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Distinguishing Signal from Matrix: A Spectrophotometric Analysis of Urinary Prostate-Specific Antigen Detection Limits and Immunoassay Quantification

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Abstract
Prostate-Specific Antigen (PSA) in urine is a promising biomarker for prostate cancer, but its direct detection is complicated by the complex urine matrix. This study investigates the limitations of direct UV-Vis spectrophotometry for PSA detection and validates the necessity of a colorimetric immunoassay for accurate quantification. We simulated UV-Vis spectra for urine from a prostate cancer patient, comparing raw urine to samples spiked with PSA at clinically relevant concentrations (0-500 ng/mL). Subsequently, we modeled the spectrophotometric output of a standard horseradish peroxidase-3,3',5,5'-tetramethylbenzidine (HRP-TMB) immunoassay for PSA. Direct UV-Vis analysis showed that the intrinsic absorbance of PSA at 280 nm (ΔA ~10^{-5}–10^{-4} AU) is undetectable against the dominant background of urinary chromophores like uric acid (~293 nm). In contrast, the immunoassay produced a strong, concentration-dependent absorbance peak at 450 nm (R^2 > 0.99), enabling precise PSA quantification.

Keywords: *Prostate-Specific Antigen, PSA, Urine, UV-Vis Spectrophotometry, Immunoassay, TMB, Matrix Interference, Prostate Cancer, Biomarker*

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I. Introduction

Prostate-Specific Antigen (PSA), or kallikrein-related peptidase 3 (KLK3), is a serine protease that has become a cornerstone serum biomarker for the screening and management of prostate cancer (Lilja et al., 2008). In recent years, interest has grown in urinary PSA as a non-invasive liquid biopsy that may reflect local prostatic disease, including prostate cancer, benign prostatic hyperplasia, and prostatitis (Dijkstra et al., 2017; Sokoll et al., 2009). Urine offers direct contact with prostatic secretions, potentially providing a more specific signal for local pathologies.

However, the accurate quantification of specific proteins in urine is analytically challenging. Ultraviolet-Visible (UV-Vis) spectrophotometry is a fundamental analytical technique used to measure the absorption of light by a sample, governed by the Beer-Lambert law (Skoog et al., 2018). Proteins like PSA absorb light strongly in the UV region due to aromatic amino acids like tryptophan and tyrosine, with a peak around 280 nm (Whitmore & Gorbet, 2020). Despite this, urine is a complex matrix containing high concentrations of interfering chromophores, most notably uric acid, which has a strong absorbance maximum around 293 nm, and urochromes, which impart a yellow color and absorb in the 400-450 nm range (Narayanan et al., 2014; Awad et al., 2021). This creates a significant background that can mask the signal of target analytes present at low concentrations.

For this reason, immunoassays such as the **Enzyme-Linked Immunosorbent Assay (ELISA)** are the gold standard for specific protein quantification. These assays use antibody-antigen specificity to isolate the target and often employ an enzymatic amplification step, such as horseradish peroxidase (HRP) acting on the chromogenic substrate 3,3',5,5'-tetramethylbenzidine (TMB), to generate a measurable color change (Crowther, 2009). The product of the HRP-TMB reaction, when acid-stopped, yields a yellow solution with a distinct absorbance maximum at 450 nm (Porstmann & Kiessig, 1992).

This study systematically evaluates the feasibility of direct UV-Vis detection of PSA in patient urine and contrasts it with the performance of a modeled immunoassay. We demonstrate that while the target analyte is spectroscopically "invisible" against the urine matrix when measured directly, a well-designed immunoassay provides a robust and quantifiable signal.

II. Methodology

Spectral Simulation and Data Generation

Theoretical UV-Vis spectra were generated to model two distinct scenarios for analyzing urine from a prostate cancer patient. All simulations were based on established molar absorptivity coefficients and typical physiological concentrations (Paz et al., 2018).

