# Role of liquid biopsy in prostate cancer diagnosis: A systematic review and meta-analysis

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Received October 24, 2025; Accepted January 28, 2026

DOI: 10.3892/wasj.2026.443

**Abstract.** Prostate cancer (PCa) is the second most prevalent type of malignancy and one of the lead causes of cancer-related mortality among the male population, accounting for almost 48% of all incident cases in men. The present study aimed to evaluate the specificity and sensitivity of blood and urine biopsies as tools for diagnosing prostate cancer in a systematic review and meta-analysis. A comprehensive search and selection of studies (written in the English language and published between 2013 and 2025) on the use of liquid biopsy in the diagnosis of PCa was conducted from four electronic databases: PubMed, Cochrane Library, OVID Medline and Science Direct. Following the search, three studies on blood-based biopsies and nine studies on urine-based biopsies were included in the present meta-analysis after screening and excluding 1,876 studies. For blood biopsy, the pooled sensitivity was 0.40 [95% confidence interval (CI), 0.25-0.57], with a high heterogeneity ($I^2$=96.5%). The pooled specificity for blood biopsy was 0.98 (95% CI, 0.93-0.99), also exhibiting significant heterogeneity with $I^2$=94.6%. The pooled sensitivity for urine biopsy was 0.83 (95% CI, 0.77-0.88) with a heterogeneity of $I^2$=91.8%. The pooled specificity for urine biopsy was 0.62 (95% CI, 0.42-0.79) with $I^2$=98.9%. The random effects model demonstrated a pooled specificity of 0.87 (95% CI, 0.74-0.94). On the whole, the present study demonstrates that blood biopsy has potential for use as a tool for confirming diagnoses (rule-in), although this biopsy may have a low to moderate sensitivity. By contrast, urine biopsy provides a significant advantage with a higher and more reliable sensitivity, although with greater variability in specificity.

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*Key words:* prostate cancer, liquid biopsy, specificity, sensitivity, diagnosis

## Introduction

Prostate cancer (PCa) is the second most prevalent type of malignancy and one of the leading causes of cancer-related mortality among the male population, accounting for almost one half (48%) of all incident cases in men (1). Current clinical techniques for the diagnosis of PCa include a digital rectal examination (DRE), prostate-specific antigen (PSA) level detection, imaging [transrectal ultrasonography (TRUS) and multiparametric magnetic resonance imaging (mpMRI) of the prostate] and prostate biopsies. Although DRE is affordable and simple to perform, its effectiveness, with a positive predictive value ranging from 5 to 30%, is dependent on the skills of the examiner (2). Moreover, PSA is not a cancer-specific marker. It is affected by age and its levels can increase in non-malignant circumstances. MRI is particularly effective in diagnosing PCa due to its high accuracy in detecting clinically significant cancers. This helps reduce unnecessary biopsies, guides targeted biopsies and facilitates monitoring during active surveillance. Key tools, such as the Prostate Imaging Reporting and Data System (PI-RADS) enhance these capabilities. However, there are some limitations, including high costs, variability in interpretation, the potential to miss aggressive cancers (resulting in false negatives), and challenges related to accessibility and the standardization of readings (3). However, mpMRI is not used for the diagnosis of PCa, but rather for biopsy guidance, local staging, post-treatment assessment, and as an adjunct tool for active surveillance. Prostate biopsy is the only surgery that enables a specific diagnosis and is currently conducted transperineally or transrectally under ultrasound guidance. A hybrid method combining TRUS and mpMRI has increased the overall accuracy of PCa diagnosis to 85% (4). Nonetheless, this method has disadvantages, including the risks of hematuria, infection and urine retention, in which in severe cases, could lead to sepsis and long-term hospitalization (5). Therefore, less invasive approaches are required.

