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FEATURE ARTICLE
UNLOCKING THE VALUE OF A SCIENTIFIC ADVISORY BOARD

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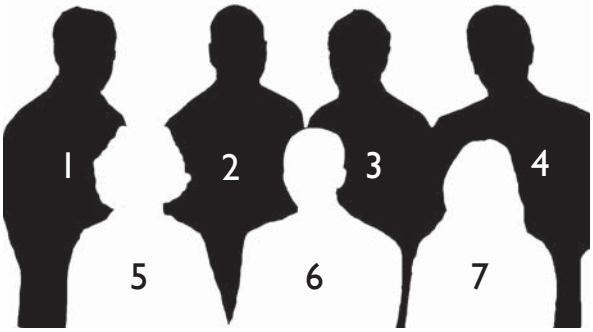

On the front cover: BioLife's Scientific Advisory Board
For a complete bio of each advisor, please visit www.biolifesolutions.com/about/advisors.htm.

1 - Darin Weber, Ph.D.
2 - Shelly Heimfeld, Ph.D.
3 - Scott Burger, MD
4 - Dayong Gao, Ph.D.
5 - Lizabeth Cardwell, MT (ASCP), RAC, MBA
6 - Andrew Hinson
7 - Colleen Delaney, MD, MSc
8 - Erik J. Woods, Ph.D. - *Unavailable During Photo Session*

UPCOMING EVENTS

Phacilitate Cell & Gene Therapy Forum http://www.phacilitate.co.uk/pages/cgtherapy/index.html	Washington, D.C.	Jan 26-28, 2009
Molecular Medicine Tri-Conference http://www.tri-conference.com/	San Francisco, CA	Feb 25-26, 2009
Stem Cell Partnering Series http://stemcellpartnering.org/	San Diego, CA	Feb 26-27, 2009
GTCbio 5th Annual Conference http://gtcbio.com/conferenceDetails.aspx?id=145	Boston, MA	Mar 9-10, 2009
SOT | ToxExpo http://www.eshow2000.com/toxexpo/2009/	Baltimore, MD	Mar 15-19, 2009
ISCT 15th Annual Meeting http://www.celltherapysociety.org/Meetings/Annual_Meeting/	San Diego, CA	May 3-6, 2009

WHAT IS YOUR VIABILITY ASSAY REALLY SAYING?

by Aby J. Mathew Ph.D.

In the post-preservation assessment of cells, we often utilize an array of "viability" assays. Yet each assay is a snapshot in time of the cells, viewed through a lens colored by the limiting parameters of the assay. Therefore, a moment should be taken to consider "What is my viability assay really saying that allows me to have confidence in my assessment of the health of the cell population?"

The cursed standard – Trypan Blue. Much of how we view viability is based on how we were first trained to assess this as part of our introduction to cell culture. Almost everyone learning cell culture first learns to assess viability with Trypan Blue and a hemacytometer. Cells that exclude Trypan Blue are "viable" and cells that stain blue are "dead". For basic cell culture, this is mostly adequate. At least two specific pitfalls exist when relying on Trypan Blue as the sole viability assay: when cells are in a condition where their membranes are transiently more permeable (this condition might stain blue but the cells are likely to ultimately survive – i.e. false positive) and when cells have intact membranes, but have initiated apoptotic pathways that have not manifested yet to the completion of cell death (this condition might not stain with Trypan Blue but the cells are likely to ultimately die – i.e. false negative).

How do we define viability? Is it a cell with intact membranes, a metabolically active cell, or a cell that is able to undergo cell division? All of these parameters are used by many to assess "viability" and each criteria has pros and cons as an individual method, especially depending on the timing of the assay in a preservation assessment model. As our group began unlocking new aspects of understanding the cellular response to preservation, we began proposing a new paradigm of assessing post-preservation cellular recovery that was based on multiple assay parameters and multiple time points of post-preservation assessment. In this manner, our view of the post-preservation cell became less of a singular snapshot and more of a dynamic characterization.