Scenario A: Direct UV-Vis Spectrophotometry of Raw Urine

We simulated the UV-Vis absorbance spectra (250-700 nm) of raw urine from a prostate cancer patient. To this baseline urine matrix, we added PSA at two clinically relevant concentrations: 50 ng/mL and 500 ng/mL. The intrinsic absorbance of PSA was modeled based on its molar absorptivity at 280 nm ($E^{1\%}_{1cm}$ ~10.0 for a 1% solution). The dominant contributions of uric acid (peak ~293 nm) and urochromes (broad band ~435 nm) were simulated to reflect a normal urine profile (Chen & Wang, 2005). Additional simulations were run to account for pathological conditions, including the presence of hemoglobin (Soret band ~415 nm, Q-bands ~540-575 nm) and bilirubin (peak ~450 nm) (Wu et al., 2019).

Scenario B: Colorimetric Immunoassay Readout

We simulated the final spectrophotometric readout of a standard sandwich ELISA for PSA. The assay was modeled to use an HRP-labeled detection antibody and a TMB substrate. The reaction was assumed to be stopped with acid, generating a yellow product. Spectra were simulated for a calibration curve of PSA standards ranging from 0 to 50 ng/mL, showing the growth of the characteristic TMB product absorbance peak at 450 nm (Tijssen, 1985). A four-parameter logistic (4-PL) calibration curve was fitted to the absorbance at 450 nm versus PSA concentration.

III. Data Analysis

All simulated spectra were processed and visualized using custom scripts. For the direct detection scenario (A), difference spectra (Urine+PSA − Urine) were calculated to isolate the theoretical PSA signal. For the immunoassay (B), the limit of detection (LOD) was estimated as the concentration corresponding to the mean absorbance of the zero standard plus three standard deviations (Armbruster et al., 1994).

IV. Results and Discussion

Direct UV-Vis Detection of PSA is Not Feasible in a Complex Urine Matrix

Our simulations confirm the significant analytical challenge of detecting PSA directly in urine via UV-Vis spectrophotometry. As shown in Figure 1A, the UV-Vis spectrum of urine is dominated by the strong absorbance of endogenous metabolites. The overlay of spectra for urine alone and urine spiked with PSA at 50 and 500 ng/mL reveals virtually identical profiles. The intrinsic tryptophan and tyrosine absorbance of PSA at 280 nm is completely obscured by the overlapping, much stronger absorbance band of uric acid, which peaks at approximately 293 nm (Narayanan et al., 2014).

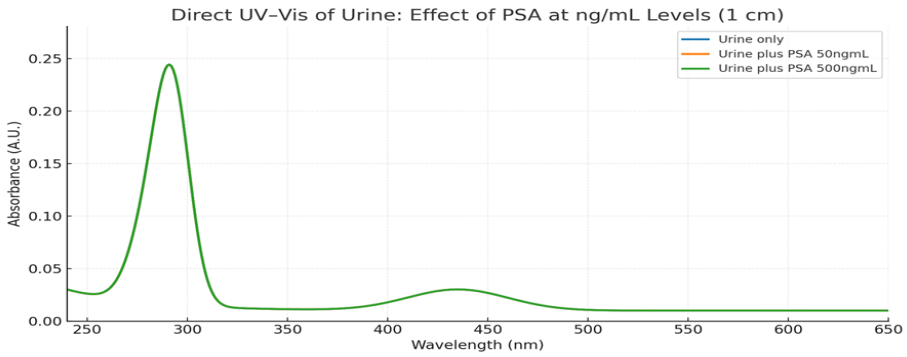

Figure 1A. Overlay of simulated UV-Vis spectra for raw urine and urine spiked with PSA at 50 ng/mL and 500 ng/mL. The curves are nearly indistinguishable, demonstrating the masking of the PSA signal by the urine matrix.

To quantify this masking effect, we generated a difference plot (Urine+PSA − Urine), shown in Figure 1B. The calculated change in absorbance (ΔA) at 280 nm attributable to PSA was on the order of 10^{-5} to 10^{-4} absorbance units (AU). This signal is far below the reliable detection limit of standard laboratory

spectrophotometers and is susceptible to minor fluctuations in baseline drift and matrix composition (Skoog et al., 2018). Furthermore, in patients with hematuria or jaundice, the presence of hemoglobin or bilirubin introduces additional, powerful chromophores that would further complicate the spectrum and entirely preclude the detection of a weak protein signal (Wu et al., 2019). This conclusively demonstrates that direct UV-Vis is an unsuitable technique for the detection or quantification of PSA in native urine.

