bpt BioPreservation Today®

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18TH ANNUAL ISCT MEETING
SEATTLE, WA

FEATURE ARTICLE

CONTINUOUS IMPROVEMENT AND BEST PRACTICES IN BIOPRESEVATION OF CELL AND TISSUE BASED PRODUCTS AND THERAPIES

VALIDATION OF IN-HOUSE QUALITY ASSURANCE ASSAYS

IN THIS ISSUE

THE SPACE NEEDLE; 605 FEET, BUILT FOR THE 1962 WORLD'S FAIR

WEB RESOURCES

www.aabb.org | American Association of Blood Banks
www.celltherapy.org | International Society for Cellular Therapy
www.asbmt.org | American Society for Blood and Marrow Transplantation
www.ishrs.org | International Society of Hair Restoration Surgery
www.alliancerm.org | Allliance for Regenerative Medicine
www.bestcollaborative.org | BEST Collaborative
www.regenerativemedicinefoundation.org | Regenerative Medicine Foundation
www.phacilitate.co.uk/pages/cgtherapy/index.html | Phacilitate

UPCOMING EVENTS

ISCT 18th Annual Meeting
Seattle, WA
June 5-8, 2012

10th Annual Umbilical Cord Blood Transplantation Symposium
June 7-9, 2012
San Francisco, CA

3rd TERMIS World Congress 2012
September 5-8, 2012
Vienna, Austria

BC's 2nd Annual Cell Therapy Bioprocessing
September 11-12, 2012
Arlington, VA

AABB Annual Meeting & CTTXPO 2012
October 6-9, 2012
Boston, MA

ISHRS 20th Annual Scientific Meeting
October 17-20, 2012
Commonwealth of the Bahamas

The 3rd North American Veterinary Regenerative Medicine Conference
November 8-10, 2012
Savannah, GA

EDITOR'S CORNER

Mike Rice, Chairman & CEO, BioLife Solutions, Inc.

BioLife customers, colleagues, suppliers, and friends,

Welcome to Seattle – the Emerald City and host city for the ISCT 2012 Annual Meeting. We're pleased to be a Gold level supporter and local host exhibitor of this critical and informative conference. Much progress in academic and clinical research, and commercialization of new cellular therapies has occurred since we last met in Rotterdam.

In this issue of BioPreservation Today® (BPT), Aby J. Mathew, Ph.D., our Senior Vice President and Chief Technology Officer, contributed the feature article titled, Continuous Improvement and Best Practices in Biopreservation of Cell and Tissue Based Products and Therapies.

Also in this issue, Mark Sandifer, Vice President of Quality at BioLife Solutions, provides some thoughts and guidance in an article focused on selecting and validating tools to perform in-house release criteria testing.

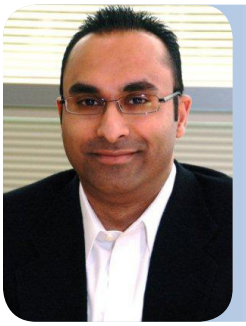
Meanwhile, here in Bothell, we're very busy with the build-out and validation of our second multi-grade cGMP clean room suite and the expansion of our corporate office. Adoption of Cryostor® and HypoThermosol® continues at a fast pace, as more clinical customers realize improved biopreservation outcomes through the use of our proprietary protein-free, serum-free freezing and storage/shipping media products.

I hope you find this issue of BPT informative and helpful. Please attend our corporate tutorial or visit our exhibit or website to learn more about our best-in-class, clinical grade biopreservation media products: http://biolifesolutions.com

Thank you,

Mike

SEATTLE 2012
18TH ISCT
ANNUAL MEETING



CONTINUOUS IMPROVEMENT AND BEST PRACTICES IN BIOPRESEVATION OF CELL AND TISSUE BASED PRODUCTS AND THERAPIES

Aby J. Mathew, Ph.D., Senior Vice President & Chief Technology Officer, BioLife Solutions, Inc.

