Temperature controlled manipulation and alcohol-free cryopreservation: A new era in sample handling and biobanking

Brian Schryver[1], Kevin J Anthony[2], Sven G. Enterlein[2] ,John Gardner[3], Catherine Fyfe[3], Aurelio Bonavia[1], Maria Thompson[1] and Rolf Ehrhardt[1]

[1] BioCision LLC, 12 East Sir Francis Drake Blvd. Suite B, Larkspur, CA 94939 USA, Tel: 1.888.478.2221, Email info@biocision.com

[2] Integrated BioTherapeutics Inc, 21 Firstfield Road, Suite 100, Gaithersburg, MD 20878, Email:info@ibtinc-usa.com

[3] Roslin Cellab, Roslin Biocentre, Roslin, Midlothian, Scotland EH25 9PP, Email: info@roslincellab.com

Abstract

Standardization of sample handling and viable long-term storage is emerging as one of the most important challenges for biobanks, diagnostic companies and research institutes. As error is inherent in laboratory processes and often the researcher is unaware of this, BioCision, through the use of thermo-conductive principles and precision-engineered tools, has developed solutions to address the lack of standardization of common sample handling, processing and storing applications.

Our research shows, for example, that when a 96-well plate is placed directly on ice, the wells do not equilibrate down to 4˚C and there is wide variability in temperature - up to 3˚C between wells. When the same plate is placed on BioCision's CoolSink™ thermo-conductive plate holder, all the wells rapidly equilibrate to the expected 0.5 - 4˚C range with <1˚C of variation between wells, thus eliminating variability and "edge effect".

With respect to rapid cryopreservation of specimens on dry ice, BioCision has developed an alcohol-free snap-freezing standardization method and the tools required to perform it. Using virus-infected samples, we show that viruses such as Venezuelan equine encephalitis (VEE) or Influenza virus (H1N1) can be snap-frozen using a CoolRack® thermo-conductive tube module on dry ice (without adding EtOH to the dry ice), resulting in no loss of viral titer. This method ensures each specimen is uniformly frozen and removes inconsistencies inherent in "user technique" or variances in shape and amounts of dry ice (and EtOH).

BioCision has also addressed the non-reproducible method of passively cryopreserving primary cells, cell lines, stem cells, yeast and other types of cells by developing a rate-controlled cell cryopreservation system - CoolCell®. The CoolCell method ensures uniform and reproducible freeze rates for all vials in the batch and yields equivalent or improved post-thaw cell viability when compared to common methods. With human embryonic stem cells (hESCs), for example, we obtain a significant increase (30-36%) in post-thaw viable cell counts compared to other bench-top freezing methods with P< 0.005.

BioCision presents here new standards for bio-specimen handling and cryopreservation, which ensures more reproducible data across multiple experiments, users and sites, while significantly improving specimen yields.

Objectives

A significant amount of effort is devoted to validating storage conditions and monitoring cold chain conditions to verify that, once frozen, precious biological samples remain below a specified temperature. Pre-analytical sample handling and storage, however, is often overlooked and represents a major source of variability in many, if not most, analytical procedures. BioCision has developed a portfolio of laboratory tools that standardizes the way temperature-sensitive samples are handled, processed and stored. One product, CoolSink thermo-conductive plate holder, is engineered from a novel alloy that rapidly adapt to source temperature and significantly reduce well-to-well temperature variation across a 96 well plate.

BioCision's CoolRack thermo-conductive tube modules organize and equalize sample cooling/freezing/thawing profiles while eliminating hazardous solvents from current methods (e.g EtOH-dry ice method commonly used to snap-freeze viruses). In collaboration with Integrated BioTherapeutics, BioCision examined the effect of snap-freezing using the common method of inserting tubes directly into EtOH-dry ice baths compared to BioCision's alcohol-free CoolRack method on 2 virus titers, H1N1 and VEE virus.

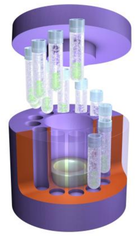
Additionally, in collaboration with Roslin Cellab, BioCision analyzed whether using the CoolCell method of cryopreservation imposed less stress upon hESCs compared to other less standardized cryopreservation methods.

Method

A) 96-well plate temperature variation: a comparison of the temperature variance among wells of a 96-well plate was made between the method of placing the plate directly onto crushed ice for cooling and resting the plate on a thermo-conductive CoolSink plate holder that is then placed on ice.