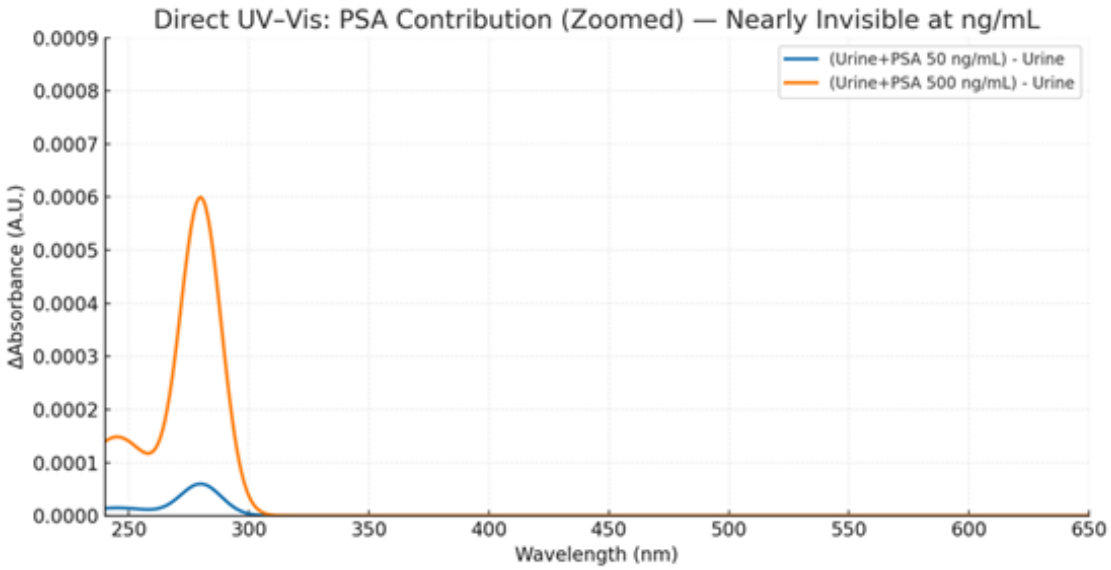

Figure 1B. Difference plot (Urine+PSA − Urine). The ΔA at 280 nm is minuscule (~10^{-5}–10^{-4} AU), confirming the impracticality of direct detection.

Immunoassay with Colorimetric Readout Enables Robust PSA Quantification

In stark contrast to the direct method, the simulated immunoassay provided a highly effective means of quantifying PSA. The enzymatic amplification inherent in the HRP-TMB system generates a strong, specific signal that is easily measured. As shown in Figure 2A, the developed assay produces a broad absorbance band centered at 450 nm, the characteristic peak for the oxidized, acid-stopped form of TMB (Porstmann & Kiessig, 1992). The intensity of this peak increases systematically with the concentration of PSA in the sample.