Liquid biopsy involves the non-invasive analysis of biomarkers in biological fluids (such as blood or urine) for the diagnosis of malignancies that avoids the disadvantages of invasive techniques and collects more molecular information than tissue biopsy (6). This test mainly analyses circulating tumor cells (CTCs), plasma cell-free genetic material, such as cell-free RNA and cell-free DNA and extracellular vesicles. Each of these biomarkers provides specific information based

on its intrinsic characteristics. Blood or urine samples can be used for analysis (7). The present performed a systematic review and meta-analysis in an aim to evaluate the specificity and sensitivity of blood and urine biopsies as tools for the diagnosis of PCa.

## Data and methods

*Search strategy*. A comprehensive search and selection of studies written in the English langauge and published between 2013 and 2025 was conducted from four electronic databases: PubMed, Cochrane Library, OVID Medline and Science Direct. The search terms and phrases used to identify the articles relevant to the study topic are listed in Table I. The present systematic review followed the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines for diagnostic test accuracy studies. Additionally, the protocol of the present systematic review has been registered in PROSPERO (RN: CRD42022338451).

*Exclusion and inclusion criteria*. Studies were included if these explored patients who were suspected of having PCa for any reason (patients) and were positive for a liquid biomarker (intervention) compared with those negative for a liquid biomarker (comparison) to assess the diagnostic accuracy for detecting PCa (outcomes) and reported sensitivity and specificity of liquid biopsy for diagnosing PCa. The following studies were excluded: Review articles, letters, editorials, case reports/series, non-human animal studies, studies on PCa with malignancy or metastases and articles not published in the English language.

*Quality assessment and risk of bias*. The risk of bias and applicability were evaluated independently by two authors (FES and FR) using the Quality Assessment of Diagnostic Accuracy Studies-2 (QUADAS-2). The following four domains were assessed: Patient selection, index text, 'reference standard, and flow and timing, and judged these as high, unclear, or low.

*Statistical analysis*. All analyses were performed using R version 4.5.1 via RS studio version 2025.09.0+387(2025; R Foundation for Statistical Computing). Heterogeneity was evaluated using the Chi-squared ($\chi^2$) test and quantified by the I2 statistic. An $I^2$ value >50% indicated substantial heterogeneity, and a P-value <0.05 was considered to indicate a statistically significant difference. The random effects model was used for the present study as a small number of studies and clinical heterogeneity are expected. Heterogeneity in the bivariate diagnostic meta-analysis was evaluated using two complementary approaches. First, the bivariate $I^2$ statistic proposed by Zhou and Dendukuri study (8) was applied which quantifies heterogeneity, while accounting for the correlation between sensitivity and false-positive rate within the Reitsma model. Second, heterogeneity was also assessed using the Holling approach, which provides both sample-size unadjusted and adjusted $I^2$ estimates to illustrate the impact of study size and design on between-study variability (9). Risk of bias assessment for specificity and specifiticity was analysed using funnel plot and Egger's test. Egger's test was used for linear regression method in the meta-analysis to detect potentials publication bias by measuring the asymmetry of the funnel plot.

True positives (TPs), false positives (FPs), false negatives (FNs) and true negatives (TNs) were recalculated using basic diagnostic formulas, considering the sample size, case prevalence, and statistical measures available from each study. Forest plots with 95% confidence intervals (CIs) were calculated and depicted. The analysis of bivariate models was performed using the Reitsma model (10-12). A summary receiver operating characteristic (SROC) curve and calculated the area under the curve (AUC) to examine the diagnostic accuracy of liquid biopsy.

## Results

*Study selection and characteristics*. The initial search for studies identified 1,876 studies; after excluding duplicates and screening for the established criteria, 12 studies were determined to be eligible for inclusion in the present meta-analysis. The PRISMA flowchart illustrating the systematic literature search is presented in Fig. 1.

The characteristics of 12 studies included in the present meta-analysis are depicted in Table II. There were three studies based on blood biopsies (13-15) and nine studies based on urine biopsies (16-24). Some studies reported more than one biomarker within the same study, leading to variations in gene targets, laboratory platforms, and sample sizes of both cases and non-cases. Blood biopsy biomarkers demonstrated a very high specificity, but exhibited a low to moderate sensitivity. By contrast, urine biomarkers exhibited higher and more consistent sensitivity, although with significant variations in specificity.

