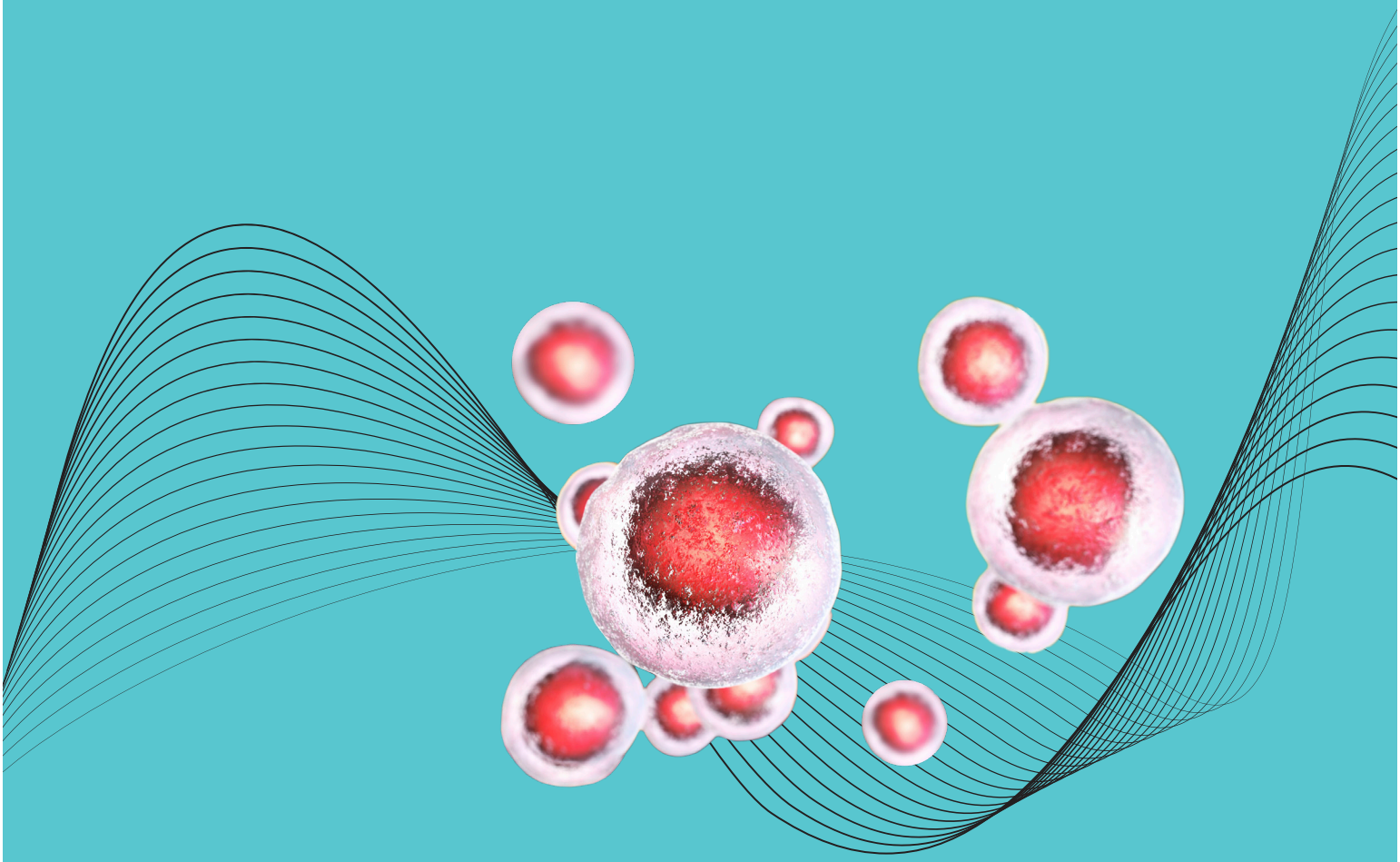
Consumables and Instruments

CASE STUDY

Cell Freezing Container PBMC Processing



AZENTA
LIFE SCIENCES

azenta.com

Method trends in PBMC cryopreservation for HIV clinical trials

Protocol enhancements over the past 10 years to improve the viability, recovery, and function of PBMCs include media formulation, the use of cold cryoprotectant, the rate of osmotic equilibration during post-thaw washing, the use of pre-chilled sample containers, and pre-cooling the alcohol-filled freezing container (AFC) prior to sample addition. By 2000 the AIDS Clinical Trial Group (ACTG) protocol (1) included working on ice and use of cold cryoprotectant prior to controlled rate freezing to -80°C. While various laboratory SOPs began to specify cold handling after isolation, the temperature of the freezing container was either unspecified or followed manufacturers instruction to use a room temperature AFC. Later revisions of the ACTG standard indicated that a pre-cooled AFC (4°C) may be used as an alternative to the room temperature starting condition (2). The 2006 HPTN specification which includes the Cross-Network SOP indicated that an AFC must be equilibrated to room temperature prior to use (3). The most recent 2009 Cross-Network SOP now indicates that ideally the AFC should be equilibrated between 2°C to 8°C prior to insertion of the vials and -80°C freezing (4). These trends seem to indicate that maintaining cold handling temperature during the liquid cryoprotectant stage may be an important component in preserving the viability and function of cryopreserved PBMC cells. This evolution in technique is persuasive because of the sensitivity and specificity of multi-center clinical trials to process-induced changes in T-cell function and phenotype.

Minimzing residence time of cells in DMSO

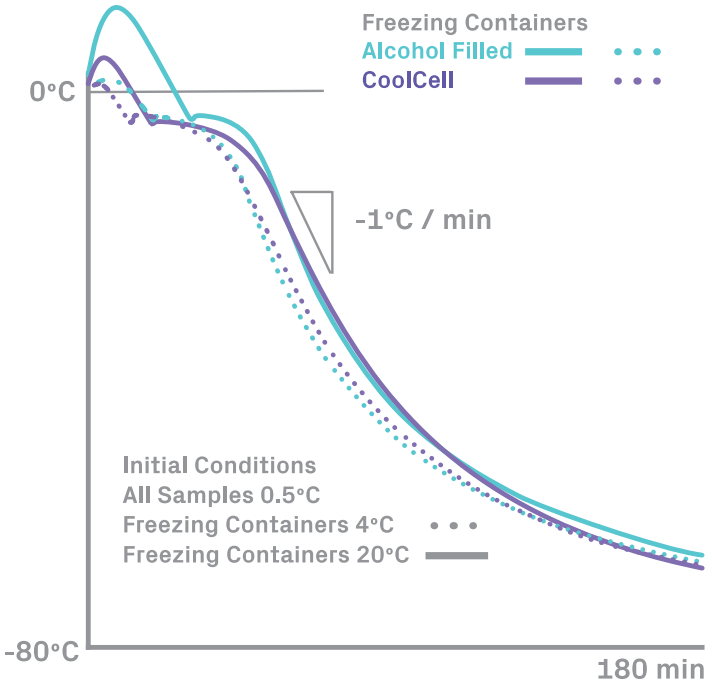