Timing is Everything! Post-preservation assessment of "viability" is often conducted immediately post-thaw. This is partially based on a lack of complete understanding for when cell death manifests itself post-preservation and/or the timing constraints necessitated by the end user application. The relative accuracy of an immediate post-thaw assessment is best illustrated by extended analysis of an adherent cell population post-preservation. If we consider for a moment, immediately upon thaw following cryopreservation, viability assessments often indicate cell survival in the range of 80-95%. Yet, if those same cells are allowed to culture under standard cell culture conditions overnight and viewed the next day through a microscope, we often see a reduced population of cells that have properly adhered to the culture surface and a significant population that are seen as dead, non-adherent cells. Therefore, the resultant population post-thaw would not seem to truly represent 80-95% viability even though that was what we measured immediately post-thaw. This phenomenon is referred to as Delayed Onset Cell Death, which articulates that cell death manifests itself post-preservation over a number of hours to days through apoptosis (programmed cell death), necrosis, and secondary necrosis. And the "True Yield" or "True Viability" of the cell population is not reflected immediately post-thaw. Assessments conducted at multiple time points indicate that cell death increases following preservation until the true cell survival reaches a nadir often ~24 hours post-preservation (varies by cell type); this is followed by re-growth (in cells still able to undergo cell division) of the cell population with subsequent later return of functional capabilities (again, dependent on cell type).

The Assays. As mentioned above, the Trypan Blue assay conducted immediately post-preservation is often misleading. It is debatable whether a single assay can provide the answer to the question of True Viability. We and others have proposed that a portfolio of assays might be a more accurate method for assessment of actual post-preservation cell population health. One component of this portfolio are Live/Dead assays that are often indicative of membrane integrity. Examples of Live/Dead assays are Trypan Blue, Calcein-AM, and Propidium Iodide (PI). A second component of this assay portfolio are assays based on cellular mechanisms; examples include metabolic indicators, such as alamarBlue, and Annexin/PI for identifying apoptosis and necrosis. A final component of the assay portfolio is functional assays. These may be dependent on the cell type being studied, but might include the Colony Forming Units (CFU) assay (used to analyze differentiation of CD34+ cells), cytochrome p450 activity (used with hepatocytes), or measures of specific protein production (as in a bioreactor system). An additional consideration can be given to methods that analyze the genome/proteome/metabolome, although the resultant information might be more for characterization and predictive value as opposed to "viability" of the cells. Again, the timing of

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UNLOCKING THE VALUE OF A SCIENTIFIC ADVISORY BOARD

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

A well-represented scientific advisory board (SAB) can be an extremely valuable resource for biotech companies if utilized appropriately to maximize the potential contributions of the members. Typical biotech and medical device firms' scientific advisors are academic and clinical researchers and practitioners with relevant experience in the scientific and clinical fields related to the company's technology. This article offers a perspective on a greatly expanded role for an SAB to directly influence the product development, quality, regulatory, intellectual property, and marketing strategies of a life sciences/biotech company. An important step in ensuring that the depth and scope of potential input from the advisors is clearly understood and agreed upon, and also to protect the company's intellectual property, is the execution of a formal consulting agreement with each advisor that specifies the nature of the relationship, identification and treatment of confidential information, ownership of ideas that emanate from the relationship, and any compensation to be paid to the advisor. If formed early enough in a company's history, the SAB can play a crucial role in the design and development of a biotech company's products and services as well as help shape key strategies. In the case of BioLife Solutions, we formed our SAB in late 2006 and initially presented the scientific foundation and intellectual property supporting our biopreservation media products branded as HypoThermosol® and CryoStor™. We intentionally selected some SAB members with relevant experience in regulatory and quality systems applicable to BioLife as well as customers and prospects in our strategic markets. In our initial meetings, we sought critical input and validation from our SAB members on product formulation, packaging, and the key features and benefits that comprise the value proposition of our products. A reasonable schedule for engaging with our SAB has evolved into a full day annual meeting. Ad hoc emails, conference calls, and smaller meetings at advisor facilities are utilized as needed.