As cellular therapies and the field of regenerative medicine continue to evolve and progress, it is somewhat of a "homecoming" that the 2012 ISCT Annual Meeting is being held in Seattle; where Nobel Laureate E. Donnall Thomas was a pioneer in the use of bone marrow stem cells for the treatment of cancer in the 1950's. As with most clinical breakthroughs, the initial evolution begins with the practice of medicine at the academic/clinical center level. However, the broad translation of medicine mandates the ability to cost-effectively transfer new medicines throughout the world. Historically, the distribution of medical evolution was often somewhat of a forked path – sharing of clinical knowledge amongst clinicians or development of medicinal products by industry. The current development of regenerative medicine (cell and tissue-based therapies) has shown that there is mutual benefit for the often-distinct circles of not-for-profit clinical centers and for-profit commercial companies to find common ground in order to further the progress of these promising therapies.

One aspect of cell and tissue-based therapy development that all "manufacturers" (whether hospital-based lab or industry) can benefit from is continuous improvement in best practices regarding the biopreservation, or stability, of their cells and tissues. This aspect of stability in process development is increasingly a system bottleneck or point of risk vulnerability for industry, as well as for clinical centers that share source material and cell/tissue products over increasing geographic distance and timeframe. The increased focus on stability/biopreservation mirrors the increased scrutiny and concern with utilizing reagents that consist of home-brew cocktails, and the increased movement to pre-formulated serum-free and protein-free GMP biopreservation media (similar to the transition of cell/tissue manufacturing to closed systems and single use disposable components). Innovation drives the development of best practices through the development of improved technology/methods from the recognition of sub-optimal methods.

COMPARISON OF BIOPRESERVATION MEDIA FORMULATIONS

COMMENT	FUNCTION	TRADITION	OPTIMIZED
Na, K, Ca, other ions	Electrolytes	Balanced for 37C	Balanced for hypothermic temperatures
Low temp pH buffter	Maintain buffering capacity throughout preservation temperature range	Typically none	Selected for effectiveness during hypothermic temperatures
Antioxidants	Scavenge free radicals generated by preservation-induced stress	Typically none	One or more
Energy substrate	Available for generation of ATP folllowing preservation	Typically none or glucose	One or more
Impermeant compounds	Reduced cell shrinking and swelling	Typically none	One or more
Serum	Variable and undefined benefits/consequences	May be included	None
Albumin	Serum macromolecules	Human Serum Albumin	None
Cryoprotectant	Manage cellular dehydration, ice crystallization, and transition to vitrified state	10% (v/v) DMSO	Cell specific; as low as 2% (v/v) DMSO

"THIS ASPECT OF STABILITY IN PROCESS DEVELOPMENT IS INCREASINGLY A SYSTEM BOTTLENECK.."

In the 1990's and early 2000's, BioLife researchers presented information primarily around the scientific mechanisms of how our novel preservation formulations improved cell viability following cryopreservation or hypothermic preservation. Our technology evolved from academic basic science to being incorporated into clinical/commercial best practices. Today, we are asked less about the science and primarily

about the quality/regulatory GMP manufacturing footprint, clinical qualification, and commercial adoption of HypoThermosol® and CryoStor®. This successful transition from novel scientific innovation to broadly adopted enabling technology should support the growth of regenerative medicine, from localized scientific promise to broad clinical application.

We often cite Table 1 as a guide for evaluating the quality and regulatory footprint of biopreservation media reagents – regardless of whether one is evaluating BioLife's intracellular-like biopreservation technology, another commercial media, or a home-brew cocktail. Last year in Rotterdam, we shared several clinical customer case studies (Athersys, Intercytex, DCPrime, and City of Hope) and tables outlining 26 clinical applications that have incorporated HypoThermosol® and CryoStor® (often as an excipient reagent included in the final cell therapy clinical product). Some of the clinical applications in the tables represent multiple customers targeting the same disorder or disease. Finally, the growing adoption of BioLife's intracellular-like biopreservation technology is also exhibited in the increasing number of citations within peer-reviewed scientific publications. The following is a brief list of some recent clinically-relevant publications citing HypoThermosol® or CryoStor®, along with paraphrased descriptions of the article contents.

