Cell stability in leukopaks at different shipping temperatures

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Introduction

Human peripheral blood mononuclear cells (PBMCs) are an integral part of gene therapy and regenerative medicine research[1]. PBMCs contain highly diverse immune cell populations, which include lymphocytes such as CD4+ and CD8+ T cells, B cells, natural killer (NK) cells, monocytes which differentiate into macrophages and dendritic cells, and a small percentage of stem cells[2,3].

Leukopaks (the product of leukapheresis collections) are the preferred source of PBMCs. They are rich in white blood cells and can reduce variability because they are derived from a single donor. The conditions used to store and transport leukopaks and other biospecimens influence the quality of the product, measured not only as total cell numbers and viability, but also immune cell population diversity.

Using fresh leukopaks as starting materials expedites critical research and development, especially when the leukopak collection and processing sites are in close proximity. However, this is not always possible, and long transit times may be required[4]. Local distribution of fresh leukopaks can take up to 12 hr for delivery, while country-wide distribution takes 24 to 48 hr, and longer for international shipping.

Previous studies analyzed the effects of temperature and time on viability, morphology, phenotype retention, metabolism (pH, glucose, O_2), mitochondrial DNA integrity, mitochondrial reactive oxygen species, membrane damage, and cytokine secretion in PBMCs and other cell lines[4–9]. PBMC activation by lipopolysaccharide and heat-killed Staphylococcus aureus was slightly affected by 24 hr ambient shipping, and completely abrogated by 48 hr[4].

Shipping fresh versus frozen products has the advantage of preventing cellular damage that occurs as a result of osmotic pressure changes during cryopreservation[8]. However delays in product delivery can lead to temperature changes in the leukopak, which may affect viability and alter metabolism and microtubule integrity[8,10]. Controlled shipping conditions that limit temperature changes in leukopak shipments will maintain the undifferentiated phenotype of white blood cells, which is important for the development of novel therapies and the success of treatments[7].

Ensuring proper storage and shipping conditions for cell and gene therapy products is paramount to the design, repeatability and reproducibility of research, development, product design and therapy outcomes. For this purpose, we studied the condition of white blood cells in leukopak products. We analyzed cell concentrations, viability, and differential leukocyte counts (DLC) during five days storage at room temperature and 4°C to mimic shipping conditions.

Study Design

Temperature to store and mimic shipping conditions.

Three healthy donors were randomly selected for leukapheresis. On the day of collection (0 hr), viability, complete blood counts (CBC) and DLC were obtained. From the remaining leukopak volume, 20 individual 1.5 mL aliquots were placed inside 2 mL vials. Ten vials were placed inside room temperature shipping boxes (Intelsius PHT (15-25) 3.5L 60 hr temperature boxes) and 10 vials inside cold shipping boxes (NanoCool™ Cooling System 2-8°C 1.05L shipping boxes) (Fig.1A-B). Cold boxes were activated and immediately closed. The temperature was monitored using ITAG Ttemperature data loggers (www.itag4.com) placed inside each box adjacent to the leukopak samples and set at 1-minute interval reads. Each day, two vials were quickly removed from each box. One vial was used for CBC analysis and one for PBMC purification and viability calculations using a standard Trypan Blue Exclusion assay.

Fig.1. Images of ambient and cold shipping boxes with temperature monitoring during 5 days of shipping-mimicked conditions.

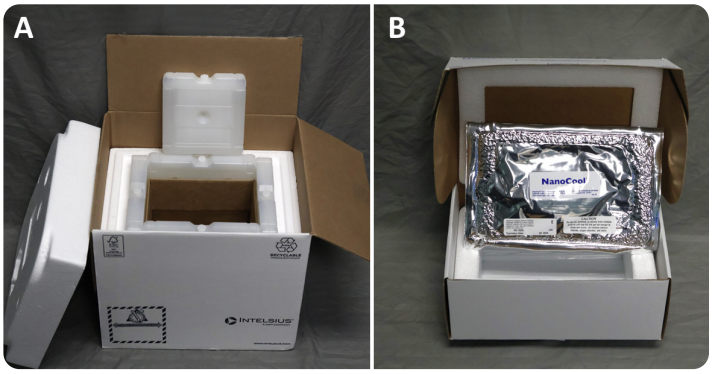

Fig. 1 (A) Intelsius PHT (15-25) 3.5L 60 hr temperature box.
Fig. 1 (B) NanoCool™ Cooling System 2 - 8°C 1.05L shipping box.

