Dried blood spot (DBS) methodology has improved sample collection workflows for clinical diagnostics targeting small molecule biomarkers. To extend these benefits to protein targets, we have developed multiple reaction monitoring mass spectrometry (MRM-MS) assays for 37 proteins using stable isotope-labeled standards. These proteins span more than 4 orders of magnitude in measured concentration from albumin (at 76 mg/mL) to apolipoprotein C-I (at 5.9 µg/mL). High precision was achieved as full process technical replicates resulted in CVs of less than 15% for all target peptides. The short-term stability of most targets in DBS samples was excellent stability as 80% remained within 20% of their original concentration, even at elevated temperature (37°C). We are now developing additional MRM assays to simultaneously quantify over 100 proteins in DBS samples for clinical biomarker screening applications.

Furthermore, we have also developed immuno-MALDI (iMALDI) technology which combines the sensitivity of immunoaffinity capture with the specificity of MS detection. We have now taken a multifaceted approach for translating our iMALDI technology into clinical laboratories for routine protein quantification. First, we have automated the sample preparation using the Agilent Bravo liquid handling robot for improved sample throughput. Secondly, we have optimized iMALDI assays for the Bruker Microflex MALDI-TOF, a bench-top instrument that is already widely used in regulated healthcare environments. Here we demonstrate iMALDI technology for the clinical measurement of plasma renin activity (PRA), an established biomarker for primary aldosteronism. The current method automates 192 iMALDI captures and analysis requires only seconds per sample. Initial validation indicates a strong correlation between iMALDI-PRA values and a clinical radioimmunoassay (R2 = 0.92). This iMALDI approach to protein quantification can be multiplexed and is applicable to a wide array of clinical peptide and protein biomarker targets.