Product Development

While our products were commercially available for three years prior to the formation of our SAB, we spent considerable time with our advisors seeking feedback to validate that our perceptions of the current unmet needs in biopreservation and the solutions our products provide to address these needs were accurate. We consider our SAB an accurate bellwether of potential market acceptance of our products. For BioLife, this meant testing our assumptions on product packaging types, fill volumes, labeling, and instructions for use. Our advisors provided valuable input on future packaging alternatives that better fit some specific use models in our strategic markets. We also present our product development roadmap annually and use our SAB to test our priorities for future R & D initiatives.

Quality Systems

As BioLife transitioned the management team in late 2006 and began to scale the company to meet demand for our products, we focused on our quality system and environment. This led us to form a relationship with Lizabeth

Cardwell, Principal, with Compliance Consulting. Liz assisted us in completing a gap analysis, qualifying and selecting a contract manufacturer, and thereafter taking responsibility for product release as our designated quality official. After a decision was recently made to resume internal manufacturing, Liz participated in the hiring process for quality and production team members.

Our internal quality team is currently focused on building a quality environment that supports internal production. At our recent SAB meeting, we shared our near and mid-term strategies and plans including facility design, workflow, validation, scale-up, raw materials qualification, final product release criteria and stability studies. Our biopreservation media products are unique in that they are regulated as excipient material when used in our customer clinical applications. Sharing our vision for a best-in-class regulatory footing elicited valuable input from our SAB members related to the quality environment required to support this goal.

Regulatory Affairs

While the current excipient product classification is well understood, we're intent on raising the bar with respect to preservation media products, and also staying ahead of pending increased regulatory oversight for our products. In this respect, our SAB proved extremely valuable in recommending two specific initiatives: 1) that we submit and maintain FDA Master Files for both product families; and 2) that we complete a series of safety studies to increase consideration of our products as intravenous carrier solutions for cell-based products. Both initiatives are now completed and are driving more awareness of our products in key market segments including cell and gene therapy and cord blood banking.

Finally, we recently presented to our SAB the next phase of our plan to become the best-in-class biopreservation media products – the transition of our products from excipient status to Class II medical devices. Again, our SAB provided great feedback on this initiative and we'll be updating the members as we navigate this process.

Marketing

Although not a typical topic for discussion with an SAB, we present our product and company marketing messages for critique by our advisors. This proves valuable in that we gain feedback that enables us to sharpen our messages and better articulate our value differentiators. Contrary to our expectations that our advisors might caution us to dial down our product claims, we were given recommendations where stronger messages supported by data could help reinforce our value drivers.

Another marketing area our SAB recommended we focus on was industry relations, specifically increased support for, and participation in scientific consortia and trade associations relevant to our strategic markets. We've taken this advice and have already seen a payoff in our ability to drive awareness with academic and clinical thought-leaders. We accomplished this, in part, by presenting at scientific sessions and by increasing study collaborations. This availed us to have our products cited in the methods and materials section of published articles on new research and clinical practices in cell therapy and peripheral and cord blood stem cell banking.

In summary, our approach to unlocking the value of an SAB started with selecting some non-traditional members whose expertise and experience was focused in quality and regulatory areas. This augmented the other members who come from clinical practice and represent the profile of customers and prospects in our key market segments. Next, we exposed nearly every functional area of the company's operations to the SAB and sought critical feedback to validate our assumptions about our markets, unmet needs, our product and company value proposition, and most importantly, how to increase product adoption at the fastest possible rate.

"...we've gained valuable insight from our advisors and have validated that our growth strategies are well articulated and can position the company for success."