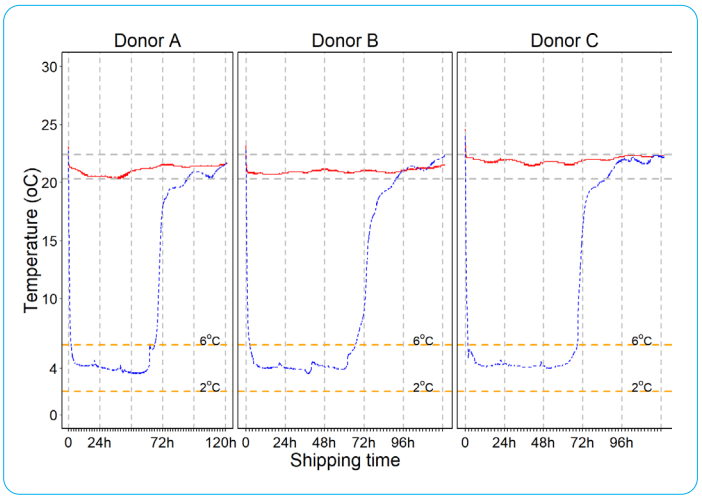

Fig. 1. Temperature curves for room temperature and cold shipping boxes captured every minute for 5 days using ITAG temperature data loggers. The ambient boxes were able to maintain constant temperatures between 20.3°C and 22.4°C during the length of the study (red lines), with a mean of 21.3°C (SD 0.52). After activation of the NanoCool contents, the temperature inside the cold boxes (blue lines) dropped from room temperature to below 6°C in 2 hr, and it was maintained below 6°C for about 2.7 days (mean 67 hr), reaching room temperature on day 3. The mean temperature during the critical shipping time (below 6°C and above 2°C) was 4.2°C (SD 0.5).

Effects of temperature storage on viability of freshly purified PBMCs

Using robust cellular starting material for research and development is critical for the successful advancement of novel therapies. The first measure for quality control (QC) is the percentage of viable cells in a product volume, and for industry standards, viabilities ≥90% are preferred. On days one through five, one leukopak sample was quickly removed from the ambient boxes and from the activated cold boxes per day and used for PBMC purification and viability analysis (Fig. 2).

Up to 24 hr, viability is relatively stable and similar between the two temperature conditions (no significant difference in the means). At 24 hr, viability starts a rapid decrease in the room temperature samples, reaching an average of 75.4% (SD 11.6) at 48 hr, while cold conditions maintained a viability of 97.3% (SD 2.3). The difference between the two conditions is significant, (p=0.018).

By 72 hr, viability remained stable in cold conditions (mean 96.6%, SD 1.4) while viability in ambient conditions dropped to even lower levels (mean 60.5%, SD 13.2). The difference between these two conditions is now strongly significant (p<0.0001). After 72 hr, the viability in the cold boxes started to drop, reaching 80.2% by 96 hr. This reduction in viability correlates with the rapid increase in temperature observed inside the cold boxes during the same period (Fig.1.). At day 5 (120 hr), viability dropped to 36.7% and 9.8% in the cold and ambient boxes respectively. Since the drop in viability is significant by day 4 (96 hr), shipping is not recommended for more than 72 hr. The statistical analysis focuses on the first 72 hr, the critical time for biological material shipping and delivery.

Fig. 2. Temperature conditions influence the viability of freshly purified PBMCs during leukopak shipping.

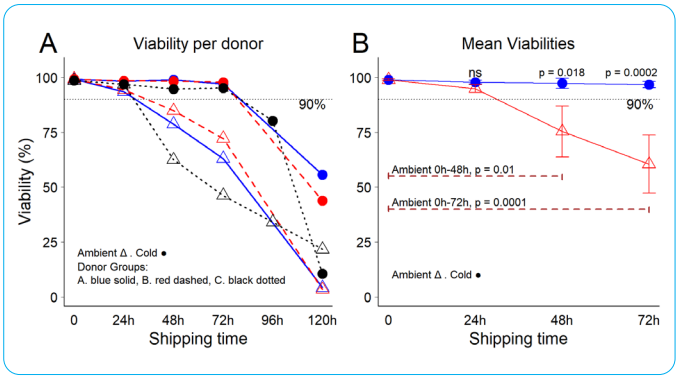

Fig. 2. (A) PBMC viability per donor and per shipping time. (B) Mean viabilities per temperature and per shipping time. Error bars represent one standard deviation from the mean. Statistical analysis performed using ANOVA with post hoc Tukey's Honestly Significant Difference Test.