Cryopreservation of adenovirus-transfected dendritic cells (DCs) for clinical use

Gülen, Maas, Julius, Warkentin, Britton, Younos, Senesac, Pirruccello, Talmadge

http://www.ncbi.nlm.nih.gov/pubmed/22465385

This study from researchers at the University of Nebraska Medical Center evaluated the effects of the cryoprotectant CryoStor, freezing and thawing, and adenovirus (Adv) transduction on the viability, transgene expression, phenotype, and function of human dendritic cells (DCs). Stability studies revealed that transduced DCs could be held in cryoprotectant for as long as 75min at 2-8°C prior to freezing with little effect on their viability and cellularity. Further, cryopreservation in CryoStor, storage, and thawing reduced the viability of the transduced DCs by an average of only 7.7%; and had no significant impact on DC phenotype and activation. In summary, cryopreservation in CryoStor, storage, and thawing had no significant effect on DC viability, function, and transgene expression by Adv-transduced DCs.

Cryopreservation of Umbilical Cord Blood with a Novel Freezing Solution that Mimics Intracellular Ionic Composition

Nicoud, Clarke, Taber, Stolowski, Roberge, Song, Mathew, Reems

http://biolifesolutions.com/biolife-investors/regenerative-medicine/improved-cord-blood/

This study from researchers at the Puget Sound Blood Center and BioLife Solutions evaluated the preservation efficacy of the novel intracellular-like cryopreservation solution CryoStor, the rate of addition of two cryopreservation solutions to cord blood units (CBUs), and reduced final dimethyl sulfoxide (DMSO) concentration of 5%. Post-thaw recoveries with CryoStor were equivalent to or slightly better than with the in-house cryopreservation solution. CryoStor also provides several advantages including reduced processing time, formulation consistency, and reduced DMSO in the frozen product (≤5%).

Evaluation of Bone Marrow-Derived Mesenchymal Stem Cells After Cryopreservation and Hypothermic Storage in Clinically Safe Medium

Ginis, Grinblat, Shirvan

http://biolifesolutions.com/biolife-investors/regenerative-medicine/independent-data-published-on-biolife-solutions/

"HYPOTHERMIC STORAGE OF CELLS IN HYPOTHERMOSOL FRS (HTS-FRS) FOR 2 AND 4 DAYS RESULTED IN ABOUT 100% AND 85% CELL RECOVERY RESPECTIVELY"

This study from researchers formerly at Teva Pharmaceutical, currently at Macrocure Ltd. and OMRIX Biopharmaceuticals, demonstrated about 95% of frozen cells were recovered as live cells after freezing in CryoStor CS5 and CS10 followed by storage in liquid nitrogen (one month storage). Hypothermic storage of cells in HypoThermosol FRS (HTS-FRS) for 2 and 4 days resulted in about 100% and 85% cell recovery respectively, less than 10% of apoptotic cells, and normal proliferation, marker expression, and osteogenic

(cont. on pg 8.)

