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At Community Blood Services
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Evaluation of a new LN_2 Heat Exchange (LNHE), Dry, Ultra-Low Freezer System

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INTRODUCTION

Heat labile coagulation molecules found in frozen plasma remain viable for 12 months if stored at temperatures of ≤ -18°C and for 7 years if stored at ≤ -65°C. Expiration dating for frozen red blood cells (RBCs) stored at ≤ -65°C is 10 years.

Hematopoietic progenitor cells (HPC) found in marrow, peripheral blood and umbilical cord blood (UCB) require frozen storage at temperatures of ≤ -150°C. Biological and chemical activity can persist as long as water activity exists; however, below -130°C all activity ceases. A temperature below the glass transition temperature of water, the temperature at which all biological activity ceases, is generally accepted as between -130°C and -135°C and is a significant temperature for long term frozen storage of hematopoietic progenitor cells . Transient Warming Events (TWE's), a rapid increase in temperature from -196°C to approximately -40°C, may result in up to 30% decrease in stem cell viability after thawing.

Mechanical freezers use compressors and refrigerants to provide the heat exchange required for reducing the temperature within a freezer. Current mechanical freezers are designed to maintain temperatures within a variety of ranges, depending upon specific product storage requirements (-40°C, -86°C, -140°C and -150°C). These types of air-cooled refrigeration systems generate a large amount of heat, to the extent that additional air conditioning may be required for rooms that have multiple units. Additionally, they require periodic defrosting and usually generate a lot of noise.

An alternative to air-cooled refrigeration units for creating low temperatures is by using nitrogen (N_2). The temperature of liquid nitrogen (LN_2) is -196°C while the vapor phase averages -140° C. Currently available LN_2 storage units consist of insulated tanks (dewars) with vertical storage racks. The racks are filled with storage boxes containing vials or bags of cells and lowered into the LN_2 filled tank. Over time LN_2 transitions into vapor which then dissipates into the atmosphere. Mechanical sensors are used to assure a continuous level of LN_2. Although this system does not require additional air conditioning or defrosting, there are safety hazards associated with LN_2 dissipated into the atmosphere as well as handling racks and cartons dripping LN_2. Computer controlled robotic systems have been developed to overcome the hazards associated with manually handling LN_2 soaked samples. Another hazard associated with storage in LN_2 is potential cross contamination between samples with either viral particles or genetic materials.

Recently a new method has been developed that using LN_2 as a heat exchange material to obtain ultra-low temperatures. Liquid Nitrogen Heat Exchange (LNHE) freezers overcome the noise and heat generation associated with mechanical freezers as well as the hazards associated with the LN_2 freezers.

We evaluated the BioStor CryoLogistic Storage unit (CLS) large capacity, -150°C, LNHE freezer and logistics system for frozen storage of Umbilical Cord Blood (UCB) units.

METHODS

Ten units of UCB donated for research were processed according to standard laboratory procedures. Each unit was red cell depleted using 1.5% HES and the supernatant containing hematopoietic progenitor cells and white cells was centrifuged at 22°C using 400g for 15 minutes. The hematopoietic progenitor cells/white cell pellet was resuspended in autologous plasma and then divided equally between two freezing bags and frozen in the presence of 10% DMSO. Half of the samples were stored for 30 days in our standard liquid phase nitrogen freezer (LPF) and the paired samples were stored in the CLS unit. The lid of the CLS was opened twice each workday (total 44 times) for 2 minutes to simulate normal activity. Automated air temperature monitoring is provided at three points in the CLS freezer: Temperature Probe Top (TPT) at the door above a thermal barrier allows for a thermal record of when the unit is opened, Temperature Probe Bottom (TPB) of the unit and Temperature Probe Center (TPC) at the center of the unit controls the addition of LN_2. All units were thawed in pairs, one set at a time. Upon thawing, units were sampled and analyzed, without washing, for CBC, Total Nucleated Cell counts (TNC) and cell recoveries, percent CD 34+ cells and 7-amino actinomycin D (7AAD) viability. Total Colony Forming Units (CFU's) were determined by plating 25,000 cells on Stem Cell Technology media 4434 per dish in triplicate and incubated 14 days under 5% CO_2 at 37 °C. All colonies with minimum 50 cells per colony were counted.

