bpt BioPreservation Today®

Volume 1 - Issue 4 | FALL 2009

published by BioLife Solutions, Inc.

FEATURE ARTICLE
CASE STUDY: MODIFIED CRYOPRESERVATION OF UMBILICAL CORD BLOOD STEM CELLS AT CRYOBANKS INTERNATIONAL

What's Inside:

In This Issue

Bourbon Street Night, New Orleans
Photographer: Richard Nowitz

RESOURCE CENTER

www.aabb.org | *American Association of Blood Banks*

www.bestcollaborative.org | *Biomedical Excellence for Safer Transfusion (BEST) Collaborative*

www.marrow.org | *National Marrow Donor Program*

www.cordbloodforum.org | *Cord Blood Forum*

UPCOMING EVENTS

CHI Science of Biobanking *http://www.healthtech.com/bnk*	Philadelphia, PA	Nov 16-17, 2009
Biobanking 2010 *http://www.biobankingconference.com/Event.aspx?id=234052*	Philadelphia, PA	Jan 20-22, 2010
Phacilitate Cell & Gene Therapy Forum *http://www.phacilitate.co.uk/pages/cgtherapy/index.html*	Washington, D.C.	Jan 25-27, 2010
Biobanking Conference 2010 *http://www.bioportfolio.com/cgi-bin/acatalog/Biobanking-Conference-2010.html*	London, UK	Feb 4-5, 2010
ISBER 2010 Annual Meeting & Exhibits *http://www.isber.org/2010.html*	Rotterdam, Netherlands	May 10-14, 2010
ISCT Annual Meeting *http://www.celltherapysociety.org/Annual_Meeting/*	Philadelphia, PA	May 22-26, 2010

BioPreservation Today® is published quarterly by BioLife Solutions, inc.®, Bothell, WA. BioPreservation Today®, BioLife Solutions®, Powering the Preservation Sciences®, HypoThermosol® CryoStor,™ DLite, Preservation Chain,™ BloodStor,™ and the three ring logo are trademarks of BioLife Solutions, Inc. All other trademarks are the property of their respective owners. Please contact us via our website at: www.BioLifeSolutions.com.

EDITOR'S CORNER

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

Greetings from the 2009 AABB TXPO in New Orleans!

We have an expanded presence at this year's event, by moderating an educational session and facilitating two Learning Labs in addition to staffing our new corporate exhibit.

This issue of BioPreservation Today features an article authored in conjunction with Donald Hudspeth, BSCLS, MT (ASCP), General Manager and International Projects Manager at Cryobanks International. Don and Aby Mathew, Ph.D., our Senior Director, Strategic Relations & Senior Scientist, report the results of the comparative evaluation completed at Cryobanks that supported their adoption of CryoStor™ CS10 as a standard cord blood cryopreservation medium.

Also in this issue, Dominic Clarke, Ph.D., our Director of Research & Development, reports the results of an evaluation of HypoThermosol®-FRS by Dr. Erik Woods' team at General Biotechnology for use in extending stability of cord blood over a 72-hour period. While HypoThermosol is not formulated as classic ACD/CPD anti-coagulants, and contains no heparin, no clotting was observed during the evaluation. Two patent applications related to this work were recently published.

Finally, we recently launched our BloodStor™ biopreservation media product line. BloodStor 55-5 is a cGMP version of the standard 55% DMSO/5% Dextran-40 cord blood freeze medium. We're pleased to leverage our Quality System and internal cGMP production capacity to offer this traditional cryopreservation medium to cord blood banks around the world. BloodStor 55-5 is formulated using WFI quality water, USP grade DMSO, and USP grade Dextran.

For those readers attending AABB this year, please stop by our corporate exhibit in space 1736, or feel free to attend our Learning Labs or Educational Session (9102-S-CT). Thank you for your interest in our products.

Best regards,

CASE STUDY: MODIFIED CRYOPRESERVATION OF UMBILICAL CORD BLOOD STEM CELLS AT CRYOBANKS INTERNATIONAL

by Aby J. Mathew, PhD, Director of Strategic Relations and Senior Scientist, BioLife Solutions, Inc. and
Donald L. Hudspeth, BSCLS, MT(ASCP), General and International Projects Manager, Cryobanks International, Inc.

