

# Isolation, characterization and stabilization of urinary Extracellular vesicles (EVs) and EV RNA

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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 contains prostate secretions and hence represents a potential valuable source for the detection and monitoring of prostate cancer. Prostate cancer is the second leading cause of cancer-related death in men and the most commonly diagnosed male malignancy worldwide, with > 1.1 million cases recorded in 2012 (<http://www.cancerresearchuk.org/>). For example, a non-coding EV RNA known as PCA3 (DD3) with an increased expression is the most characterized urine biomarker for prostate cancer. However, potential for microbial proliferation and the labile nature of extracellular analytes at the point of sample collection and transport to the lab drives 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 sample collection & downstream processing.

In this study, we have analyzed two commercially available EV extraction kits based on different principles and compared them with ultracentrifugation technique for size, concentration and specificity of the isolated EVs from human urine samples. Analysis was performed using nanoparticle tracking and western blotting for exosomal membrane markers. EV RNA contents in various urine fractions (first morning first void, random first void and midstream) collected using Colli-Pee device (Novosanis) were compared using RT-qPCR assay to provide better understanding of the urine fractionations that are ideal for EV research. We have also developed a novel and efficient sample stabilization solution for preservation of EV RNA in urine samples during an ambient temperature hold. Lastly, we have established a framework for evaluating technologies and techniques in the EV sample processing space, which can be utilized by other research groups.