Ambient temperature affects the lymphocyte to monocyte ratios during shipping

The number of white blood cells received in leukopaks is an important measure of QC in the gene therapy industry. Lymphocytes are the most important component of the immune system, including critical cell populations such as CD4+ and CD8+ T cells, NK cells, and antigen presenting CD19+ B cells. Monocytes, which differentiate into macrophages and dendritic cells[2], are another important component of the immune system involved in the phagocytosis of pathogens. Complete cell blood cell counts with DLC were obtained and analyzed from three independent leukopak samples, for each day of shipping-mimicked conditions using ambient and cold (4°C) boxes. Although white blood cell concentrations are not affected by time or temperature conditions during shipping (Fig 3), other differences in DLC were observed over time at different shipping temperatures.

At zero hour (collection day) only one read was obtained per sample. At 24 hr, 48 hr, and 72 hr, two readings were obtained per sample, one from ambient shipping boxes and one from cold shipping boxes. At 24 hr, when the differences in mean viabilities between ambient and cold shipping are not significant, we observed an increase in the percentage of lymphocytes and a decrease in the percentage of monocytes in ambient shipping (Fig.4). These tendencies continue at 48 and 72 hr, clearly separating the two temperature conditions. This is a relative percentage value that does not represent an increase in lymphocyte cell numbers (data not presented).

Following these observations, the ratio of lymphocyte percentage over monocyte percentage (Lym/Mon) was calculated per condition, donor, and shipping day (Fig. 5). The Lym/Mon ratio was stable during the first 72 hr in cold boxes, then it rapidly increases as the temperature in the cold boxes increases (Fig. 1.) and data not shown. On ambient shipping, the Lym/Mon ratio shows a small but steady increase in the first 48 hr, rapidly jumping at 72 hr. The difference is not significant at 24 and 48 hr, but it is strongly significant at 72 hr (P<0.0001). Even though the differences at 24 and 48 hr are not significant, the changes in Lym/Mon ratio suggest cellular and molecular effects[4]. These changes, albeit small, might influence the quality of the immune cells and compromise their use. This possibility needs to be further analyzed.

Fig. 3. White blood cell concentrations are not affected by time or temperature conditions during shipping.

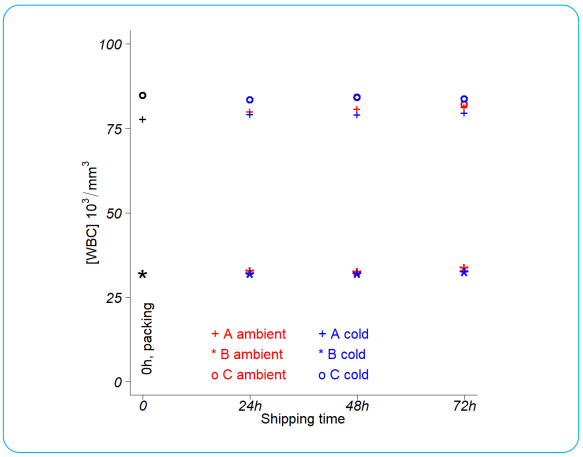

Fig. 3. Total white blood cell counts were obtained every day from three independent leukopak samples (donors A, B, and C) using a CBC Analyzer (Horiba). At zero hr (packing day) only one read was obtained per sample. At 24, 48, and 72 hr, two readings were obtained per sample, one from ambient shipping boxes and one from cold shipping boxes.

Fig 4. Temperature conditions (ambient versus 4°C) influence the lymphocyte and monocyte composition of leukopaks during shipping.

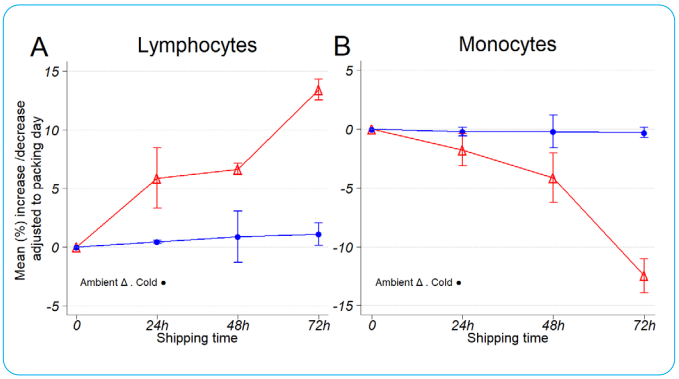

Fig. 4. CBC and DLC were obtained every day from three independent leukopak samples (donors A, B, and C). (A) Mean increase/decrease of lymphocytes per shipping day, adjusted to collection day. (B) Mean increase/decrease of monocytes per shipping day, adjusted to collection day. Error bars represent one standard deviation from the mean.