B) Snap-Freezing with CoolRack: The CoolRack thermo-conductive tube module was used to snap-freeze virus preparations from infected cell supernatants to compare its performance against the commonly-used ethanol-dry ice slurry method. The CoolRack® module was equilibrated on dry ice for 10 minutes prior to the start of the experiment. Tubes containing H1N1 virus with a titer of $2.5x10^5$ $TCID_{50}$ and VEE virus with a titer of $1.3x10^9$ $TCID_{50}$ were placed in the CoolRack module for 3-5 minutes until completely frozen. An identical sample tube was frozen in ethanol-dry ice by submersing the samples for 10 minutes. The frozen samples tubes were stored overnight (H1N1) or for three days (VEE) in a -80˚C freezer. Samples were thawed and the titers assayed by TCID50. Results represent an average of three samples.

C) Stem Cell Cryopreservation: BioCision's CoolCell passive cell freezing container is engineered to deliver controlled-rate cell freezing through:



- Radially-symmetric thermal exchange to ensure uniform cooling profiles to all vial positions
- Highly thermo-conductive core
- Synthetic materials used in aerospace industry for lightweight & insulative properties
- Conduction and/or micro-convection air control

Figure 1: CoolCell passive alcohol-free -1˚C/minute controlled-rate cell freezing container

By precisely controlling thermal energy transfer, CoolCell cell freezing container standardizes each freezing run by providing highly reproducible freezing profiles and eliminating temperature variations during cryopreservation. (Figure 2)

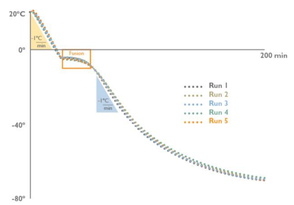


Figure 2: Five consecutive freeze cycles in -80ºC freezer using CoolCell (12-well, 2ml cryo vials). Cooling profiles and phase change are identical every time.

Roslin Cellab evaluated the CoolCell method in a human embryonic stem cell cryopreservation regime and compared three common bench-top freezing methods including freezing tubes in a styrofoam box, freezing tubes wrapped in paper towels, and freezing tubes in an isopropanol-filled freezing container. For more details on the CoolCell method see Thompson et al., 2011. Two hESC lines, RC-7 and RC-10, originally isolated from the inner cell mass of human embryos at the blastocyst stage were expanded for 2-3 weeks under standard culture conditions using STEMPRO® hESC culture medium (Invitrogen, Cat. # A1110501) to produce an adequate supply of cells for the study. A total of five vials each containing 2 x 10^6 live cells per vial in 1.0 ml of serum-free cryopreservant (Cryostor CS10, Biolife Solutions Cat. # 210102) were frozen for use in each method, and stored for two weeks. The resuscitated cells were placed into standard culture conditions and harvested at one day post-thaw and examined for morphology/attachment under the microscope x 10, and three days post-thaw for final viability analysis using alamarBlue (AbD serotec, Cat Number BUF012A/B).

Results

A) **96-well plate temperature variation:** 96-well plates placed directly on crushed ice for cooling do not achieve critical <4º C temperature in any of the wells (Figure 3i). However, by using a CoolSink thermo-conductive plate holder as an interface between the plate and the ice, all wells were brought to <4º C and temperature was evenly distributed, virtually eliminating edge effect (Figure 3ii).

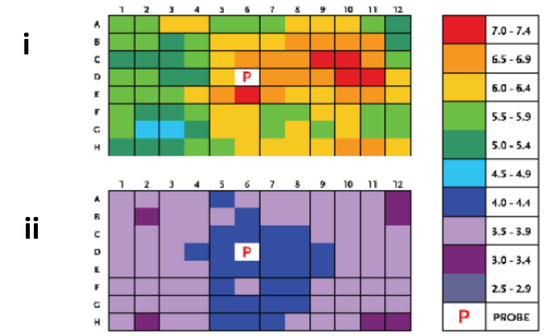


Figure 3. Final equilibrium well temperature for a 96-well flat bottom plate in direct contact with (i) crushed ice and (ii) CoolSink 96F. Colors represent 0.5˚C temperature intervals of the corresponding plate wells. The white cell represents the well that was fitted with the thermocouple probe.