Diagnostic data were not presented in a complete 2x2 format (TPs, FPs, FNs and TNs) from 11 studies in the present meta-analysis. A total of 12 studies (13-24) reported only sensitivity, specificity, predictive value, or overall accuracy percentages without explicitly stating case frequencies. To ensure consistency and enable a collaborative analysis of all studies: TP, FP, FN and TN values were recalculated using basic diagnostic formulas, considering the sample size, case prevalence, and statistical measures available from each study. The analysis revealed considerable heterogeneity in both diagnostic performance and cohort size.

A descriptive analysis of the meta-analysis revealed significant variation in diagnostic performance across the studies. Sensitivity estimates varied from 0.127 to 0.950, whereas specificity varied from 0.110 to 0.998. A homogeneity test confirmed these findings. Both sensitivity ($\chi^2$=2160.7; df=30; P<0.0001) and specificity ($\chi^2$=3981.0; df=30; P<0.0001). Additionally, the diagnostic odds ratio (DOR) values exhibited a wide range of accuracy patterns across studies, with values spanning from <1 to >1. A strong positive correlation (Rho=0.638; 95% CI, 0.367-0.809) between sensitivity and the false positive rate further indicated a structural association between these diagnostic parameters. These findings support the use of bivariate models, such as the Reitsma model, as a suitable approach, since they can account for dependencies between the diagnostic variables within their structure.

*Risk of bias assessment*. The QUADAS-2 assessment of 12 studies in the present meta-analysis (Fig. 2) revealed a good to moderate methodological quality, a low or unclear risk of

Table I. Literature search strategy.

| Databases | Key words | Results | Date of attempt |
|---|---|---|---|
| PubMed | 'Liquid biopsy' OR 'Circulating tumor cell' OR 'CTC' OR 'ctDNA' OR 'Circulating tumor DNA' OR 'Circulating tumor Deoxyribonucleic Acid' AND 'Prostate cancer' AND 'Diagnosis' | 374 | November 11, 2025 |
| Cochrane Library | 'Liquid biopsy' OR 'Circulating tumor cell' OR 'CTC' OR 'ctDNA' OR 'Circulating tumor DNA' OR 'Circulating tumor Deoxyribonucleic Acid' AND 'Prostate cancer' AND 'Diagnosis' | 717 | November 11, 2025 |
| Medline | 'Liquid biopsy' OR 'Circulating tumor cell' OR 'CTC' OR 'ctDNA' OR 'Circulating tumor DNA' OR 'Circulating tumor Deoxyribonucleic Acid' AND 'Prostate cancer' AND 'Diagnosis' | 82 | November 11, 2025 |
| Science Direct | 'Liquid biopsy' OR 'Circulating tumor cell' OR 'CTC' OR 'ctDNA' OR 'Circulating tumor DNA' OR 'Circulating tumor' AND 'Deoxyribonucleic Acid' AND 'Prostate cancer'AND 'Diagnosis' | 705 | November 11, 2025 |

