Isolation and characterization of cell free nucleic acids and extracellular vesicles from human urine samples

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Abstract

Analytes present in the extracellular fraction of bodily fluids (ex. blood, urine) have utility as a tool for uncovering the molecular landscape of tumors and hold great potential for discovery of individualized cancer medicine. Urine, being non-invasive as a sample type, has an obvious advantage over blood when used for liquid biopsy purposes. However, potential for microbial proliferation and the labile nature of host cells and extracellular vesicles (EVs) at the point of sample collection and transport to the lab drive the need for stabilization of urine samples. Development of such sample stabilization opens up capability of various biomarkers present in the extracellular fraction to be used in liquid biopsy. This is of particular interest as studies around urinary analytes for cancer diagnosis, progression and therapeutic effect are rapidly expanding in cohort sizes. Multi-site collections and at-clinic collections are increasingly prohibitive for large-scale recruitment and lead to variability in the time between collection and processing.

In this study, we have analyzed commercially available urine extraction methodologies for the analysis of cell-free nucleic acids, extracellular vesicles (EVs) and EV RNAs present in the extracellular fraction. Commercially available EV extraction kits were also compared with ultracentrifugation technique for size, concentration and characterization of the isolated EVs from human urine samples using nanoparticle tracking analysis and western blot analysis for membrane markers. EV RNA contents in various urine fractions (first morning first void, random first void and midstream) were compared using RT-qPCR assay to provide better understanding of the differences in collection techniques and fractionations and establish ideal methods for EV research work. First morning first void and random first void urine specimens were collected using Colli-pee device from Novosanis. We have also assessed various available and novel principles for the stabilization of analytes within urine samples during a room temperature hold. Lastly, we have established a framework for evaluating technologies and techniques in the urine sample processing space, which can be utilized by other research groups interested in liquid biopsy studies.