Aby J. Mathew, PhD

Donald L. Hudspeth, BSCLS, MT(ASCP)

Umbilical cord blood, which is typically discarded after birth, is a rich source of stem cells that have been transfused into thousands of patients to cure numerous fatal diseases. In recent years, cord blood transplants (CBT) have become widely recognized as a safe and effective alternative to traditional bone marrow transplants (BMT). Currently, umbilical cord blood stem cells can be used to treat over 75 life-threatening diseases, including leukemias, anemias, lymphomas, and inborn errors of metabolism. As scientists continue to discover new uses for umbilical cord blood stem cells, many other diseases could potentially be treated using these unique cells. There are currently over 700 clinical trials being performed for diseases such as type I diabetes, Alzheimer's, Parkinson's, stroke, paralysis, heart disease, and wound repair.[1]

The cryopreservation protocols for the process of long-term storage of cord blood derived stem cells have remained relatively unchanged since the first cord blood banking program commenced in 1992.[2] In general, after a cord blood unit (CBU) is red cell depleted, a concentrated leukocyte fraction is cryopreserved by gradual addition of a concentrated DMSO solution to obtain a final DMSO concentration of 10% by volume. The DMSO solutions often contain an osmotic stabilizer such as dextran and possibly a serum component (i.e., human serum albumin). Current CBU cryopreservation practices typically include preparation of a concentrated cryopreservation solution (2X or 5X) followed by a gradual (perhaps up to 15 minutes for each unit) addition of the concentrated solution to the final CBU. A slow, gradual addition is used to protect the cells from damaging osmotic stress due to high DMSO concentrations and the high exothermic reaction which occurs as the concentrated DMSO solution mixes with the cell solution. While traditional methods provide "acceptable" results, new approaches and methods for cryopreservation of CBU's should be considered which could result in: decreased preparation and processing time, improved post-thaw recovery and viability, improved consistency, reduced toxic DMSO concentrations, and elimination of serum and protein.

Cryobanks International, Inc., located in Altamonte Springs, Florida is a leader in the collection, processing, and banking of stem cells derived from umbilical cord blood. Cryobanks provides both public donation and personal storage programs. Cryobanks is a National Marrow Donor Program Network Cord Blood Bank, and is AABB accredited, FDA registered and cGMP/cGTP compliant. Cryobanks is also licensed by the states of New York, New Jersey and California for cord blood collection and/or processing. In addition, Cryobanks provides consulting and management

services for those interested in developing their own cord blood banking operations. **For more information about Cryobanks International call 1-800-869-8608 or visit their website at www.cryo-intl.com.**

Cryobanks evaluated the utilization of the intracellular-like cryopreservation media CryoStor CS10 (contains 10% DMSO) in comparison to the traditional 55% DMSO/5% Dextran-40 cryopreservation solution. This study also investigated a process modification of 1:1 dilution of CryoStor CS10 with the buffy coat (final DMSO concentration as 5%) as a comparison to the traditional 5:1 dilution of 55-5 DMSO-Dextran (final DMSO concentration as 10%). Figure 1 represents some of the data resulting from this study comparing CBU's processed with CryoStor CS10 1:1 method with historical data of CBU's processed with 55-5 DMSO-Dextran 5:1 method.