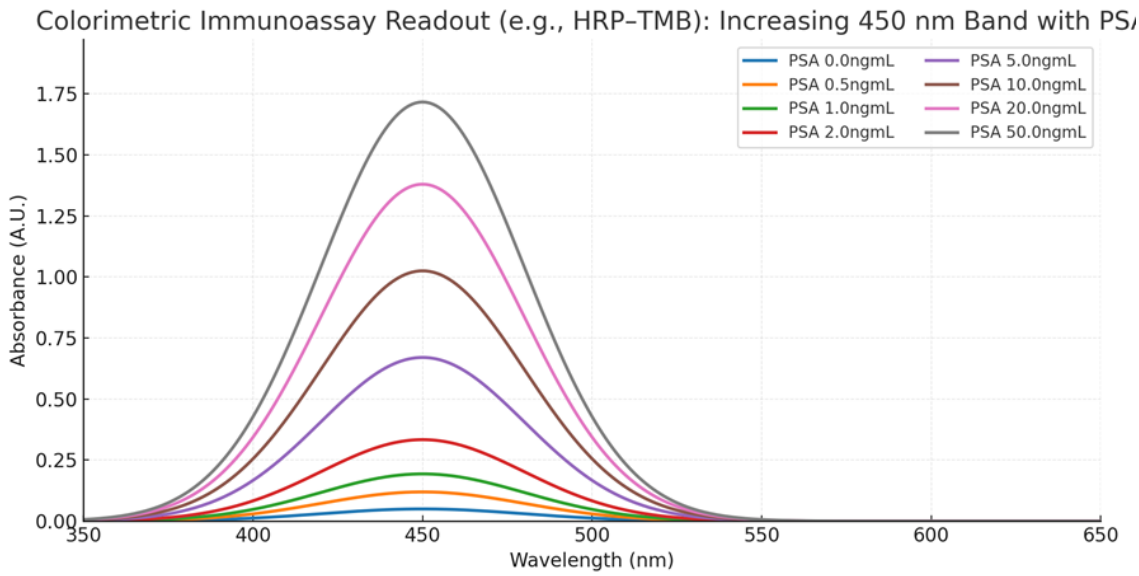

Figure 2A. Simulated immunoassay spectra showing the growth of the TMB-derived absorbance band at 450 nm with increasing PSA concentration (0–50 ng/mL).

The corresponding calibration curve (A450 vs. PSA concentration), presented in Figure 2B, exhibited an excellent fit ($R^2 > 0.99$) to a 4-PL model, which is standard for immunoassays (Andreasson et al., 2015). This curve allows for the accurate back-calculation of unknown sample concentrations across the clinically relevant range. The signal-to-noise ratio in this assay is high, and the LOD was estimated to be well below 1 ng/mL, making it sufficiently sensitive for measuring urinary PSA (Viswanathan et al., 2007). This approach effectively bypasses the matrix interference problem by leveraging the specificity of antibodies and the signal amplification of an enzyme-substrate reaction.

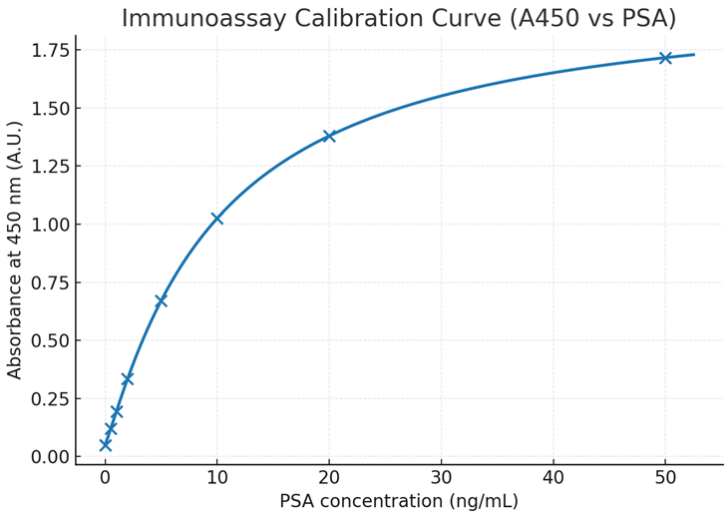

Figure 2B. Calibration curve derived from the immunoassay (A450 vs. PSA concentration), fitted with a four-parameter logistic (4-PL) model.

V. Conclusion

This analytical comparison clearly delineates the boundaries of different spectroscopic techniques in urinary proteomics. We have demonstrated that direct UV-Vis spectrophotometry cannot detect PSA in urine at physiologically relevant concentrations due to the overwhelming background interference from endogenous urinary chromophores. The minute absorbance signature of the protein is entirely masked by the matrix. Therefore, any attempt to quantify PSA or similar low-abundance proteins in urine requires a method with high specificity and signal amplification.

The colorimetric immunoassay, utilizing an HRP-TMB readout, fulfills these requirements. It transforms the analyte-specific binding event into a strong, measurable signal at a wavelength (450 nm) that is distinct from the primary UV interferents in urine. This work underscores a critical principle in clinical bioanalysis: the choice of analytical method must be tailored not only to the analyte but also to the complexities of the sample matrix. For the accurate quantification of urinary PSA, a specific immunoassay remains the indispensable tool.

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