B) Snap-Freezing Virus with CoolRack: The use of the CoolRack tube module on dry ice without alcohol to freeze viruses was equivalent to that of the classic method of using ETOH/dry ice (Figure 4).

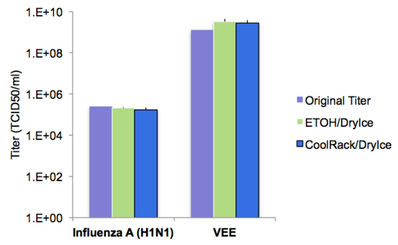


Figure 4: Graph showing the Titer (TCID50/ml) of two different viruses H1N1 and VEE - using the two freezing methodologies compared to the original titer value.

C) hESC Cryopreservation: Cryopreservation and recovery of the hESC lines RC-7 and RC-10, using a CoolCell passive controlled-rate freezing container provided a significant increase in viable cell yield post-thaw when compared to other common freezing methods tested (P<0.005; determined by 1 way ANOVA) (Figure 5).

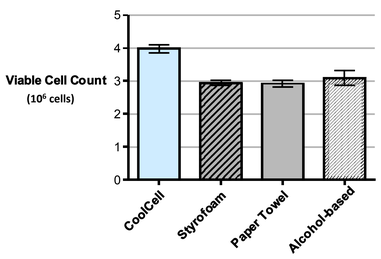


Figure 5: Two hESC lines were frozen/thawed using four different methods and viable cell count after three days post-thaw in culture was measured (n≥6).

Results cont.

The CoolCell freezing container provided a significant increase in cell attachment to standard coated tissue culture plates at one day post thaw compared to other freezing methods tested (Figure 6).

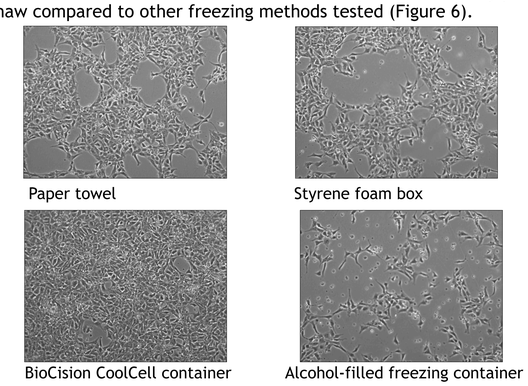


Figure 6: hESC line RC-10 was observed at day one post resuscitation under x10 magnification using four different freezing methods.

Conclusion

The research presented in this poster demonstrates the need for standardized techniques during the pre-analytical stage of research when temperature variability and irreproducibility affect sample expression. We have shown three significant examples of how common methods affect experimental outcomes:

A. 96-well plates placed directly on ice do not reach the critical 4˚C temperature in any of the wells and the well-to-well temperature varies depending upon factors that are difficult, if not impossible to control, such as the size of the ice crystals used, the position of each well in relation to the ice, and the rate at which the ice thaws. CoolSink thermo-conductive plate holders provide a temperature-equalizing interface that eliminate these factors. The CoolSink plate holder brings each well to at least 4˚C in an evenly distributed manner, ensuring uniformity across all wells and eliminating "edge effect."
B. Using the CoolRack module as a vehicle for snap-freezing viruses in dry ice, allows the process to be standardized and reproducible. The virus titers recovered from the CoolRack method were equivalent to conventional freezing in ETOH/dry ice. Additional benefits include eliminating ethanol from the process which minimizes risk of contamination, eliminates hazardous solvent handling and waste and reduces the risk of tube markings being inadvertently erased by the EtOH.
C. BioCison's CoolCell provides highly reproducible cell freezing profiles and also outperforms the traditional passive freezing methods in terms of viability, attachment and proliferation of hESCs, post-thaw. CoolCell technology also eliminates the need for isopropanol, as well as the costs and hazards associated with its use and disposal.

In summary, the data presented in this poster clearly shows that using BioCision's products can improve pre-analytical sample handling processes and introduces standardization and reproducibility which will result in better data and improved scientific discovery.

References and Acknowledgements

Thompson, M., Nemits, M., Ehrhardt, R. (2011) Rate-controlled cryopreservation and thawing of mammalian cells. *Nature Protocol Exchange* 03/05/2011 doi: 10.1038/protex.2011.224

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