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Temperature Standardization Gets the Cold Shoulder

BioCision, Larkspur, Calif.

Laboratories remain slow to adopt protocols and technologies that can improve and standardize temperature control in biomaterial sample handling and preservation.

BioCision's CoolCell alcohol-free cell freezing containers ensure standardized controlled-rate -1°/min cell freezing in a -80°Cfreezer—without alcohol or any fluids.

While research involving temperature-sensitive biomaterials has been conducted for centuries, efforts to standardize temperature control in sample processing and handling have not been widely adopted. The situation continues despite the fact that research involving temperature-sensitive biomaterials has grown exponentially in recent years. Drug developers are now increasingly targeting biologics and other therapies that are highly temperature-sensitive. Out of the top 10 global pharma products positioned for launch in 2014, seven are biologics that have stringent requirements for cold chain handling. In addition, the introduction of new assays and other diagnostic tools based on analysis of blood, plasma, tissue and other biomaterials means that more laboratories than ever are now handling biological samples. In many cases, samples must be shipped to different locations for processing or analysis, making temperature control even more challenging.

Temperature control is one aspect of a larger issue that is having a profound effect on both research and patient care around the world: sample standardization. Many laboratories use different and often independently generate standards and procedures to process samples. This practice is widely recognized as a major factor contributing to diagnostic errors that affect both the quality of patient care and healthcare costs. According to a recent study, these errors are most apparent during the pre-analytical phase, which is much more susceptible to uncertainties and accidents, leading to inappropriate care. Another study reported that 20 percent of errors in pre-analytical testing are not caught by lab professionals, resulting in unnecessary testing and an unjustifiable increase in costs. A study conducted by Frost & Sullivan found that the cost of pre-analytical errors represents between 0.27 and 0.57 percent of the total operating cost of a hospital. For a healthcare facility with an operating budget of $1 billion, reducing the risk of sample errors could present a savings of up to $2.7 million or more annually.

Another concern is the reproducibility of research. Without access to well-documented sample handling SOPs or protocols, researchers may find it difficult or near impossible to reproduce the results. Scientists at Amgen recently worked to recreate research results reported in the peer

review journal Cell in an effort to develop a new oncology drug. After six months of work, the research team was forced to shut down the program because they could not replicate the results of the original research effort. Additionally, Bayer recently reported that its internal preclinical programs were unable to replicate published research findings in 65 percent of cases.

In an effort to address these problems, some regulatory agencies and academic institutions in the U.S., including the NIH, National Cancer Institute (NCI), Critical Path Institute (C-Path) and the College of American Pathologists Biorepository Accreditation Program, have recently taken steps to develop guidelines to improve sample standardization. In Europe, a consortium of public and private research organizations and industry representatives also recently joined together in a similar effort. While some progress is being made, on a global scale novel molecular diagnostics continues to lack clear consensus on quality assurance protocols for pre-analytical procedures, including sample collection, handling, transport, processing and storage.

The impact of temperature

Among the variables that can affect sample integrity, fluctuations in temperature and the inability to maintain optimal temperatures for different biomaterials at all stages of research are the most common. The problem is so pervasive that it can affect both research and diagnostics in many therapeutic areas. One example is Alzheimer's disease. Prof. Kevin Barnham from the Mental Health Research Institute (MHRI) recently published a paper calling for improved standardization in blood collection and processing methods to address a critical challenge in the diagnosis of Alzheimer's. His position is based on findings related to the use of a blood-based diagnostic to detect the presence of amyloid beta peptide, the main component of amyloid plaques found in the brains of patients with Alzheimer's disease. The test could potentially be more widely used to confirm a diagnosis of Alzheimer's disease, but blood-based diagnostic markers can produce widely variable results when exposed to variations in temperature. Regarding the use of this diagnostic tool, some protocols suggest processing blood samples at 4 C while others suggest room temperature. Some call for samples to be stored at -80 C while others call for storage at -180 C. The rate at which samples are frozen and the number of freeze-thaw cycles can also vary from lab to lab. Individually and collectively, these variables can reduce the accuracy of these tests, making results unreliable.

Current standards in cryopreservation, the process where cells and tissue are preserved by cooling to sub-zero temperatures, highlight the continuing challenges. Around the world, laboratories routinely employ various technologies and procedures to freeze specimens, with options including cooling sample tubes on crushed ice or holding tubes in a dry ice and alcohol bath. The use of different technologies often means that the time necessary to complete a freezing process can vary. For many samples, delay or improper execution of the freezing process can negatively impact cell structure, function or biological activity. In the development of many drugs and vaccines, especially those that involve the use of stem cells or other biological materials, precise temperature control at every stage of the production process is essential to maintain sample integrity and develop therapies without the risk of degradation, which can reduce

or completely eradicate patient benefit.

Improving reproducibility

Recent efforts to improve reproducibility of research results are gaining momentum, though there is much work still to be done. Several research organizations including Science Exchange, PLOS ONE, figshare and Mendeley joined together to launch a program called the “Reproducibility Initiative” specifically to address the low rate of reproducibility in scientific research caused by poor standardization. One goal of this program is to match interested researchers with an independent research group that will attempt to confirm the reproducibility of original studies.

While many organizations are involved in efforts to improve standardization, progress will also require the participation of individual researchers and laboratories around the world. A survey of scientists at a prominent cancer center found that more than half were unable to reproduce published scientific findings at least one time. Among the scientists who contacted original researchers for information or clarification to determine the challenges in reproducibility, 62 percent reported that the original study authors responded negatively, indifferently or not at all.

Choosing the right temperature control solution

Decisions regarding the use of temperature control technology are often influenced by multiple factors, including a lab technician’s established practices and personal preferences, the range of available products on site, the use of preferred vendors and service providers, budget and other considerations. This situation prevails despite the fact that ensuring reproducible temperature profiles and standardizing temperature-sensitive lab procedures are clearly positioned as essential goals in a wide array of scientific disciplines, including cell and tissue culture, cryopreservation, histology, immunohistochemistry, viral and bacterial research, molecular biology, cell therapy, pharmaceutical manufacturing and biofuels research.

Advances in technology are positioned to play an important role in standardizing temperature control in the years ahead. Many labs continue to use outdated options, including Styrofoam boxes and alcohol-filled freezing containers, both of which require multiple steps and present a risk of human error. More recently, insulated alcohol-free freezing containers were introduced to streamline the freezing process with the potential to improve efficiency and temperature control standards. A recent test comparing the use of alcohol-filled containers versus alcohol-free containers found that pre-chilled samples stayed warm for longer periods before freezing when stored in alcohol-filled containers. In some cases samples showed a warming trend for 22 minutes at a temperature of up to 12 C, a variation that could affect overall sample viability and function.

Looking ahead, the effort to improve temperature control procedures and standards on a global scale will require continued action on multiple fronts. Sample standardization guidelines must be adopted by laboratories and hospitals on a global scale. More researchers and other laboratory professionals must have access to advanced technologies, products and protocols that can help them improve temperature control and make the freezing process almost error-proof. In addition, laboratory and research professionals at every level must recognize the essential benefits and impact that these efforts will have on research and patient outcomes, and actively support the use of protocols and technologies that can improve and standardize temperature control.