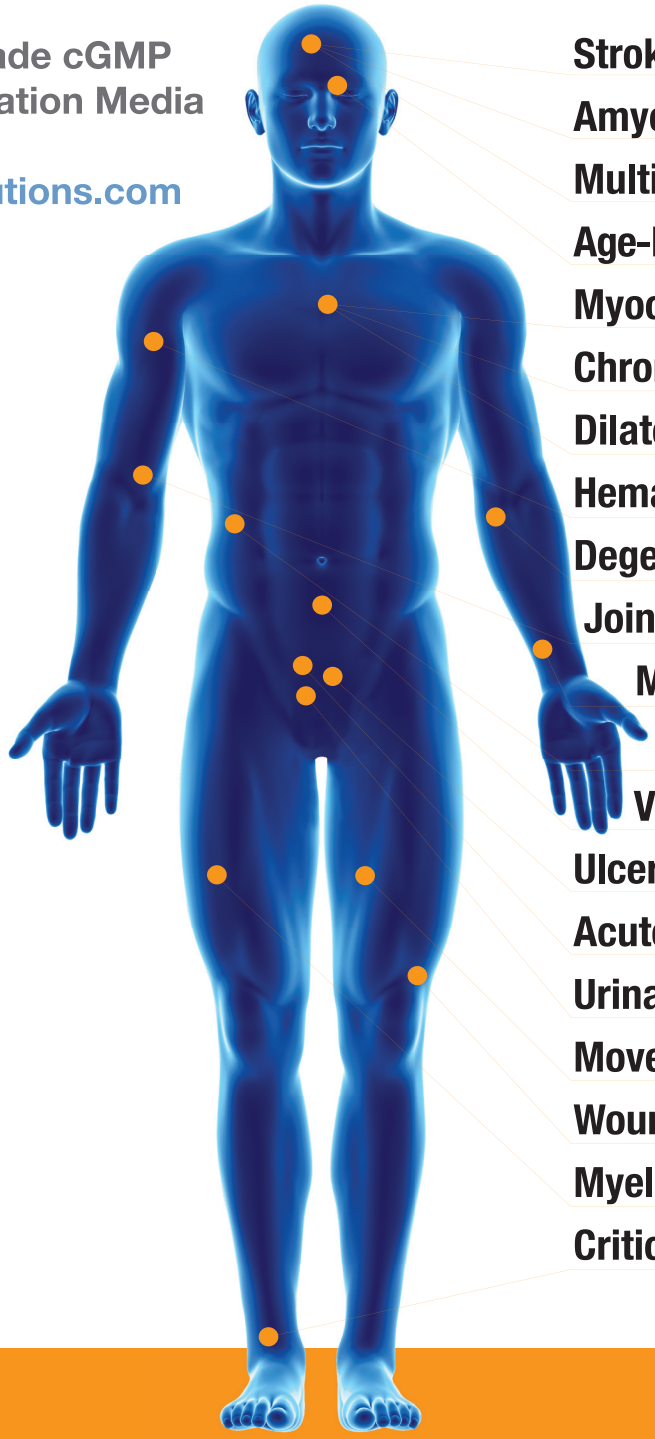
EXTEND CELL STABILITY

IMPROVE CELL VIABILITY

HypoThermosol® & CryoStor® in Customer Clinical Trials

Clinical Grade cGMP
Biopreservation Media

BioLifeSolutions.com



- Stroke
- Amyotrophic Lateral Sclerosis
- Multiple Sclerosis
- Age-Related Macular Degeneration
- Myocardial Infarction
- Chronic Heart Failure
- Dilated Cardiomyopathy
- Hematologic Malignancies
- Degenerative Joint Disease
- Joint Repair
- Melanoma
- Lymphoma
- Various Cancers
- Ulcerative Colitis
- Acute Myeloid Leukemia
- Urinary Stress Incontinence
- Movement Disorders
- Wound Repair
- Myeloma
- Critical Limb Ischemia

(cont.; Continuous Improvement and Best Practices in Biopresevation of Cell and Tissue Based Products and Therapies)

potential. Results demonstrate that human MSC could be successfully cryopreserved for banking and clinical applications and delivered to the bedside in clinically safe protective reagents.

Hyaluronan-Supplemented Buffers Preserve Adhesion Mechanisms Facilitating Cryopreservation of Human Hepatic Stem/Progenitor Cells

Turner, Mendel, Wauthier, Barbier, Reid

http://www.ncbi.nlm.nih.gov/pubmed/22472355

This study from researchers at the University of North Carolina, Chapel Hill, evaluated an effective method for serum-free cryopreservation of the cells, allowing them to be potentially stockpiled and stored for use as an off-the shelf product for experimental or clinical programs. Using methods that include CryoStor, as well as hyaluronans (HA) that preserve adhesion mechanisms, cells were able to demonstrate high viability, cell attachment and formation of expanding colonies of cells that stably maintain the stem/progenitor cell phenotype.

A double-blind, randomized, controlled, multicenter study to assess the safety and cardiovascular effects of skeletal myoblast implantation by catheter delivery in patients with chronic heart failure after myocardial infarction

Povsic, O'Connor, Henry, Taussig, Kereiakes, Fortuin, Alan Niederman, Schatz, Spencer, Owens, Banks, Joseph, Roberts, Alexander, Sherman

http://biolifesolutions.com/biolife-investors/regenerative-medicine/independent-data-published-on-biolife-solutions/

This study from a team of clinicians and researchers from Duke University Medical Center, Florida Hospital Center and Cardiovascular Institute, Minneapolis Heart Institute, The Christ Hospital Heart and Vascular Center, Mayo Clinic, Scripps Clinic, Columbia University Medical Center, and Bioheart reports results from the MARVEL clinical trial to determine the safety and preliminary efficacy of transcatheter intramyocardial administration of myoblasts in patients with heart failure (HF). HypoThermosol FRS (HTS-FRS) was utilized as the transport media for the cells, as well as for the placebo condition (no cells).