Multiple configurations are available. Cascade design allow liquid nitrogen vented by –150°C to support –80°C and then –40°C freezers

RESULTS

1. Percent recovery of Total Nucleated Cells was almost identical and not statistically significant when comparing the two different storage systems.
2. Recoveries of CD34+ cell were 75.5% and 73.4 % in LPF and LNHE respectively when compared to the pre-freezing results, but ware not statistically significant.
3. Viability of the cells stored in the CryoLogistic freezer was no different then those stored in liquid nitrogen for this same period of time.
4. The difference in number of colonies generated by the cells under these two different conditions was not statistically significant.

Table I.
Results of Post Thaw Cell Recovery and Viability

Test Method	LPF Storage*	CLS Storage*	Statistical Difference
TNC (%) Recovery	98.5 ± 1.8	98.6 ± 1.6	NS
CD 34+ (%) Recovery	75.3 ± 12.3	73.4 ± 0.6	NS
Viability-7AAD (%)	86.6 ± 3.96	86.1 ± 4.3	NS
Total CFU x 10^8	0.49 ± 0.31	0.53 ± 0.33	NS

* mean ± sd

Table II. Temperature Monitoring
Results of the CLS Unit

	Temp Probe Top (TPT)	Temp Probe Bottom (TPB)	Temp Probe Center (TPC)
Ave**	-151.38	-156.43	-156.42
Max.	-113.33	-149.58	-149.40
Min.	-168.81	-173.45	-174.06

** 47,493 data points

DISCUSSION

The BioStor CLS series of deep storage freezers, available in -40°C, -80°C and -150°C configurations, are designed to reduce temperature fluctuation of stored materials. This is accomplished in four ways. First, the CLS units use a liquid nitrogen heat exchange dry storage environment. Second, the rotating drum containing the storage cassettes allows quick and easy access to samples/products. Third, the computer controlled product management program allows rapid access to samples/products. And fourth, the units have been designed to use a "thermal barrier" or "thermal break" at the access door. The thermal barrier is a layer of super-cooled air that prevents warmer, room air, from affecting the temperature of any stored material. The break is accomplished by positioning the sample/product storage drum below the open plane of the door. The door, angled to reduce the air space above the thermal barrier, allows easy access to the storage cassettes in the drum. The Temperature Probe Top, one of three temperature probes in the CLS units, records the temperature above the thermal barrier. This allows for a thermal record of when the unit is opened. The top probe is intentionally exposed to the affects of ambient room air when the unit is opened. It will record a rise in temperature corresponding to the time that the door to the unit is opened. At the same time, the middle and bottom probes (which record the temperature below the thermal barrier where the material is stored) will measure temperature fluctuations of the air around the samples/product. In addition to adding to the temperature stability of the stored material, the thermal barrier reduces the risk of condensation entering the storage vessel, which prevents frost and ice accumulation. The CLS should not have to be defrosted in normal usage.

CONCLUSIONS

1. The BioStor CLS large volume storage freezer meets required criteria for frozen storage of Hematopoietic Progenitor Cells (HPC) and other biological materials.
2. The BioStor CLS can accommodate approximately 11,000 to 12,000 samples of 25 ml Pall freezing canisters or equivalent.
3. CLS units are cooled using liquid nitrogen. An 110V electrical supply is necessary to support the computer and barcode reader, the tracking function and the automatic drum rotation system. A 24V battery back-up system is sufficient to support the electromagnetic valve and the internal temperature-recording device. Absent an 110V supply, the drum can be rotated manually with a hand crank. In the event of a total electrical failure, the CLS has a by-pass valve that can feed LN_2 directly into the heat exchanger. Cooling can continue indefinitely so long as a supply of LN_2 is available. The unit will stay at temperature for a prolonged period without an LN_2 supply depending upon climate, capacity and usage.
4. While LN_2 consumption was not a focus of this study, in a worst-case scenario (an un-insulated LN_2 delivery system using 180 liter tanks hooked to a manifold system configured more for ease of tank replacement than LN_2 usage optimization), the CLS consumed approximately 3000 gallons in 60 days at -155C. The usage rate of LN_2 varies directly with the design, size and configuration of the LN_2 storage and delivery system.