

The International Society for Biological and Environmental  
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**Due North: Aligning Biobanking Practice  
with Evolving Evidence and Innovation**

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The abstracts that follow demonstrate the broad range of timely issues  
addressed in the contributed oral and poster presentations  
at ISBER's 2017 Annual Meeting & Exhibits.



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**Background:** Dried blood spotting on filter paper cards has been a reliable well-established technique to screen newborn babies for congenital metabolic diseases. Previous reports have also described reproducible quantitation of small molecules, DNA, and high abundance proteins from these papers with success. However, protein preservation has not been fully evaluated for downstream proteomic applications. We are investigating if dried plasma spots (DPS) are a viable and cost effective alternative to collect and preserve proteins for downstream biomarker analysis using multiple analytical endpoints to monitor degradation and oxidation.

**Methods:** Under consent, plasma was collected from 20 healthy volunteers, pooled, aliquoted, and stored using different DPS conditions like nitrogen-flushed bags and pre-stabilized by chemical or heat methods. Control samples were stored at  $-80^{\circ}\text{C}$ . Quantitation accuracy and protein stability will be evaluated for a period of 24 months. A targeted assay (SIS-CAPA-SRM) for protein quantitation is used to follow proteins of interest via analysis on a Waters Xevo TQ-S. Untargeted protein analysis is completed by enriching low abundance proteins with hydrogel nanoparticles then analyzing the digested peptides by LC-MS (LTQ Orbitrap Lumos). Lipopolysaccharide binding protein (LPS-BP) and soluble transferrin receptor (sTfR) were evaluated by SISCAPA-SRM. The selected DPS method(s) will be utilized on a clinical validation study (phase 2). Results will be normalized for comparison against control and in between DPS arms.

**Results:** Protein extraction optimization from filter paper yielded  $97\% \pm 2.7\%$  of total starting protein. At 18 months, the number of unique proteins identified by shotgun analysis showed a progressive decrease from 3% to 25%, depending on the DPS condition, when compared to 0 months. The CERES cards demonstrate the best protein recovery over this storage period. Preliminary findings also indicate a reduction in oxidation rates for nitrogen-flushed DPS cards when compared to samples stored at room air. Nearly 100% yields for LPS-BP and sTfR was obtained on heat stabilized DPS cards after 6 months of ambient storage.

**Conclusions:** 1) Improvements are needed in the recovery of low abundant proteins and oxidation rates before DPS is considered a viable alternative to freezing for the long-term preservation of plasma proteins; 2) DPS CERES and a home-brew chemically stabilized filter paper have been selected for phase 2.

### BRS-33 Banking of Human Pluripotent Stem Cells

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**Abstract Body:** The generation of human pluripotent stem cell banking networks ensures that well-characterized and quality controlled stem cell lines of different origin (adult, fetal, embryonic, induced pluripotent) and grade (research, clinical) are broadly accessible to researchers worldwide for therapy and drug development. The Isenet stem cell biobank, by participating in a number of European and National Research Projects and by joining stem cell biology academic laboratories, provides a unique resource for human and animal biospecimens and by applying "high quality management system" ensures long-term cell storage and preservation of the cell's original features. The establishment of well-characterized panels of induced pluripotent stem cells lines from mature cells in the body holds

great potential for applications in regenerative medicine, drug screening, disease modeling, and medical treatments. While there is growing demand for new stem cell resources, our priority is to make available to the scientific community high quality research-grade pluripotent stem cells that have been generated by academic laboratories.

Isenet represents a fundamental source of highly-controlled biomaterial that fulfills the most stringent standards since it participated in several European and National Research Projects in collaboration with academic stem cell laboratories. Isenet acquires, cryopreserves, characterizes, and distributes well-documented biospecimens since it applies sequentially and systematically a high-quality management system for long-term cell cryopreservation by following a quality control stem cell pipeline. Cells are cryopreserved in culture medium containing 10% dimethyl sulfoxide (DMSO) and/or in CryoStor<sup>®</sup> CS10, a Good Manufacturing Practice cryoreagent containing 10% DMSO, free of animal proteins. This DMSO-based and serum free solution gives optimal results in term of cell viability. All cell line batches are stored in liquid nitrogen containers at  $-196^{\circ}\text{C}$ .

### BRS-34 Total Proteome and Phosphoproteome Analysis and Comparison of Cryopreservation in Liquid Nitrogen and Dry Ice Versus PAXgene-Fixed Tissues at Varying Post-Mortem Intervals Using LC-Mass Spectrometry

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Precise identification and quantitation of proteins in tissue specimens is a highly desirable for accurate downstream study of systems biology, clinical diagnosis, prognosis, and personalized medicine for any disease. In this study, critical preanalytical variables, namely post-mortem interval across three different time points (0, 4, and 12 hours from time of death or clamp time) and four different preservation methods and formats (cryopreserved in LN2, cryopreserved in dry ice, PAXGene-fixed paraffin-embedded and PAXgene fixed in PAXgene stabilizer solution [PFS]) were examined. The study examined differences in total proteome and phosphoproteome expression analysis in 20 cases (organ or tissue transplant donor) for two different tissue types (skeletal muscle and thyroid). Standard protocols for total protein and phosphoprotein enrichment from cryopreserved samples were employed, while modifications needed for PAXgene-fixed samples were developed for the study. Compared to cryopreserved specimens, PAXgene-fixed samples show promising results for total proteome and phosphoproteome analysis for biomarkers discovery and analysis utilizing LC-MS analysis. This study is designed to elucidate the usefulness and potential applications of PAXgene-fixed tissues at various post-mortem intervals compared to traditional means of fixation and long storage for complex analysis of proteins and phosphopeptides for the advancement in biomarker discovery and expression analysis.

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### BRS-35 Comprehensive Analyses of Long Non-Coding RNA Expression Profiles in NSCLC Identified AFAP1-AS1 as a Prognostic Biomarker

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