Transmitting Warming Rates of 250mL Cryopreservation Bags

OVERVIEW

Exposure of cryopreserved biological material to ambient temperatures may reduce the functionality and viability of the stored material, particularly when sample temperature rises above the glass transition temperature (TG) of the material. Most commonly, samples experience exposure to ambient temperatures during processing, shipment, and transfer between storage containers. It is important to understand how different types of samples warm and at what rate they warm to inform best practices for sample handling and storage. The effect of ambient temperature exposure on non-targeted samples (innocent samples) contained in a shared rack is also of interest as the rack must be both removed and replaced into storage, leading to two transient warming events (TWEs) experienced by the non-targeted samples. Previous experiments have been conducted to characterize the warming rate of various sizes and types of vials, but little information was found regarding warming rates in cryopreservation bags commonly used in CAR-T therapies. This work will demonstrate the warming rate of cryopreservation bags and cassettes when removed from LN2 vapor storage and replaced after removing a targeted cassette. It will also illustrate the elastic warming rates for cassettes after they are replaced into an LN2 vapor environment.

OBJECTIVES

1. Characterize the temperature profiles of 250mL blood bags filled with 70mL of liquid during sample extraction using automation and manual methods.
2. Quantify the warming rates for non-targeted cassettes in a shared rack or frame.
3. Compare and contrast the temperature profiles of samples extracted manually to those extracted with automation.

METHODS

Omega brand Thermocouples were placed inside of OriGen GS250 cryopreservation bags. The bags were filled with 70mL of colored water using a syringe to simulate blood or other biological fluids. The thermocouples were then sealed inside of the fluid-filled bags using hot glue. Each bag and thermocouple were then placed inside a Custom BioGenics ZC021 cassette. All probes were plugged into a data logger and temperature data was taken every second for the duration of each test.

Automated Extraction: A plot of the temperature over time during a manual extraction shows how the non-targeted sample in the frame is exposed twice to ambient temperatures: Once when the frame is first removed from the freezer and placed into the sample preservation cart, and once more when the frame is removed from the preservation cart and replaced into the freezer (Fig. 3). From the time the freezer lid was opened to when the samples were placed into the sample preservation cart, there was a rise in temperature of 16.3 °C. From the time the samples were removed from the cart and the lid was closed, there was an additional ΔT of 9.5 °C. After the samples were returned to the freezer and the lid was closed, there was a final rise in temperature of 1.1 °C due to elastic warming. In total, there was a maximum ΔT of 26.9 °C from the moment the freezer lid opened to when maximum temperature was reached.

CONCLUSIONS

Temperature Profiles: As shown in figure 5, the total change in temperature for a non-targeted sample extracted using automation in an extreme use case was approximately 85% lower than that of a non-targeted sample extracted using standard manual methods. This is likely due to the second exposure of non-targeted samples during the return to the freezer from the preservation cart and to the increase in time required to extract the rack manually. It was also found that the elastic warming of a non-targeted sample was greater using automation than using manual methods. This is likely due to the difference in the two styles of racks. The manual frame has a much lower thermal mass due to its smaller size and lower number of shelves. The greater thermal mass of the automation-friendly rack led to an increased elastic warming, but also a decreased total ΔT for the sample.

Warming Rates: The warming rate for a non-targeted sample during a standard manual extraction was 12.0°C/min in the linear region, with an additional average elastic warming rate of 3.69 °C/min. The warming rate for a non-targeted sample during normal automated extraction was 0.75°C/min in the linear region, with an additional average elastic warming rate of 0.62°C/min. Examining the linear region of the temperature profile, it will take a sample extracted using automated methods more than 16 times as long to reach TG than a sample extracted using standard manual methods. The average elastic warming rate for cryopreservation bags is also smaller than that measured using other sample types often used in cryopreservation storage, such as a rack of vials. This is likely due to the large size of the sample and the thermal conductivity of the cassette the sample is stored inside.