Fig: 5. Effects of time and temperature on lymphocyte to monocyte ratios.

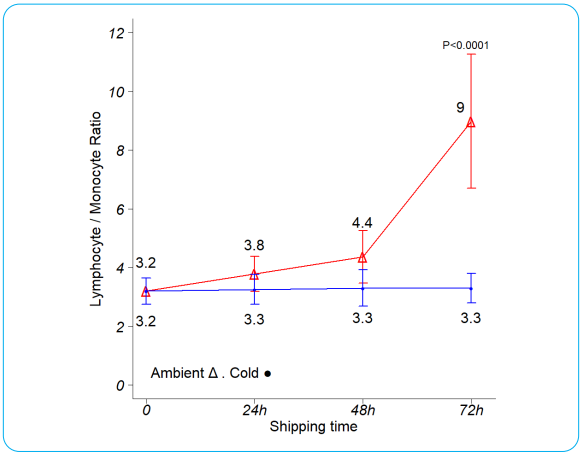

Fig. 5. Shipping conditions were mimicked using boxes stored at ambient temperature and 4°C. Analysis was conducted using three independent leukopak samples. CBC and DLC were obtained every day, and the Lym/Mon ratio was calculated per donor, per shipping time, and per storage temperature. The data points on the graph display the mean Lym/Mon ratio obtained during the study, error bars represent one standard deviation from the mean. ANOVA with post hoc Tukey's Honestly Significant Difference Test.

Conclusions

In the present study, we analyzed the effects of temperature during shipping of freshly collected leukopaks. Two shipping conditions were tested: ambient and 4°C. Fig. 1 shows that the ambient boxes hold a stable temperature of approximately 21°C during the five days of the shipping-mimicked study. The cold boxes rapidly equilibrated and remained at 4°C for roughly 72 hr, followed by a rapid increase to ambient levels at later time points.

This study also demonstrates that the viability of PBMCs remains above 95% for 72 hr in the cold shipping boxes. However, the viability quickly drops to 80% at 96 hr, and further decreases were observed after that time point (Fig. 2. A and 2. B, circles). At ambient temperature of 21°C, the viability of PBMCs dropped to 94% by 24 hr, and then to 75% by 48 hr, followed by further decreases after that time point (Fig. 2. A and 2. B, triangles). Due to the rapid decrease in viability in the first two days, the ambient shipping method should be carefully considered and may not be the ideal condition to ensure proper cell health. A drop of ~4% in viability was observed at 24 hr; however, the boxes were maintained at a constant temperature in the laboratory (mean of 21.3°C). During the actual shipping process, temperature conditions may vary due to transportation and environmental conditions, box manipulation, etc.

This study also shows the effects of temperature and time on the Lym/Mon ratio. Even with a small drop in viability at 24 hr, a 5.9% increase in lymphocytes and a 1.77% decrease in monocytes was observed in leukopaks maintained at ambient temperature. A similar pattern was observed after PBMC purification (1.53% increase in lymphocytes and 0.42% decrease in monocytes, data not shown). These changes are more evident at 48 hr (Lym/Mon ratio = 4.4), and especially at 72 hr (Lym/Mon ratio = 9).

Ambient shipping should only be considered for same day local destinations. For overnight and longer shipping times, cold shipping is strongly recommended to avoid negative effects from unexpected transit delays. The decrease in monocyte percentages at 24 hr of ambient shipping may be an indication of unhealthy cells, or it could also be due to adhesion to plastic surfaces. In blood, monocytes adhere to epithelia to migrate to tissues where they are activated by CD4+ Th1 and CD8+ T cells[2]. Further studies comparing ambient and cold shipping with an assessment of lymphocyte and monocyte activation, via cytokine and chemokine release, signaling pathways activation, and/or RNA profiling, could be considered for next steps. When using cold shipping, we observed that viability, cell counts, and Lym/Mon ratios were well maintained up to 72 hr.

As supported by these studies, BioIVT endeavors to preserve the quality of our cell products for up to 72 hr when shipped using our NanoCool™ Cooling System shipping containers. Even with overnight shipping, we highly recommend cold conditions for optimal cell health. Following these recommendations, researchers can prevent unexpected changes to leukopak cell populations and avoid testing failures.

References

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