

# Benefits of Plasma Over Other Body Fluids for Circulating Tumor DNA Detection in Genitourinary Tumors

Manishkumar S. Patel, Liang Wang 

Department of Tumor Biology, H. Lee Moffitt Cancer Center and Research Institute, Tampa, United States

Liquid biopsies have been established as remarkable alternatives to conventional biopsies because of their numerous benefits, including early-stage cancer detection, monitoring disease progression, and predicting treatment response and survival through a minimally invasive approach[1]. Cell-free DNA (cfDNA) consists of short DNA fragments (peak size = 167 bp) released into the bloodstream or other body fluids after cell apoptosis, carrying genome-wide DNA from parent cells. Among cfDNA, circulating tumor DNA (ctDNA) is derived from tumor cells and can be analyzed from various sources, including blood, urine, saliva, pleural effusions, and cerebrospinal fluid (CSF). Plasma-based ctDNA analysis has shown advantages over other biofluids, thanks to standardized blood collection and plasma isolation techniques.

Targeted therapies require continuous monitoring and quick analysis of evolving tumor profiles using minimally invasive methods. Early cancer diagnosis and early detection of disease recurrence also require easily accessible material for genomic analysis, making the detection of ctDNA in body fluids crucial in this process. Generally, ctDNA constitutes < 0.01% to 10% of the total cfDNA, a low yield, necessitating sensitive assays for its detection. Challenges such as hidden micrometastasis, low DNA-shedding tumors, and the influence of different biological variables like mucinous histology contribute to false negatives. All these factors make the detection of ctDNA in body fluids a significant challenge for clinical applications.

Although plasma is widely used for ctDNA analysis, serum and other body fluids are also significant components of large biorepositories. A recent study demonstrated that variant allele frequency was reduced by approximately half in serum when compared to plasma samples[2]. Serum contains higher levels of non-tumor cfDNA, primarily due to leukocyte lysis during serum preparation. This can adversely affect the detection of mutations in ctDNA, especially the ones with low allele fraction mutations[2]. Overall, plasma minimizes ctDNA dilution and enhances the sensitivity of ctDNA analysis compared to serum.

For genitourinary (GU) tumors such as prostate, bladder, and renal cancers, the major sources of ctDNA are plasma, urine, and seminal fluid. However, blood-based ctDNA analysis is well established for clinical implementation due to routine blood collection as part of clinical management of patients. Plasma-derived ctDNA enables the identification of prognostic, predictive, and response biomarkers in prostate cancer[3]. In a recent study, investigators reported an epigenetic classification of renal cell carcinoma (RCC) in patients across all stages of the disease[4]. The study demonstrated that urine-based classification was less accurate than plasma; however, technical and computational optimization could improve urine-based analysis. Another study demonstrated that plasma ctDNA can be used for predicting treatment response in metastatic castration-resistant prostate cancer[5]. In metastatic urothelial carcinoma, mutation concordance between plasma ctDNA and matched tumor tissue was found to be 83.4%([6].

## Abbreviations

cfDNA cell-free DNA  
ctDNA circulating tumor DNA  
GU genitourinary

In non-muscle-invasive bladder cancer, ctDNA from plasma was reported to predict disease recurrence, with a high concordance of somatic variants with tumor DNA[7]. Molecular profiling of prostate cancer using urine and blood revealed a significant association between mutation allele frequencies in plasma and patients with metastatic prostate cancer[8]. Additionally, while urine exhibited more mutations compared to blood, the number of mutations was not found to be associated with clinical characteristics of prostate cancer patients. However, studies have shown that urine cfDNA may be a better source than plasma for early detection of and predicting treatment response in bladder cancer [9–11]. In patients with bladder cancer, urinary ctDNA exhibited a high concordance rate with tumor DNA compared to plasma ctDNA[12], highlighting the importance of urine as a surrogate source of ctDNA for diagnosis, disease monitoring, and personalized medicine.

The major challenges associated with using liquid biopsies for ctDNA detection include gaps in technology development, issues with clinical research methodologies, and the optimal integration into the clinical workflow[13]. The origin of ctDNA circulating in biofluids can vary across tissue sources[14], hence, the best source of ctDNA for a particular cancer depends heavily on the tissue of origin. While plasma has been the most universally accessible, extensively studied, and technologically advanced source for ctDNA detection, the challenge for ctDNA detection in plasma is low ctDNA

content (low ctDNA/cfDNA ratio). In recent years, urine has shown promising results over plasma as a major source of ctDNA for diagnosis and monitoring of some GU cancers, especially bladder cancer. Compared to plasma, urine is a completely noninvasive source of ctDNA, characterized by ease of sampling, a high ratio of ctDNA/cfDNA, and high sensitivity for renal cell and urothelial carcinomas due to proximity to the tumors. However, large volume requirements of urine pose challenges for sample storage. Additionally, ctDNA yield may depend on time since the previous void, with glomerular filtration potentially limiting transrenal ctDNA content[15,16]. Seminal fluid may provide a prostate-specific source of cfDNA for prostate cancer, allowing for noninvasive longitudinal sampling at multiple time points. Seminal fluid has demonstrated reduced DNase activity[17] and high DNA concentrations[18]. However, collecting seminal fluid can be challenging after surgical removal of the key organ and/or during ongoing androgen deprivation therapy.

Despite some theoretical benefits, urinary and seminal fluid ctDNA assays are less developed than plasma-based ctDNA alternatives. Challenges associated with the clinical application of non-plasma-based ctDNA detection include the lack of commercial availability and difficulties in the standardizing pre-analytical factors. Overall, plasma remains the more validated source for clinical applications of liquid biopsies in most GU tumors, although urinary ctDNA assays may substitute for plasma assays in bladder cancer.

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## Key Words

ctDNA, cfDNA, plasma, GU tumors, liquid biopsy

## Competing Interests

None declared.

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