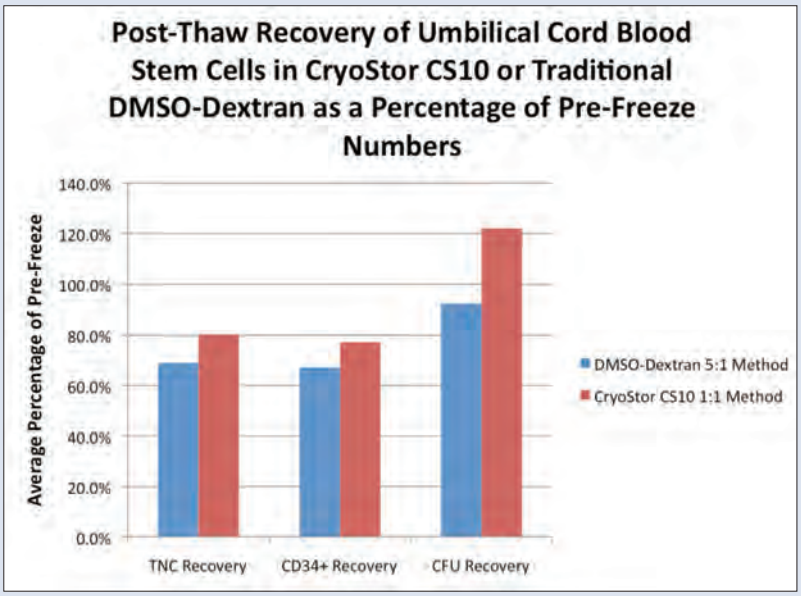


Figure 1 *Post-Thaw Recovery of Umbilical Cord Blood Stem Cells in CryoStor CS10 or Traditional DMSO-Dextran as a Percentage of Pre-Freeze Numbers. For all CryoStor CS10 parameters, n=39. For DMSO-Dextran, sample size for the following parameters was: TNC (n=71), CD34+ (n=49), CFU (n=14).*

As indicated in Figure 1, samples processed via the intracellular-like CryoStor CS10 1:1 method demonstrated equivalent/improved post-thaw recovery in comparison to the traditional extracellular-like DMSO-Dextran (55% DMSO/5% Dextran) 5:1 Method. Intracellular-like formulations are balanced to cells specifically under low temperature conditions such as hypothermic storage and cryopreservation, thereby reducing osmotic gradients leading to the onset of cell death via apoptosis, necrosis, and secondary necrosis. Improved control of osmotic gradients, as well as reduced DMSO concentrations, may also allow further process modification to eliminate the slow infusion of a high concentration DMSO such as in the traditional 55-5 DMSO-Dextran 5:1 Method. Further insight toward the rate of cryoprotectant infusion is discussed elsewhere in this publication, as well as presented at the AABB Annual Meeting & TXPO 2009 by Nicoud et al., Evaluation of Novel Cryopreservation Media and Methods for Cord Blood Stem Cell Banking. This reports the results of a study conducted in conjunction with the Puget Sound Blood Center (Seattle, WA). For review of improved cryopreservation using intracellular-like cryopreservation solutions, see publications by Clarke et al.[3] and Stylianou[4].

Methods continue to be developed for improved shipment, processing, and eventual therapeutic utilization of blood-derived cell products. These developments include evaluation of the shipment of the source blood (i.e., temperature, time, preservation media), isolation of cell products (i.e., Manual - hetastarch, Ficoll™, Percoll™, PrepaCyte®; Automated – Thermogenesis AXP™, Biosafe Sepax®), cell expansion protocols, cryopreservation solutions (i.e., 100% DMSO, 55% DMSO/5% Dextran, BloodStor™, CryoStor™), cryopreservation dilution (i.e., 5:1, 1:1, others), and including which parameters are most appropriate for therapeutic release (i.e., TNC, MNC, CFU, CD34+, recovery, viability, others). Currently, a fair level of variability exists in the methods and outcomes for each of the steps in the processing/application of cell products derived from cord blood (and even peripheral blood and bone marrow), and further understanding of the efficacy of each step towards the establishment of best practices or updated standards would be worthwhile for investigation.

1. *www.cryo-intl.com*
2. *Rubinstein et al., Processing and cryopreservation of placental/umbilical cord blood for unrelated bone marrow reconstitution. Proc. Natl. Acad. Sci. 92:10119-10122, 1995.*
3. *Clarke et al., Improved post-thaw recovery of peripheral blood stem/progenitor cells using a novel intracellular-like cryopreservation solution. Cytotherapy. 11(4):472-479, 2009.*
4. *Stylianou et al., Novel cryoprotectant significantly improves the post-thaw recovery and quality of HSC from CB. Cytotherapy. 8 (1): 57-61, 2006.*

BIOPRESERVATION LEARNING OPPORTUNITIES AT THE 2009 AABB ANNUAL MEETING & TXPO

Saturday, Oct 24 | 10:30am–12:00pm

EDUCATIONAL SESSION & DISCUSSION

(9102-S-CT) ROOM 288/289

FUNDAMENTALS/PITFALLS IN BIO-PRESERVATION OF CELL PRODUCTS AND NOVEL NEW TECHNOLOGIES

Director/Moderator
Aby J. Mathew, PhD

Faculty
Jason Acker, PhD; Erik Woods, PhD; Aby J. Mathew, PhD

Intended Audience
Physicians, Scientists, Managers/Supervisors

Event Description
The expanding arena for cell therapies utilizes cell products sourced from blood, bone marrow, and tissues. The success/efficiency of the cell therapy is potentially affected by the periods of preservation, which include transport of the source material and preservation of the final cell therapy product. This workshop will address the science behind current biopreservation, the pitfalls that are both recognized and often unrecognized, and new technologies that improve the final cell therapy product in terms of efficacy, quality/regulatory and cost efficiency.

