**Considerations for Preservation of Sample Quality**

Mass cytometry allows simultaneous measurement of multiple patient samples collected at multiple time points, processed by multiple researchers, using a multiplex panel identifying multiple immune cell subsets and their functional attributes. Capturing the essence of biologically significant physiology necessitates minimizing technical variability stemming from sample processing, storage conditions, antibody staining fluctuations, and run-to-run differences. Several techniques are available to ensure the quality of the measurements and control for technical inter- and intra-patient sample variation.

A key aspect of any study for which multiple samples are obtained is ensuring sample stability, integrity, and consistency across the entire cohort. In general, biological samples are best preserved when stored cryogenically (-80°C or liquid nitrogen), allowing long-term “banking” of numerous samples while minimizing sample degradation. Consequently, the importance of optimal functioning of storage equipment, detailed personnel training, and careful attention to sample handling cannot be overstated.

Special care should be exercised when selecting and maintaining storage equipment. The equipment should be serviced and maintained by qualified personnel prior to embarking on sample acquisition. It is recommended to seek advice from colleagues who have experience with cryogenic preservation regarding which manufacturers and models offer the most reliability. It is also advisable to install a temperature monitor alarm system that can contact key personnel in case of a power failure or equipment malfunction, particularly after regular work hours.

After suitable storage equipment is selected, the next step is ensuring that all samples are processed in the same manner, preferably by the same personnel where possible. It is of critical importance to establish a clear and detailed SOP for sample processing, particularly for multi-center collaborations and multi-personnel endeavors. Personnel should be trained in-person by an expert in the field, and the training should be recorded and disseminated. A critical tool in assessing consistency in sample processing across multiple sites and multiple personnel is acquisition and analysis of a set of QC (Quality Control) samples obtained at each site. These samples should be processed according to the SOP to be used for the main cohort, and should be identical across the sites/personnel (i.e., the same sample “types”). Analysis of QC samples should be performed prior to analysis of the main cohort so any problems can be quickly identified and solutions can be implemented as quickly as possible for the main cohort.

Storage of samples from large cohorts occasionally necessitates reorganization and relocation of samples. Furthermore, equipment break-downs may require samples to be temporarily transferred to a new piece of equipment during repair. Cryogenically preserved samples should only be handled on dry ice, and should be covered by as much dry ice as possible while outside of the storage equipment. Many types of preservation media are extremely temperature-sensitive, and the integrity of biological samples preserved in such media is easily jeopardized by careless handling. Therefore, personnel training should necessarily contain a portion focusing on proper handling of cryogenically preserved samples. Personnel should also be provided with appropriate equipment, including suitable polystyrene/polypropylene containers, cryogenic gloves, and any other relevant PPE. Emphasis during training should be applied regarding the necessity for rapid handling of cryogenically preserved samples and the importance of constant contact with dry ice while minimizing contact with ambient air and laboratory equipment and surfaces.

Proper handling of cryogenically preserved samples is possibly nowhere more crucial than when it comes time to ship the samples to a site that will be processing them for single cell analysis. To minimize variability in single cell data acquired from biological samples, it is highly desirable to perform all sample analysis at a single site populated by experts in the field, preferably utilizing the same analysis platform for the entire cohort. For multi-site collaborations, this requires shipment of cryogenically preserved samples to the analysis site. All shipments should be contained within dry ice, and expedited (preferably overnight) shipping should be employed. Where possible, it is preferable to organize samples within cardboard freezer boxes, to secure the boxes to prevent them from opening during shipment, and to completely surround each box with at minimum 5 times the box volume in dry ice, distributed evenly on all sides. Furthermore, since dry ice will sublime during shipment, it is important to focus on placing the boxes toward the bottom of the polystyrene shipping container (atop a layer of dry ice) and covering them with the majority of the dry ice at the top of the container, ensuring that the boxes will not become exposed during shipment while the dry ice at the top sublimes. The polystyrene shipping container should then be completely filled with dry ice prior to sealing it for shipment. These recommendations are especially important for international shipments, where the samples can be delayed while passing through the destination country’s customs offices. This also necessitates utilizing a trusted and reliable shipping courier that provides regular dry ice replenishment during shipment. It is crucial that personnel at the destination site are provided with tracking information and are readily available to immediately transfer the samples to proper storage equipment. It is also a good idea to include a temperature monitoring device within the shipment so that any deviations in temperature can be tracked and catalogued.