This strategy has proven incredibly effective. We have gained valuable insight from our advisors and have validated that our growth strategies are well articulated and can position the company for success. Growing biotech companies interested in maximizing SAB contributions should consider an expanded, non-traditional member profile and a broader role for this advisory board to help shape the company for future growth.

Optimizing Biopreservation Yield

by Dominic M. Clarke, Ph.D.

PART 1: STEPS TO IMPROVING CRYOPRESERVATION OUTCOMES

To many, the cryopreservation aspect of their potentially invaluable product is often considered an afterthought and is simply a step involved in the fabrication of their final product with the assumption that what you put in essentially equals what you get out. To this notion, current cryopreservation protocols applied by many groups have been largely developed through the imitation of others. As such, these conventional cryopreservation protocols can often result in significant cell loss (> 50% in many cases). This loss is not often appreciated, nor is the associated loss in quality of the cell product. A loss of this scale translates to loss in product value and in the case of a cell therapy product, an impending uncertainty in therapeutic dose. With the continued rapid expansion in the areas of cell therapy, drug discovery, and cell banking, effective cryopreservation strategies are required.

The question is – what is an effective cryopreservation strategy?

Is an effective process based on yield determined by immediate post-thaw analysis? What about the function of the cells? A common solution by the standard researcher is simply to factor in the anticipated post-preservation cell loss and compensate by cryopreserving a significantly greater number of cells/samples. Does this affect the efficacy of the sample or therapeutic product? What about the associated costs? All of these components should be considered when preparing and performing your cryopreservation protocol.

Most cryopreservation procedures consist of a standard set of steps that one follows to obtain a frozen, preserved product. These steps generally consist of the following: 1) preparation of your cryopreservation cocktail, 2) addition of the solution to the cellular product, 3) freezing of the sample, 4) storage of the frozen sample, 5) thawing of the sample, 6) dilution or washing of the sample, and 7) yield or viability assessment of the sample (see Optimization Steps inset). While it would be difficult to completely change the process, some simple changes and adjustments to the current set of steps can result in significant improvements to cell recovery and product yield. The steps followed and the care taken when performing the steps plays an important role in dictating the true preservation of the product.

Suggestions to consider to improve cryopreservation process and product yield are listed in the Optimization Steps inset. The typical "in-house" cryopreservation cocktail traditionally consists of a cryoprotectant (DMSO, glycerol, etc.) within a liquid media (saline, culture media), sometimes supplemented with a non-defined serum of animal origin (fetal bovine serum). As a vehicle solution, cell culture media was developed for culturing cells, not preservation. Therefore, using a vehicle solution formulated for the preservation of cells can lead to improved cell recovery and yield. The concentration of the cryoprotectant used is another step that should be considered. For many, the standard application consists of a 10% DMSO concentration. DMSO is toxic to cells and the toxicity varies from one cell type to the next. A minimal range of concentrations can be evaluated, but the optimized concentration can result in significant improvements. The standard slow-step freezing rate is routine and generally effective, but a sometimes overlooked or unknown component is the ice seeding step. A seeding step should be included in all freezing protocols as this will decrease arbitrary ice-nucleation events and sample-to-sample variability. This results in overall improved sample viability. Lastly, a general step to improve overall cryopreservation yield is to take care when performing cryopreservation – this will aid significantly to sample consistency, reliability, and recovery.