It is generally agreed that exposure time to DMSO in the liquid state suspension should be minimized. Protocols often specify keeping the exposure after DMSO prior to freezing to as short a time as practical. The HANC SOP (4) emphasizes “work quickly after the freezing medium is added and allow no longer than 10 minutes before placing cells in the freezer”. The additional time and warming temperature of a room temperature AFC works against efforts to minimize this exposure time. Likewise, reducing the exposure time is supported by general recommendations to wash cells quickly after thawing. However, to minimize osmotic shock, the ACTG Cryopreservation Working Group specifies that the samples should be thawed quickly to 0°C then diluted with washing medium in a slow (2-3 min) drop-wise process rather than a bolus 5ml first-step dilution. This method improved viable cell recovery by a mean of 32% (5) and suggests that efforts to minimize delay in the wash step should not include a bolus dilution.

Methods to reduce freezing lag time

In an attempt to characterize the time lag and temperature rise of samples undergoing the freezing step, the following experiment was performed using a standard alcohol-filled freezing container and an insulated low-thermal mass freezing container. These two containers were continuously monitored for sample temperature in both room temperature and pre-chilled starting conditions. In both cases the sample loads were chilled on ice to simulate the final aliquoting step of current best practice PBMC freezing protocols. The measured lag before freezing is the elapsed time that sample temperature remains above its initial iced starting temperature.

Experiment

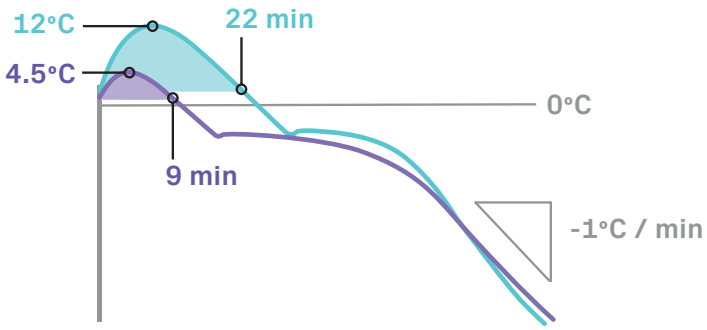
Twelve 1ml samples of DMEM, 10% DMSO, 20% fetal calf serum in a 2 ml cryotube were pre-cooled to 0.5°C. The alcohol-filled container and CoolCell at either 20°C or pre-cooled to 4°C were loaded with samples and placed in a -75°C environment. Sample temperature was measured with a thermocouple probe suspended in the sample fluid of one of 12 tubes for each combination of conditions.



