

# Advancing Early Detection of Kidney Cancer (Year 3): A Novel Automated Workflow for High-Throughput Proteomic Profiling of Urinary Extracellular Vesicles

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Renal Cell Carcinoma (RCC) is the world's deadliest urological disease. Although detection before metastasis can raise a patient's 5-year survival rate by over 75%, current diagnostic methods are impractical for early tumor identification (MRI, CT) and invasive (tissue biopsies). Extracellular vesicles (EVs) are a promising reservoir of disease biomarkers for non-invasive diagnoses, but a bottleneck in EV sample processing restricts their immense potential. Current methods have low EV yield, slow processing speeds, and low sample capacity. I aimed to address these issues with a high-throughput, automated workflow for EV isolation, EV lysis, protein extraction, and protein denaturation. The automation results in protein-covered beads ready for various analytical methods, including immunoassays, protein quantitation assays, and mass spectrometry. When I applied the workflow to clinical RCC patient samples, I identified a total of 3,793 unique proteins and 40,380 unique peptides, with 992 significantly upregulated proteins in RCC patients versus healthy controls. These potential biomarkers were involved in several important kidney cancer metabolic pathways also identified with a manual control. Compared to the standard manual protocol for contamination levels, efficiency, and consistency of EV isolation, the automated workflow shows reproducible and robust proteomic quantitation with less than 10% median coefficient of variation. The automation also holds 4X sample capacity, reduces manual labor by 6-10X, and expedites total processing speed by 2-3X. This hands-free workflow represents a practical EV extraction and profiling approach that can benefit both clinical and research applications, streamlining biomarker discovery, tumor monitoring, and early cancer diagnoses.