

THE URINARY EV METAL FINGERPRINT: A CELL-DERIVED BIOMARKER ALTERNATIVE TO CONVENTIONAL URINE ANALYSIS

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Extracellular vesicles (EVs) are nanoscale particles released by cells that carry proteins, nucleic acids, lipids and trace elements which reflect their cell of origin. Their stability and cellular specificity make them promising biomarkers for minimally invasive diagnostics. Unlike whole urine, a composite excretory fluid influenced by hydration, diet, and renal filtration, urinary EVs (uEVs) represent a structured biological compartment that may better capture cell-level metal regulation^[1-3]. To explore this potential, uEVs were isolated from nine healthy female volunteers (23–31 years old). Seventeen metals were quantified in both uEVs and total urine using inductively coupled plasma mass spectrometry (ICP–MS). The uEVs were analysed using an optimised in-house protocol (our method), while total urine metals were measured with the PerkinElmer clinical methodology for hospitals^[4-5]. In a second experience, one volunteer (volunteer A) provided samples at three independent timepoints to assess biological repeatability and intra-individual variability, and uEVs were characterised by nanoparticle tracking analysis (NTA), confirming typical concentrations and size distributions. Principal component analysis (PCA) and partial least squares discriminant analysis (PLS-DA) both revealed a consistent separation between uEV and urine metal profiles, indicating that the uEV metalome constitutes a distinct and regulated compartment rather than a subset of urinary excretion. PCA loadings and PLS-DA variable importance in projection (VIP) scores identified the metals most responsible for group discrimination, underscoring the coordinated and cell-regulated nature of the uEV metal composition compared with the heterogeneous urinary matrix. In volunteer A, coefficients of variation (%CV) across timepoints were comparable between matrices; in fact, the limits of detection (LoD) and quantification (LoQ) achieved with our uEV method were between 2- and 9600-fold lower than those of the clinical assay, demonstrating far greater analytical sensitivity. Collectively, these results show that uEVs provide a stable and biologically meaningful representation of metal homeostasis, supporting their potential as a sensitive source of biomarkers for physiological and pathological processes^[6].

References

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Figures

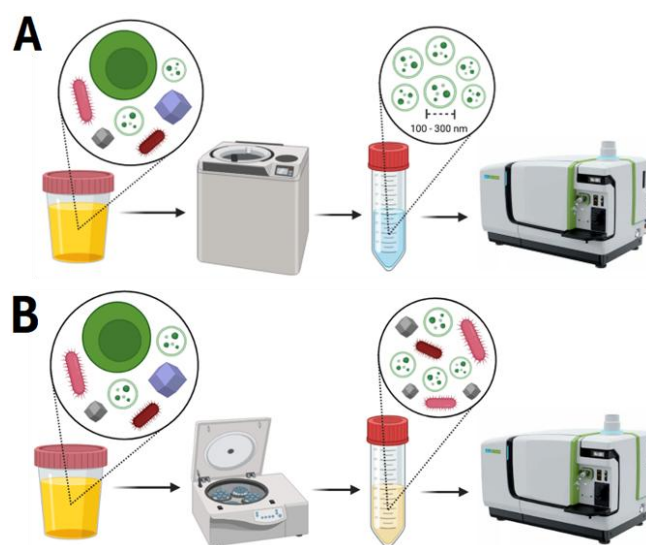


Figure 1. Schematic workflow comparing our method (A) and the current hospital clinical method (B).