OPTIMIZATION STEPS

Cryopreservation Vehicle Solution: *Replace standard culture media with intracellular-like solution*

Cell Specific DMSO Concentration: *10% is not always optimal – compare other concentrations (e.g. 2%, 5%, 10%)*

Pre-freeze Sample Holding Time: *DMSO is toxic to most cells so minimizing holding time can reduce cell stress*

Freezing Rate: *Apply a "seeding" or ice-nucleating step to reduce supercooling and intracellular-ice formation which improves sample consistency*

Post-thaw Sample Holding Time: *Samples should be processed immediately after the point of ice dissipation*

Post-thaw Analysis of Sample: *Immediate post-thaw analysis results in false positives; 24-hour measurements are ideal*

Summary

Cryopreservation is a necessary process and effective strategies are required to achieve optimal recovery and function. Unfortunately, standard methods are not effective for all biologics and some consideration for the cryopreservation aspect in product development should be performed. Current cryopreservation protocols can be significantly improved with limited optimization and appreciation for the steps and techniques involved. Remember, what you put in is more than likely not what you get out unless you take the time to consider the steps. Many steps should be considered to maximize the yield of your cryopreserved product.

EVOLUTION OF BEST PRACTICES IN BIOPRESERVATION

by Ian B. Nicoud, Ph.D.

PART 1: ORGAN PROCUREMENT AND PRESERVATION FOR TRANSPLANTATION

Perhaps the most well known application for preservation of biologic materials occurs in the setting of organ collection and transport for transplantation. The harvest and storage processes for each of the six major solid organs that are frequently harvested for transplantation (kidney, pancreas, liver, heart, lung, and intestines) have similarities and differences. This article is intended as a brief and general review of the best practices in the field, with a specific focus on the evolution of optimized preservation solutions.

The majority of cadaveric donor kidneys are collected during a multi-organ retrieval operation. This collection procedure has evolved from time consuming fine dissection and removal of individual warm organs in vivo to the current best practice of asanguinous, pre-chilled, no-touch en bloc removal of multiple organs. Individual procurement teams are assembled for the collection of each organ; a complex orchestration, as each team member performs initial dissection and isolation of the organ of interest.

Removal of the heart takes precedence over explantation of the other organs, followed by lungs, liver, small intestine or pancreas, and finally the kidneys. Exsanguination and arrest occur as the heart is perfused with a cold cardioplegic solution and vented through a small incision in the left atrial appendage. Pulmonoplegic solution is simultaneously introduced directly through the pulmonary artery and iced saline slush is applied topically to the thoracic cavity to enhance organ hypothermia. Once excised, the heart-lung is rinsed with cold saline, the lungs are inflated and the heart-lung is transported on ice. Once the thoracic organs are removed, the abdominal organs can all be perfused through the aorta, though the portal system is often flushed separately. The liver and pancreas are perfused via the superior mesenteric vein, and the gallbladder is often incised and flushed as well, since residual bile can damage the biliary epithelium. The organs are often removed en bloc and separated on the back table. Individually isolated organs are packed in preservation solution and transported on ice.

The perfusate for flushing and storing donor organs has been of particular interest to researchers because it affects the safe preservation time and has a direct effect on delayed graft rejection and long-term graft survival rates of organs following transplantation. Many solutions have been developed for this purpose and though there may be individual optimal solutions for each organ, it is widely accepted in the field that the most universal solution for organ perfusion and preservation during cold storage is the University of Wisconsin solution (also labeled as UW, Belzer solution, and Viaspan™). This solution has been shown to extend the safe preservation time for kidney, liver and pancreas; though in the heart and lungs, preservation efficacy is relatively unchanged[1,2]. The ability to extend safe cold storage times has a significant impact on the field, as it increases the available donor organ pool; however, it is important to note that the best practice remains using the donor organ as soon as possible after collection.

The improved preservation observed using UW is attributable to an optimized design, which was created in consideration of multiple observed mechanisms of preservation injury (Table 1)[2,3]. This concept of optimizing a preservation solution based on specific cellular and molecular events might seem obvious, perhaps due to the significance that preservation has on the efficacy of transplanted organs, and the associated magnitude of therapeutic benefit that transplantation offers. However, this mentality has only recently begun to resonate with a level of importance for the collection and preservation of tissues, blood products, and reproductive cells.

