

## ABSTRACT

We have assayed the quality of biofluids at several time points following thawing at different temperatures to assess the effects of sample handling on pristine new plasma samples and 12 year old clinical samples. A variety of methods, including MesoScale Discovery and Luminex MagPix immunoassays, and mass spectrometric techniques including Accurate Mass Tags (AMT), Isobaric Tags for Relative and Absolute Quantitation (iTRAQ), and Selective Reaction Monitoring (SRM), were used to monitor the effects of time and temperature on the proteome in biofluids.

Using immunoassay methods, markedly different behaviors were observed for some classes of plasma proteins. While pro-inflammatory cytokines persisted, even in very old samples, if kept frozen or at 4°C, they were surprisingly unstable at room temperature. In contrast, some metabolic markers are exquisitely more sensitive to poor storage conditions. For example, glucagon-like peptide 1 is markedly unstable in thawed samples, even those kept at 4 °C.

Quantitative proteomics was also studied using iTRAQ and AMT (LC MS/MS) mass spectrometry-based workflows to identify nearly 300 human plasma proteins in depleted plasma samples, and the majority of them (>93%) maintained steady levels over 24 hours while sitting on ice. However, several complement factors diminished with time, as did fibrinogen beta chain, hemoglobin-a1, ITIH4, ITIH3, fibronectin, attractin, hypoxia inducible factor 1 alpha and others. Furthermore, using the SRM method, which does not require antibody reagents, on an Agilent TripleQuad mass spectrometer, we discovered and developed maternal peripheral blood markers that accurately predict labor in the term and preterm periods, particularly within 48 hours of delivery. In a blinded validation study of 150\* samples, our new biomarker panel ("BirthStat") outperformed the standard fetal fibronectin (fFN) level assay.