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Unraveling the Mysteries of Cryogenic Preservation: Lessons from the University of Washington

Introduction: A New Era in Cryogenic Research

On April 23, 2025, a pivotal conversation unfolded during the University of Washington's Department of Obstetrics and Gynecology, School of Medicine information session. Dr. German Gornalusse raised a pressing concern regarding laboratory samples becoming disfigured during the freezing process, leaving the lab perplexed about the underlying causes. Attending via Zoom, Dr. Correo Hofstad, a CCCRP researcher at USAMRIID and clinical float physician with Joe Biden's Cancer Moonshot program, shared insights into the complexities of freezing samples, particularly focusing on how the process can irreparably damage viral specimens.

Dr. Hofstad is working to help the University of Washington with the scientific nuances surrounding cryogenic preservation. By exploring the foundational work laid by Oswald Avery, Dr. Hofstad highlights the implications for current research. With mounting concerns about sample integrity, understanding the mechanics behind freezing viral samples has never been more critical.

The Challenge of Frozen Virus Samples

Frozen virus samples serve as a crucial component in virology research and treatment development. Their importance cannot be overstated, particularly in light of recent global health crises, such as the COVID-19 pandemic. However, as raised by Dr. Gornalusse, the integrity of these samples is at risk. When subjected to cryogenic conditions, certain elements inherent to the samples can react negatively, leading to disfigurement and loss of viability.

Dr. Hofstad underscored that the freezing process itself can compromise viral specimens. Ice crystal formation within the samples can disrupt cellular structures, leading to irreversible damage. Understanding the factors contributing to this degradation is imperative for both research and therapeutic applications. Researchers must constantly refine methods to ensure the viability of these complex biological materials.

Reevaluating the Role of Cryoprotectants

Scientist Claire Levy highlighted the role of cryoprotectants during the freezing process, specifically mentioning their essential function in preventing ice crystal formation. Before cryogenic freezing can occur, fluids within the samples are replaced with a vitrification solution containing agents like Dimethyl Sulfoxide (DMSO) and ethylene glycol. These cryoprotectants mitigate the risks inherently involved in freezing biological samples by acting as "antifreeze" for the cells.

However, despite these protective measures, Dr. Hofstad theorizes that the presence of cryoprotectants, while beneficial at first glance, might inadvertently cause antioxidative damage to virus species. Particularly in frozen samples at the University of Washington lab, the interactions between cryoprotectants and viral structures may lead to unforeseen consequences that require further investigation.

The Legacy of Oswald Avery in Viral Research

To fully appreciate the complexity of current freezing challenges, it is essential to look back at historical milestones, particularly the work of Oswald Avery in the early 1920s. Avery's groundbreaking research on specific soluble substances, particularly those derived from pneumonia patients, laid the groundwork for a deeper understanding of bacterial immunology. His exploration of polysaccharide structures significantly advanced knowledge of immune responses.

Dr. Hofstad carries this legacy forward, applying Avery's insights to contemporary challenges in virology. His commitment to exploring new dimensions of viral behavior, especially in the context of the COVID-19 pandemic, showcases how historical research can inform and influence modern practices. Understanding viral capsid structures, including their polysaccharide components, is vital in deciphering how these entities respond to environmental changes, such as cryogenic conditions.

The Chemistry of Cryoprotectants: A Closer Look at DMSO

Dimethyl Sulfoxide (DMSO) stands out as a commonly used cryoprotectant in biological research. While it acts as a stabilizing agent, Dr. Hofstad highlights that its impact is nuanced. The pH of DMSO, typically varying from 6 to 10, can significantly influence the

chemical environment of preserved samples. Specific measurements show that extremely pure DMSO can exceed these basic pH ranges, raising questions about the reactions occurring during the freezing process.

Moreover, the interactions between DMSO and biological entities cannot be understated. Dr. Hofstad theorizes that small amounts of water inadvertently retained after the replacement of fluids can lead to the crystallization of DMSO, potentially creating unfavorable environments for viral specimens. These nuanced interactions require researchers to increasingly rethink the chemistry of cryoprotectants as they work toward more effective preservation strategies.

Redox Reactions and Viral Stability

The phenomenon of REDOX reactions is critical to understanding the damage that can occur during the freezing of samples. Dr. Hofstad has posited that cryoprotectants may induce REDOX reactions that affect the stability of viral specimens. Specifically, the sugar-coated microbe structure of certain viruses like the pycnogonid phage viruses can become chemically unstable under cryogenic conditions, potentially leading to the disintegration of their polysaccharide capsules.