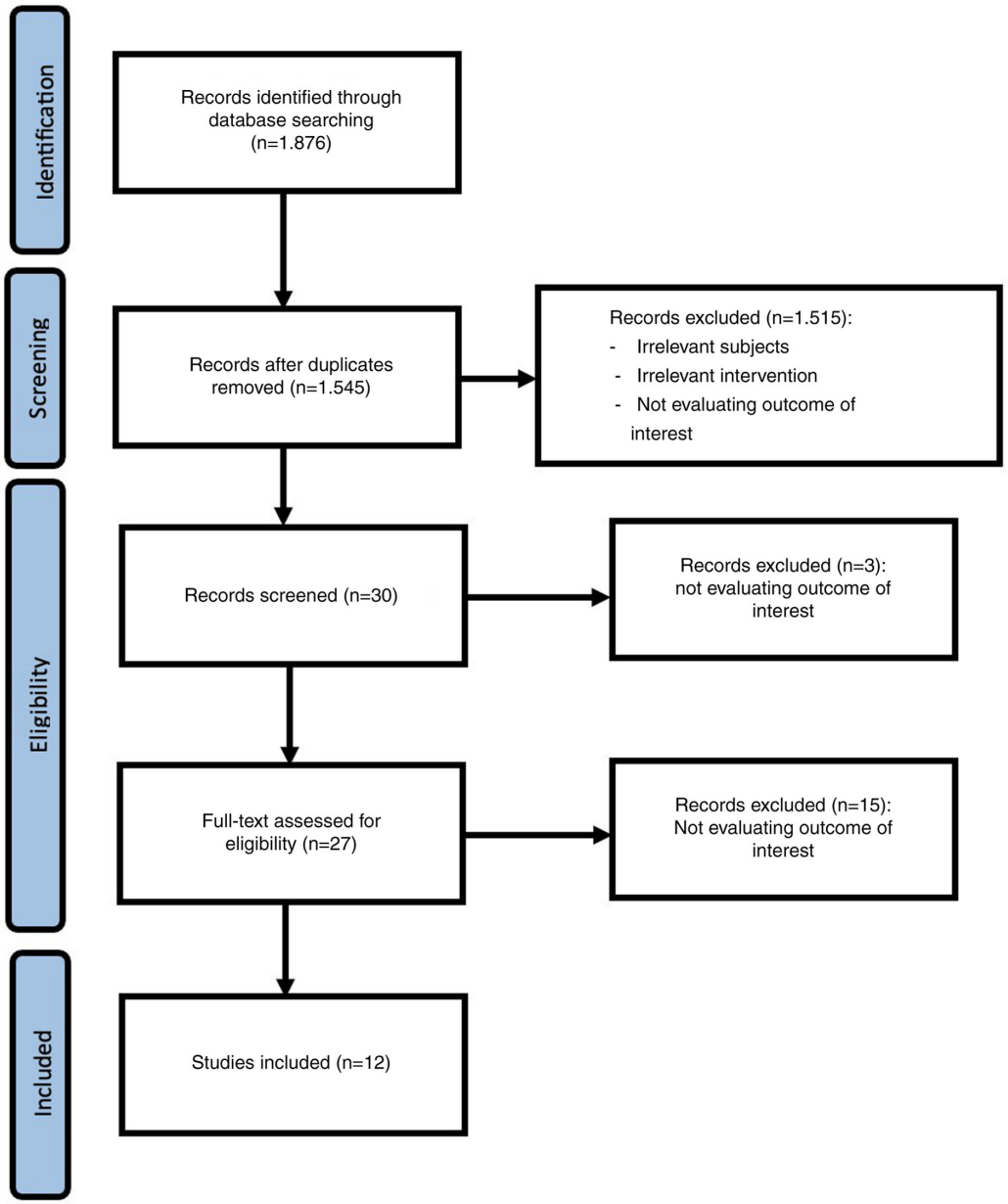


Figure 1. PRISMA flowchart of the studies included in the present systematic review and meta-analysis.

Table II. Characteristics of the studies included in the present systematic review and meta-analysis.