Table 1: Optimized Preservation Solution Design

MECHANISM OF INJURY	SOLUTION COMPONENT
Cell Swelling	Large moleclue impermeants *(e.g. hydroxyethyl starch, impermeant sugars, lactobionate)*
Interstitial Expansion	
Energy Depletion	Energy substrates *(e.g. adenosine, mannitol)*
Intracellular Acidosis/pH	pH buffers *(e.g. phospate, histidine, HEPES)* No glucose *(metabolizes to lactic acid)*
Reactive Oxygen Species	Free radical scavengers/inhibitors *(eg. allopurinol, glutathione, vitamin E derivatives)*

In the next issue of BPT, part 2 of this article series will focus on how the mindset and techniques applied to preservation of organs for transplantation can impact the current best practices for tissue collection and storage.

1 Norman and Suki. Primer on Transplantation. 1998 American Society of Transplant Physicians, Thorofare, NJ. Ch.24 Pre-Transplant Preparation of the Cadaver Donor/Organ Procurement, P. 201
2 Southard JH, Belzer FO. Organ Preservation. Annu Rev Med. 1995;46:235-47
3 Maathuis, M-H., Leuvenink, H., Ploeg, R. Transplantation. 2007;83(10):1289-1298

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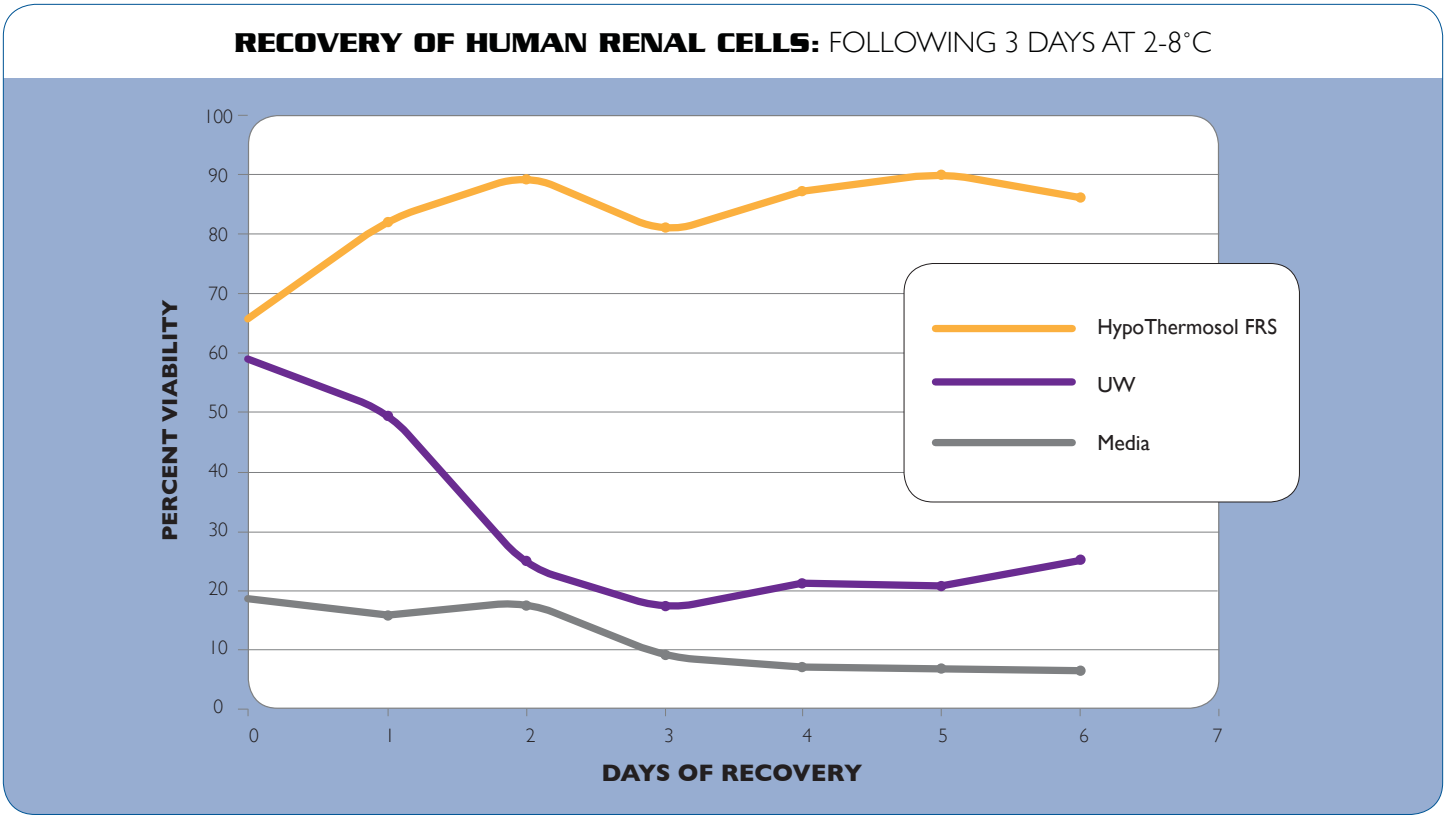

