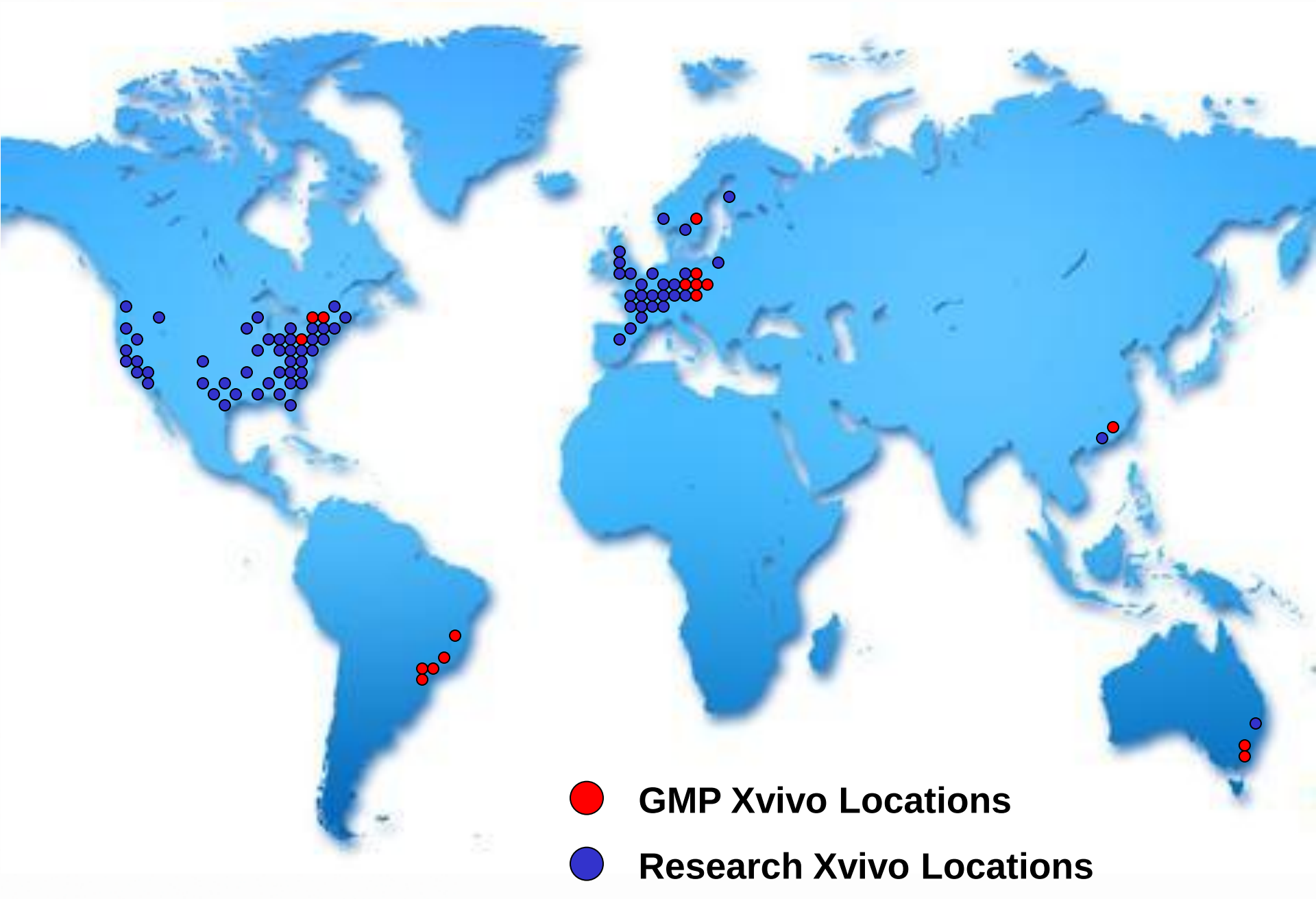
Research → GMP








Research
GMP


GMP Xvivo Locations
Research Xvivo Locations

Thank You



Est. 1982

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www.biospherix.com