Cryopreservation of body cavity fluids for diagnostic cytology

Veterinary Research Scholars Program
University of Missouri

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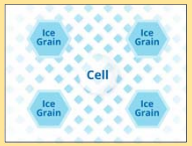
life in nano ice

Background Information

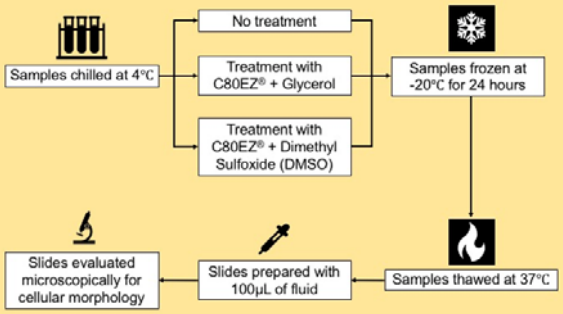
Freezing cells disrupts cellular morphology because of both intra- and extracellular ice formation, which pierce cellular membranes. For this reason, it is traditionally recommended that only fresh samples be submitted for diagnostic cytology. This limits the use of diagnostic cytology to patients that are within range of a diagnostic laboratory. It is known that a commercially-available cryopreservative medium, C80EZ® (Cryocrate Labs, LLC, USA), maintains cellular viability during mid-term storage at -20℃ by minimizing cryo-damage through the formation of cubic nano ice crystals instead of large, damaging hexagonal ice pieces.



The principle objectives of this study were to examine the effect of freezing on cellular morphology of several biological fluids and to evaluate how treatment with C80EZ® affected cellular structure.

Methods

- Highly cellular fluid samples were obtained through submissions for diagnostic cytology at the University of Missouri Veterinary Medical Diagnostic Laboratory-Clinical Pathology Laboratory.
- Four fluids were included in this study: bronchoalveolar lavage (BAL) fluid, peritoneal fluid, pericardial fluid, and urine (results not all pictured).
- Samples were routinely processed for cytologic exam: slide preparation involved cytocentrifugation or direct smear followed by staining with Modified Wright-Giemsa Stain.
- Samples treated with C80EZ® medium were mixed in a 1:1 (v/v) ratio.



C80EZ® Treatment of BAL Fluid

This sample was from a 5–year-old, spayed female German Shepherd Dog diagnosed with lymphoma. The total nucleated cell count was 29,265 cells/µL.

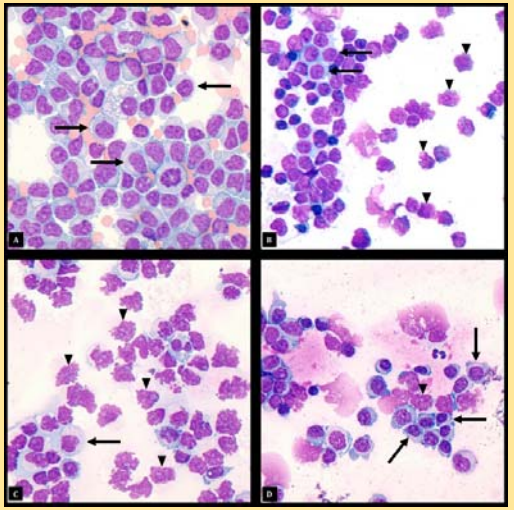


Figure 1. Cytocentrifuged slide preparations of BAL fluid, images captured at 600x **A**. Fresh sample, no C80EZ® treatment **B**. Sample frozen at -20℃ for 24 hours, no C80EZ® treatment **C**. Sample frozen at -20℃ for 24 hours, treated with C80EZ® + Glycerol **D**. Sample frozen at -20℃ for 24 hours, treated with C80EZ® + DMSO. **Arrows indicate intact cells. Arrow heads indicate ruptured cells.**

C80EZ® Treatment of Peritoneal Fluid

This sample was from a 10-year-old male Labrador Retriever diagnosed with septic neutrophilic inflammation. The total nucleated cell count was 291,200 cells/µL.

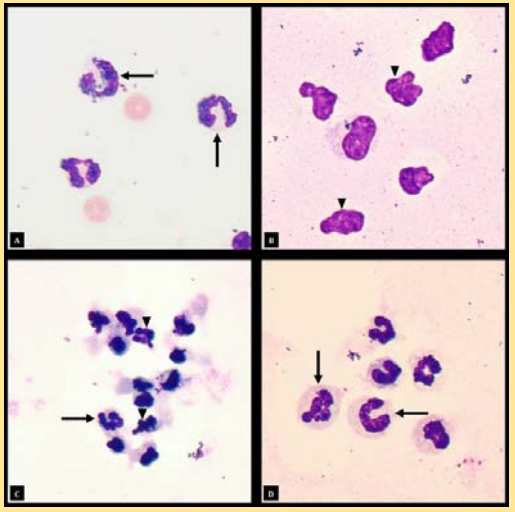


Figure 2. Direct smear slide preparations of peritoneal fluid, images captured at 1000x **A**. Fresh sample, no C80EZ® treatment **B**. Sample frozen at -20℃ for 24 hours, no C80EZ® treatment **C**. Sample frozen at -20℃ for 24 hours, treated with C80EZ® + Glycerol **D**. Sample frozen at -20℃ for 24 hours, treated with C80EZ® + DMSO. **Arrows indicate intact cells. Arrow heads indicate ruptured cells.**

Conclusions

- Untreated frozen samples experienced cellular degradation, determined by loss of cellular membranes and swollen nuclei.
- Samples treated with C80EZ® + DMSO maintained their cellular and nuclear borders better than samples treated with C80EZ® + Glycerol.

Overall, cryopreservation of bodily fluids can be achieved with this methodology while maintaining cellular morphology necessary for diagnosis.

Future Goals

- Explore additional variables:
  - Different bodily fluids: transudates, modified-transudates, exudates
  - Effects of glycerol-only and DMSO-only cryopreservation
  - Longer freezing periods

Acknowledgments

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