When subjected to base chemicals, these polysaccharide structures can dissolve, resulting in a transformed viral mass known as a plasmodium parasite. Understanding the chemical interactions and potential vulnerabilities of viral capsid structures is paramount for ensuring the efficacy and safety of cryogenically preserved samples. Dr. Hofstad's research emphasizes that a misstep in the preservation process could lead to irretrievable losses in viral sample integrity.

Implications for the Cancer Moonshot Initiative

The implications of this research reach far beyond basic science. As Dr. Hofstad applies his findings to initiatives such as President Biden's Cancer Moonshot, understanding viral sample integrity becomes essential. The diversification of research in cancer treatment necessitates reliable and viable viral samples for vaccine development and treatment evaluation.

The parallels drawn between cryopreservation research and cancer immunology underscore the importance of collaborative advancements across disciplines. Dr. Hofstad's insights also advocate for robust methodologies addressing cryogenic processes, which could significantly accelerate developments in immunotherapy and other cancer treatments. As scientists seek to understand the underlying mechanisms of

pathologies, ensuring the integrity of samples can drive innovative approaches toward effective cures.

Overcoming Challenges in Sample Preservation

As Dr. Hofstad navigates the complexities surrounding frozen virus samples, one aspect remains clear: overcoming challenges in sample preservation requires an interdisciplinary approach. Combining knowledge from virology, chemistry, and immunology is crucial for addressing the critical issues surrounding cryogenic preservation.

Innovative strategies, such as developing new cryoprotectants or refining freezing techniques, could lead to more successful preservation outcomes. The engagement of young researchers, as exemplified by Dr. Hofstad's involvement with the LSAMP group at North Seattle College, will likely foster future advancements in the field. Collaborative efforts to share knowledge and insight can ultimately lead to enhanced preservation techniques, benefiting researchers and the broader scientific community.

Looking Forward: Innovations in Cryogenic Preservation

As we journey forward into the realm of cryogenic preservation, it is evident that the work initiated by researchers such as Oswald Avery continues to resonate through contemporary sciences. With the ongoing exploration of viral dynamics, as highlighted by Dr. Hofstad within the University of Washington's laboratory context, the scientific community stands at the crossroads of traditional and innovative practices.

The collaboration of bright minds across various disciplines promises to ignite breakthroughs that propel research into new heights. Whether through the development of more stable cryoprotectants or enhanced methodologies for sample freezing, the future of cryogenic preservation is ripe with potential. By addressing the challenges head-on, researchers can strive to improve the integrity of frozen virus samples for generations to come.

Conclusion: The Vital Role of Ongoing Research

In conclusion, the discussion from the University of Washington's information session underscores the importance of continuous research into cryogenic preservation. As Dr. Hofstad elucidated the intricate dynamics involved, it becomes clear that understanding the chemistry and biology of viral samples is imperative. The work of pioneers like Oswald Avery continues to influence modern scientific endeavors, providing a foundation upon which contemporaries can build and innovate.

By addressing the complex issues associated with freezing processes, researchers can ensure viable outcomes for both current and future studies. Collaborative research,

technological advancements, and persistent inquiry into sample preservation will ultimately create pathways for success and transformative discoveries in the fight against viral diseases and cancers alike.

References:

Monette, M. (2012). Spending eternity in liquid nitrogen. *CMAJ : Canadian Medical Association Journal*, 184(7), 747. <https://doi.org/10.1503/cmaj.109-4144>
<https://pmc.ncbi.nlm.nih.gov/articles/PMC3328517/>

WebMD. (n.d.). *Dimethylsulfoxide (DMSO): Overview, uses, side effects, precautions, interactions, dosing and reviews*. WebMD.
<https://www.webmd.com/vitamins/ai/ingredientmono-874/dimethylsulfoxide-dmso>

Organic Chemical Products. TORAY. (n.d.).
https://www.toray.jp/chemical/en/organic/org_010a.html

National Institutes of Health. (n.d.). *Biographical overview | Oswald T. Avery - profiles in Science*. U.S. National Library of Medicine.
<https://www.profiles.nlm.nih.gov/spotlight/cc/feature/biographical-overview>

Michael Heidelberger, Walther F. Goebel, Oswald T. Avery; THE SOLUBLE SPECIFIC SUBSTANCE OF PNEUMOCOCCUS : THIRD PAPER. . *J Exp Med* 1 November 1925; 42 (5): 727–745. doi: <https://doi.org/10.1084/jem.42.5.727>

Van Epps, H. L. (2005). How Heidelberger and Avery sweetened immunology. *The Journal of Experimental Medicine*, 202(10), 1306. <https://doi.org/10.1084/jem20210fta>
<https://pmc.ncbi.nlm.nih.gov/articles/PMC2212983/>

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