Objectives

- Review the science behind current cryopreservation/ biopreservation methodologies.
- Explain current practice of red blood cell and cord blood stem cell preservation, and potential issues resulting in sub-optimal systems with potential clinical impact on cell therapies.
- Present new technologies that may potentially improve the landscape of cell preservation for cell therapy.

Monday, Oct 26 | 10:30-11:30am
Monday, Oct 26 | 3:30-4:30pm

LEARNING LAB SESSIONS

ALTERNATIVE CRYOPRESERVATION MEDIA AND METHODS FOR CORD BLOOD STEM CELL BANKING

Facilitator
Dominic M. Clarke, PhD

Event Description
Historical cryopreservation of blood-based stem cell products has typically utilized the slow addition of 50% DMSO solutions to achieve a final concentration of 10% DMSO in a volume of 25ml. Although DMSO is an essential component of cryopreservation media, the vehicle for DMSO is just as important. Optimized biopreservation media that are pre-formulated, serum and protein free, and fully-defined are commercially available, but are not amenable to a traditional 5:1 dilution scheme.

This interactive learning lab will introduce concepts of biopreservation and demonstrate processing methods for the incorporation of optimized biopreservation media that can improve post-preservation recovery and viability while streamlining workflow.

Commander's Palace Jazz Brunch, New Orleans
Photographer: Richard Nowitz

IMPROVED METHOD FOR COLLECTION AND STABILITY OF UMBILICAL CORD BLOOD PRIOR TO PROCESSING

by Dominic M. Clarke, Ph.D., Director of Research and Development, BioLife Solutions, Inc.

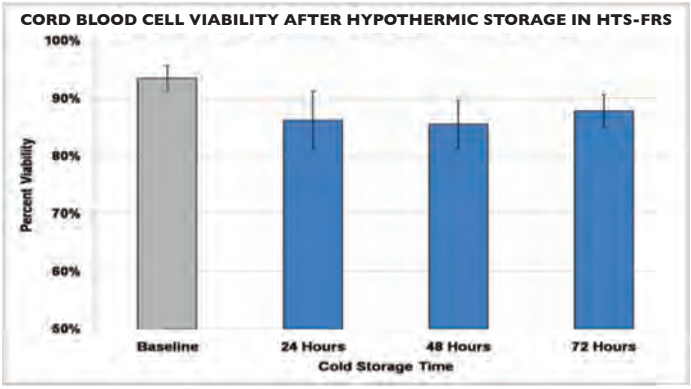
Biological integrity and usability of birth tissue such as placental and umbilical cord blood and tissue begin to decline postpartum, thereby rendering them essentially unusable as a source of viable tissues and/or cells within minutes to a few hours. Therefore, in the many cases where trained collection personnel are not on staff or must travel to be on site at the time of delivery, improved the collection and stability methods for preservation of these birth tissues are required to improve stem cell recovery and/or tissue viability.

Current collection and preservation processing practices for blood cell products, including umbilical cord blood units, typically include collection into a bag containing ACD/CPD followed by transport to a processing center. Without the addition of an appropriate transport/storage solution, rapid processing of the units is required to prevent further degradation of the cellular components needed for eventual transfusion. Therefore, improving collection and hypothermic storage practices can have a significant effect on blood and tissue units in the following aspects:

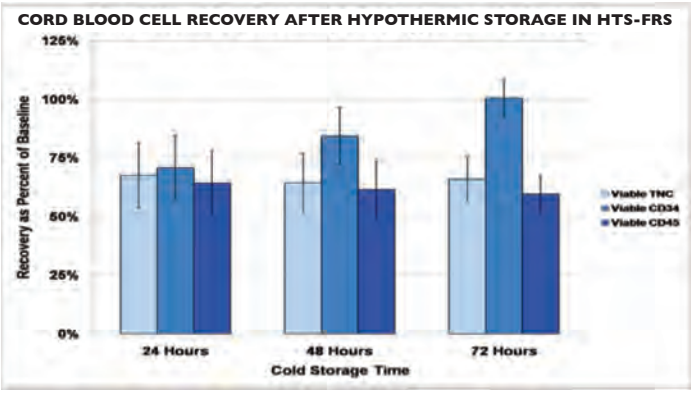
- improved sample storage and stability
- improved collection logistics
- improved yield and viability of important stem cells
- increased blood unit availability
- enhanced safety and quality improvement
- decreased sample loss due to expiration and transport