Results

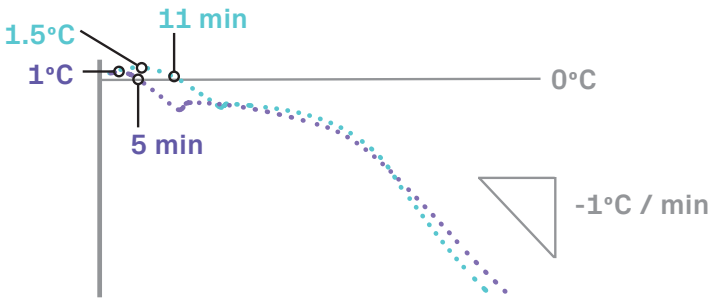
Room temperature containers 20°C

The alcohol-filled system samples (red solid) warmed to a maximum of 12°C and remained above initial sample starting temperature for 22 minutes before continuing to freeze. The CoolCell samples (blue solid) warmed to a maximum temperature of 4.5°C and remained above their initial starting temperature for 9 minutes before continuing to freeze.



Pre-cooled containers 4°C

The alcohol-filled system samples (red dotted) warmed to a maximum of 1.5°C and remained above initial sample starting temperature for 11 minutes before continuing to freeze. The CoolCell samples (blue dotted) warmed to a maximum temperature of 1°C and remained above their initial starting temperature for 5 minutes before continuing to freeze.



Alcohol-filled freezing container thermodynamics

Alcohol-filed systems rely on a large thermal mass and high heat transfer to slow sample cooling rates to about -1°C per minute. The heat lost from the alcohol (250ml) is about 10 times greater than the heat removal required for sample freezing. The high heat transfer rate between the room temperature alcohol and pre-chilled sample tubes will first warm samples before freezing. This large heat release will also increase local temperature in the -80°C freezer and will warm nearby frozen samples even when using pre-cooled containers. The combination of high thermal mass and high heat transfer creates a warming sample temperature "bounce" before freezing commences.

Cell Freezing Container freezing container thermodynamics

The Cell Freezing Container is an insulated container with a small solid core thermal ballast. The core has a total heat capacity of about one-fourteenth (7%) of the alcohol system. This design relies on radially symmetric placement of samples with low heat transfer (high insulation) to achieve the same controlled rate of -1°C per minute. With high insulation and low thermal mass, Cell Freezing Container sample warming is significantly reduced.

Conclusion

In both starting conditions the alcohol-filled system warmed the pre-chilled samples more and longer than Cell Freezing Container before freezing overtakes initial sample temperature. This warming step of up to 22 minutes at a temperature of up to 12°C in the room temperature alcohol-filled container may influence overall sample viability. The insulated Cell Freezing Container system had significantly lower heat stress on the samples and kept sample temperature below 5°C with a room temperature Cell Freezing Container starting condition. To minimize the time cells are exposed to liquid freezing medium and minimize re-warming of cells above their iced processing temperature, using a Cell Freezing Container will provide both a shorter time lag and a lower peak temperature before freezing than that provided by an alcohol-filled freezing container.

References

1. Weinberg A. et. al., 2000 Viability and Functional Activity of Cryopreserved Mononuclear Cells. Clinical and Diagnostic Laboratory Immunology 7:714-716
2. ACTG Consensus Protocol for PBMC Cryopreservation and Thawing , Laboratory Technologists Committee of the Adult AIDS Clinical Trials Group version 3.0 released July 1, 2001
3. HIV Prevention Trials Network Microbicide Trials Network Laboratory Manual, version 1.0 release date Nov 15, 2006
4. HANC Cross-Network PBMC Processing Standard Operating Procedure HANC-LAB-P0001, v2.0, effective date July 7, 2009
5. Weinberg A. et. al., 2009 Optimization and Limitations of Use of Cryopreserved Peripheral Blood Mononuclear Cells for Functional and Phenotypic T-Cell Characterization. Clinical and Vaccine Immmunology 16:1176-1186

AZENTA
LIFE SCIENCES

azenta.com

© 2022 Azenta US, Inc. All rights reserved. All trademarks are property of Azenta US, Inc. unless otherwise specified. **43001-CS 1221**