Interim analysis results from the RESTORE-CLI, a randomized, double-blind multicenter phase II trial comparing expanded autologous bone marrow-derived tissue repair cells and placebo in patients with critical limb ischemia

Powell, Comerota, Berceli, Guzman, Henry, Tzeng, Velazquez, Marston, Bartel, Longcore, Stern, Watling

http://biolifesolutions.com/biolife-investors/regenerative-medicine/independent-data-published-on-biolife-solutions/

This study from a team of clinicians and researchers from Dartmouth-Hitchcock Medical Center, Jobst Vascular Center, Malcolm Randall VAMC and University of Florida, Vanderbilt University Medical Center, Minneapolis Heart Institute at Abbott Northwestern, University of Pittsburgh, Miller School of Medicine at the University of Miami, University of North Carolina Medical School, and Aastrom Biosciences reports results from the RESTORE-CLI clinical trial to assess the safety and clinical efficacy of intramuscular injection of autologous tissue repair cells (TRCs) for the treatment of critical limb ischemia (CLI). HypoThermosol FRS (HTS-FRS) is utilized as part of the hypothermic transport of the cells.

Juvenile Chondrocytes May Facilitate Disc Repair

Kim, Adkisson, Wendland, Seyedin, Berven, Lotz

http://biolifesolutions.com/biolife-investors/regenerative-medicine/independent-data-published-on-biolife-solutions/

This study from a team of researchers from the University of California, San Francisco and ISTO Technologies evaluated the in vivo disc regeneration potential of juvenile chondrocytes. Transplantation of juvenile chondrocytes resulted in a significant positive outcome compared to that of fibrin glue alone and may provide an ideal allograft for future human studies. Chondrocytes were cryopreserved in CryoStor.

Isolation, propagation, and characterization of human umbilical cord perivascular cells (HUCPVCs)

Sarugaser, Ennis, Stanford, Davies

http://www.ncbi.nlm.nih.gov/pubmed/19089362

This chapter from a team at Tissue Regeneration Therapeutics (TRT) describes the isolation, culture, and characterization of perivascular cells derived from umbilical cord tissue. CryoStor is utilized for cryopreservation of the isolated cells.

A Phase IIa Open-Label Dose-Escalation Pilot Study Using Allogeneic Human Dermal Fibroblasts for Nasolabial Folds

Lowe, Lowe, St Clair Roberts

http://www.ncbi.nlm.nih.gov/pubmed/20722660

This study from a team of clinicians and researchers reports results from a clinical trial to evaluate allogeneic human dermal fibroblasts (HDFs) for the treatment of nasolabial folds as an alternative strategy to improve the structure, texture, and quality of the skin. The cell suspensions consisted of the fibroblasts suspended in HypoThermosol FRS (HTS-FRS) delivered via intradermal injections.

"CELLS WERE ABLE TO DEMONSTRATE HIGH VIABILITY, CELL ATTACHMENT AND FORMATION OF EXPANDING COLONIES OF CELLS THAT STABLY MAINTAIN THE STEM/PROGENITOR CELL PHENOTYPE"

VALIDATION OF IN-HOUSE QUALITY ASSURANCE ASSAYS

Mark Sandifer, Vice President of Quality, BioLife Solutions, Inc.

Most manufacturers have a choice of performing analytical testing in-house or using a contract testing laboratory. The primary advantage of in-house testing is that results are available sooner; however, the cost of acquiring equipment and performing validation can be obstacles. Using contract laboratories can also be challenging. The testing laboratory must be qualified as an approved service provider, laboratories may have backlogs that delay testing, and investigations can be lengthy when there are suspect results.

Some instruments, such as pH meters, are relatively simply to qualify for in-house use. But even a simple qualification requires that the instrument being purchased be of high quality. Reading reviews and listening to the recommendations of colleagues is invaluable when selecting the instrument. Also important is the proper selection of peripheral devices. In the case of a pH meter, choosing the right electrode is of extreme importance. The manufacturer of the electrode will be able to advise if the electrode is suitable for the type of sample, the sample size, and the conditions of use. The electrode must also be carefully maintained to ensure accurate measurements, fast response time, and a longer lifetime.