Figure 1. Human renal cells were subjected to 3 days of cold storage in preservation solutions and allowed to recover at 37°C for 6 days post-preservation. Cells were assayed for metabolic activity with alamarBlue during the recovery.

each of these assay methods will be critical to understanding the data that is produced.

OUR Standard. For many years, BioLife Solutions' scientists have implemented a system utilizing multiple assay methods conducted at multiple time points to better understand the health of cells as influenced by the biopreservation process. By conducting assays at multiple time points post-preservation, we identified a delayed decline in cell viability in certain conditions (Figure 1, UW *(ViaSpan)*) that would not have been detected immediately post-preservation (Figure 1, Day 0).

In addition, utilizing assays for membrane integrity that also allow for visual analysis provides qualitative information in addition to quantitative data (Figure 2).

Regardless of the specific methods used to assess "viability", the underlying function of the assays is to provide a means to accurately assess the health of the cell model. The validation of your viability assessment system, and its ability to reflect the efficacy of your biopreservation process, should be of critical

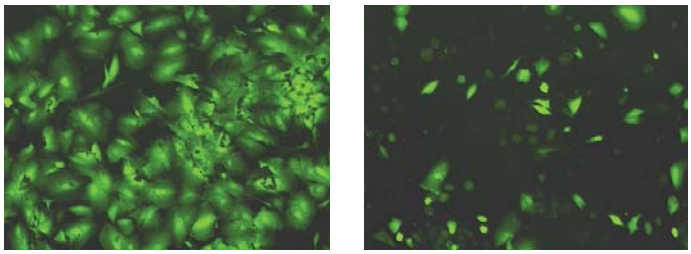

Figure 2. Human hepatocytes were subjected to 2 days of cold storage at 2-8°C in preservation solutions and allowed to recover at 37°C for 1 day post-preservation. Cells were assayed with the fluorescent membrane integrity indicator Calcein-AM. Cells in the left panel were preserved in HypoThermosol-FRS. Cells in the right panel were stored in UW/ViaSpan.

importance whether you are a basic science researcher or involved in the scale up of a commercial cell therapy product. Basic science is affected by lost time and lost cells from sub-optimal biopreservation. Cell therapy companies and transfusion labs may find that sub-optimal biopreservation can have a significant impact on the success of the clinical therapy, as well as affect the cost-efficiency of the delivery model. So again ask yourself, "What is my viability assay really saying"?

BioLife Solutions develops and markets patented hypothermic storage/transport and cryopreservation media products for cells, tissues, and organs. The Company's proprietary HypoThermosol® and CryoStor™ platform of biopreservation media products are marketed to academic research institutions, hospitals, and commercial companies involved in cell therapy, tissue engineering, cord