BENCHMARKING PERFORMANCE OF COMMONLY USED PROTEOMICS BIOMARKER DISCOVERY PLATFORM – TOWARDS CLINICAL PROTEOMICS STANDARDIZATION

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Protein biomarker discovery is the unbiased, semiquantitative process by which the differential expression profile of specific proteins between two or more states is determined. Platforms combining removal of high abundance proteins using multi-component immunodepletion systems (IDS) followed by peptide fractionation and LC-MS/MS analysis are currently the mainstay of unbiased proteomic biomarker discovery. Despite its rapid technological developments and widespread use, proteomics has also raised major concerns regarding its reproducibility, sensibility and throughput. To address these concerns, we explored several relevant quality assurance benchmarks of immunodepletion and LC-MS performance. Our first goal was to evaluate the impact of immunodepletion on the observed dynamic range and the experimental repeatability. We found that immunodepletion columns afforded highly repeatable; nevertheless, despite the observed improvements in dynamic range, we argue that the use of plasma-designed IDS may not be suitable for other biofluids such as CSF. We also provide the most extensive, to the best of our knowledge, analysis of nontargeted plasma proteins captured by the depletion columns. Finally, a MS-based quantitative test is provided to help individual laboratories to benchmark performance and reliability of their own IDS. The second goal in this study was to evaluate repeatability and reproducibility across platforms and LC-MS systems in a multicenter study setup. To this end, we derived a number of LC-MS performance metrics from replicate experiments from different laboratories and estimated that the median coefficient of variation (CV) at the peptide MS1 level signal varied from 22 to 66% across platforms. We also observed that the median CV value was significantly reduced in less complex samples. In an attempt to identify source of irreproducibility in proteomics analysis, we carried out a systematic evaluation of variability of each component of LC-MS platforms separately. Our results indicate that most of the variability is attributable to the LC-MS injection.