Supplies are also an important consideration. The most important of these are the standards that are used for qualification and daily standardization of the device. In the case of pH meters, IUPAC/NIST buffers are a must to ensure high accuracy and consistency. Storage of standards is of utmost importance. In most cases, the expiration date on the label is for the unopened container. Once opened, the length of time the

"READING REVIEWS AND LISTENING TO THE RECOMMENDATIONS OF COLLEAGUES IS INVALUABLE WHEN SELECTING THE INSTRUMENT."

standard may be used can be quite short. It is important to check with the manufacturer of the standard for open-time stability data. When in doubt, it is always best to employ single-use standards. Many manufacturers provide standards in this type of packaging.

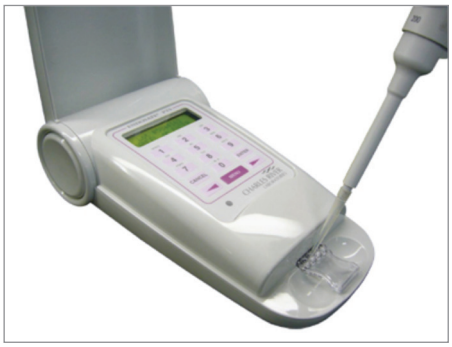
The quality of the instrument is not always proportional to price due to considerations of instrument accuracy and use. Determining the conditions of use and the accuracy required for analytical results can result in substantial savings. For example, an instrument for use under adverse environmental conditions normally is more expensive than one for use under standard laboratory conditions. Instruments that deliver more precise results are also usually more costly. If the measurement being taken does not require the degree of accuracy that the instrument can deliver, it is worthwhile to evaluate an instrument with slightly less accuracy.

It is always best to select at least two candidate instruments from different manufacturers. Having quotations in hand from both manufacturers is an excellent tool for price negotiation. It is also an opportunity to include periodic calibration, peripherals, and supplies in the price that would otherwise need to be paid for separately. During the negotiation process, it is worth asking if the manufacturer provides on-site validation or if a validation package that can be executed in-house is available. Often, installation qualification (IQ) and operational qualification (OQ) are included in these packages, but performance qualification (PQ) may need to be developed and conducted in-house. Negotiating for a free loaner device during calibration or repair of the in-house device is also possible.

BioLife's most recent experience in bringing testing in-house is the addition of an osmometer, densitometer, and the Endosafe® Portable Testing System™ (PTS™) for endotoxin testing. In the case of the Endosafe® PTS™ device, we determined that a more rapid turnaround time was required for receiving endotoxin results. Using a contract testing laboratory, the time of sampling to the time of receiving results was approximately three days. In-house testing has reduced that time to approximately 30 minutes. The per-sample cost, including the calculation for the purchase and maintenance of the device, has decreased by forty percent.

BioLife first became interested in the Endosafe® PTS™ device because of an article in the PDA Journal of Pharmaceutical Science and Technology, "Evaluation of the Endosafe® Portable Testing System™ (PTS™) for the Rapid Analysis of Biopharmaceutical Samples." (PDA J Pharm Sci and Tech 2010, 64 211-221). BioLife obtained a loaner device from the manufacturer to evaluate the device, and then proceeded to purchase it. The device included a set of supplies and standards in addition to an on-site qualification and service package. The performance qualification of the Endosafe® PTS™ device included testing with endotoxin standard solutions ranging from 1 to 0.01 EU/mL and comparing results of testing to the traditional Bacterial Endotoxin Test (BET) using the kinetic chromogenic method. Multiple experiments were conducted to ensure the accuracy, precision, and repeatability of the testing.

BioLife's experiences with bringing testing in-house have been positive; however, in some cases we have decided to continue with contract testing due mostly to an unfavorable return on the investment in the equipment and ancillary support required for the testing. An important aspect of making the determination to bring testing in-house is to include representatives from all affected departments on the return-on-investment analysis team. Having a dynamic team will ensure that testing and quality requirements are met, and that all user requirements have been considered.