In a recent study, the use of the hypothermic storage solution, HypoThermosol® FRS (HTS-FRS; BioLife Solutions, Bothell, WA) was investigated to determine the feasibility and efficacy for collection and to extend stability of human cord blood. Umbilical cord blood was collected into collection bags (Pall Medical) that had been pre-filled with 35 ml of cold (4°C) HTS-FRS. The units were stored for up to 72-hours at 4°C and were monitored every 24-hours for coagulation by visual determination of clot formation. After 24, 48 and 72-hours storage at 4°C, a 25 ml sample of the cord blood was removed for processing.

Results of this study demonstrate that the hypothermic storage solution, HypoThermosol, can be used effectively for both collection and storage of umbilical cord blood and for subsequent sedimentation methods. No external CPD/anticoagulant was used during collection and no clotting was observed over the entire investigated time course. Minimal decrease in viability was observed when compared with baseline and overall viability; TNC, CD34+, and CD45+ recoveries were maintained over the 72-hours of storage. In summary, cord blood units can be stored for extended lengths of time and the stability of the cellular components is maintained. The described method may be used to improve blood unit availability and overall quality.



Maintenance of cellular viability during hypothermic (2-8°C) storage of cord blood in HTS-FRS without anti-coagulant over 72 hours. Umbilical cord blood was collected at the time of C-section into collection bags that had been pre-filled with 35 ml of HTS-FRS at 2-8°C. No clotting was observed and viabilities were maintained over 72 hours of cold storage.



Recovery of viable nucleated cells, and CD34+ and CD45+ cells from cord blood units following hypothermic storage in HTS-FRS. A 25 ml sample was removed from cold storage (2-8°C) units at 24, 48, and 72 hours; TNC, viability, CD34+ and CD45+ were assessed by flow cytometry. Compared to baseline (time of collection), viable TNC, CD34+, and CD45+ cell counts decreased slightly within the first 24 hours, but stabilized for the remainder of the 72 hour cold storage.

PRESERVATION CHAIN™ MEDIA PRODUCTS

Source Material — Cell Products

Make BloodStor,™ CryoStor,™ and HypoThermosol® Your Strongest Links!

INTRODUCING BLOODSTOR™ 55-5

BioLife Solutions recently announced the launch of its BloodStor biopreservation media product platform. The product family includes BloodStor™ 55-5 for cryopreservation of umbilical cord blood stem cells, as well as other variants for peripheral blood derived stem cells. BloodStor 55-5, packaged in standard, single-use sterile vials of various fill volumes, is formulated with 55% USP grade DMSO and 5% USP grade Dextran-40 in injection quality water (WFI) and supports a common cord blood processing protocol.

Mike Rice, BioLife's chairman and CEO, noted, "The launch of our BloodStor product family supports our mission to become the leading provider of preservation tools for cells, tissues and organs. We anticipate that BloodStor will be widely utilized to address the increasing demand for preservation media products in the rapidly growing cord blood banking industry. BioLife's quality system and internal manufacturing facility's capacity can offer additional standard and custom products to strategic markets."

BioLife's manufacturing facility and quality system are compliant with 21 CFR part 820 - Quality System Regulation for Good Manufacturing Practices (GMP) of medical devices, 21 CFR parts 210 and 211 covering GMP for Aseptic Production, Volume 4, EU Guidelines, Annex 1 for the Manufacture of Sterile Medicinal Products, and ISO14644 for Clean Rooms and Associated Controlled Environments. The Company expects to achieve the ISO13485 medical device quality management systems certification by the end of 2009.

All BloodStor products are tested for sterility to USP <71>, endotoxin to USP <85>, pH, appearance, and cell-based preservation efficacy.

VISIT BIOLIFE AT THE AABB ANNUAL MEETING & TXPO 2009!
Look for **Booth #1736**, and for the BioLife **Interactive Learning Labs**:
Monday, Oct 26 | 10:30-11:30am and 3:30-4:30pm

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.

www.biolifesolutions.com | For more information, please call: 425.402.1400.

BioLife Solutions
POWERING THE PRESERVATION SCIENCES