EndoSafe®-PTS™ Rapid Endotoxin Detection System from Charles River Laboratories

BIOLIFE SOLUTIONS COMMENCES BUILD-OUT OF SECOND GMP PRODUCTION SUITE AND CORPORATE OFFICE EXPANSION

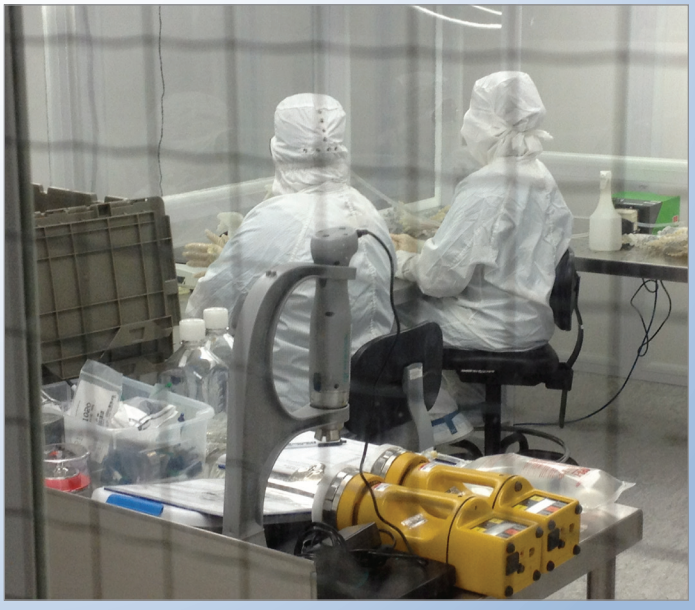
Demand for HypoThermosol® and CryoStor®, and New Contract Manufacturing Agreement Driving Growth and Creation of New Jobs

BioLife Solutions, Inc. (OTCBB: BLFS), a leading developer, manufacturer and marketer of proprietary clinical grade hypothermic storage and cryopreservation freeze media for cells and tissues, recently announced that it is doubling the square footage of its existing facilities. The additional space will be dedicated to the build-out of an additional GMP manufacturing clean room suite and space for additional team members, whose jobs are being created by increasing demand for the Company's biopreservation media products and also a high value contract manufacturing agreement that was executed in late 2011. BioLife's operations are located in Monte Villa Farms, a Bothell biotech and data center campus.

Mike Rice, Chief Executive Officer, commented on the outlook for BioLife by stating, "We're very pleased to see demand for HypoThermosol and CryoStor continuing to increase. Our best-in-class proprietary products are now recognized by key opinion leaders and a growing customer base in our strategic market segments of regenerative medicine, biobanking, and drug discovery. This growth, along with a new contract manufacturing customer we acquired late in 2011, will enable BioLife to create up to 10 additional jobs in manufacturing, quality assurance, sales, and marketing. We estimate ending 2012 with 25 team members and having significantly increased revenue over 2011."

BioLife's existing already cleanroom suite adheres to, and the additional clean room suite will adhere to, very stringent quality requirements including the US FDA Good Manufacturing Practice guidelines for Sterile Drug Products Produced by Aseptic Processing, and Annex 1 – Manufacture of Sterile Medicinal Products – of Volume 4 of the European Guidelines to Good Manufacturing Practice of Medicinal Products for Human and Veterinary Use.

"OUR BEST-IN-CLASS PROPRIETARY PRODUCTS ARE NOW RECOGNIZED BY KEY OPINION LEADERS AND A GROWING CUSTOMER BASE IN OUR STRATEGIC MARKET SEGMENTS OF REGENERATIVE MEDICINE, BIOBANKING, AND DRUG DISCOVERY."



BioLife Solutions develops and markets patented hypothermic storage/transport and cryopreservation media products for cells, tissues, and organs. BioLife's proprietary HypoThermosol®, CryoStor®, and BloodStor® platform of biopreservation media products are marketed to academic research institutions, hospitals, and commercial companies involved in cell therapy, tissue engineering, cord blood banking, drug discovery, and toxicology testing. BioLife products are serum-free and protein-free, fully defined, and formulated to reduce preservation-induced, delayed-onset cell damage and death. BioLife's enabling technology provides research and clinical organizations significant improvement in post-preservation cell and tissue and viability and function.