| Authors | Year of publication | No. of samples | Biomarker | Sample | Sens | Spec | No. of patients with disease | No. of patients without disease | TPs | FNs | TNs | FPs | (Refs.) |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Constancio *et al* | 2019 | 323 | FOXA1 | Blood | 0,61 | 0,77 | 121 | 136 | 74 | 47 | 105 | 31 | (13) |
| | | | GSTP1 | Blood | 0,15 | 0,98 | 121 | 136 | 18 | 103 | 133 | 3 | |
| | | | HOXD3 | Blood | 0,8 | 0,43 | 121 | 136 | 97 | 24 | 58 | 78 | |
| | | | RARB2 | Blood | 0,22 | 0,96 | 121 | 136 | 27 | 94 | 131 | 5 | |
| | | | RASSF1A | Blood | 0,13 | 0,96 | 121 | 136 | 16 | 105 | 131 | 5 | |
| | | | SEPT9 | Blood | 0,12 | 0,99 | 121 | 136 | 15 | 106 | 135 | 1 | |
| | | | SOX17 | Blood | 0,23 | 0,99 | 121 | 136 | 28 | 93 | 135 | 1 | |
| | | | Panel (4 genes) | Blood | 0,72 | 0,72 | 121 | 136 | 87 | 34 | 98 | 38 | |
| Haldrup *et al* | 2018 | 37 | CCDC181 | Blood | 0,26 | 1 | 212 | 307 | 55 | 157 | 307 | 0 | (14) |
| | | | HAPLN3 | Blood | 0,44 | 1 | 212 | 307 | 93 | 119 | 307 | 0 | |
| | | | ST6GALNAC3 | Blood | 0,3 | 1 | 212 | 307 | 64 | 148 | 307 | 0 | |
| | | | ZNF660 | Blood | 0,22 | 1 | 212 | 307 | 47 | 165 | 307 | 0 | |
| | | | Panel (3 genes) | Blood | 0,67 | 1 | 212 | 307 | 142 | 70 | 307 | 0 | |
| Ried *et al* | 2020 | 47 | CTC + PSA | Blood | 0,97 | 0,99 | 29 | 18 | 28 | 1 | 18 | 0 | (15) |
| Davey *et al* | 2020 | 56 | 7mRNA+ 2miRNA | Urine | 0,79 | 0,89 | 28 | 28 | 22 | 6 | 25 | 3 | (24) |
| | | | 7mRNA | Urine | 0,75 | 0,84 | 28 | 28 | 21 | 7 | 24 | 4 | |
| Kim *et al* | 2021 | 38 | miR-6090 | Urine | 0,95 | 0,68 | 19 | 19 | 18 | 1 | 13 | 6 | (16) |
| McKiernan *et al* | 2018 | 503 | EPI | Urine | 0,83 | 0,26 | 369 | 166 | 306 | 63 | 43 | 123 | (17) |
| Van Neste *et al* | 2018 | 905 | DLX1 | Urine | 0,83 | 0,16 | 393 | 512 | 326 | 67 | 82 | 430 | (18) |
| | | | HOXC4 | Urine | 0,91 | 0,22 | 393 | 512 | 358 | 35 | 113 | 399 | |
| | | | HOXC6 | Urine | 0,91 | 0,33 | 393 | 512 | 358 | 35 | 169 | 343 | |
| | | | PCA3 | Urine | 0,91 | 0,2 | 393 | 512 | 358 | 35 | 102 | 410 | |
| | | | TDRD1 | Urine | 0,9 | 0,11 | 393 | 512 | 354 | 39 | 56 | 456 | |
| | | | HOXC6+ DLX1 | Urine | 0,91 | 0,36 | 393 | 512 | 358 | 35 | 184 | 328 | |
| Ochiai *et al* | 2013 | 633 | PCA3 | Urine | 0,67 | 0,72 | 264 | 369 | 177 | 87 | 266 | 103 | (19) |
| Wang *et al* | 2017 | 42 | Flotillin2 | Urine | 0,88 | 0,94 | 16 | 16 | 14 | 2 | 15 | 1 | (20) |
| | | | Flotillin2+ PARK7 | Urine | 0,68 | 0,93 | 16 | 16 | 11 | 5 | 15 | 1 | |
| | | | PARK7 | Urine | 0,42 | 0,93 | 16 | 16 | 7 | 9 | 15 | 1 | |
| Yu *et al* | 2024 | 155 | WWP1 + RAB5B | Urine | 0,81 | 0,89 | 43 | 92 | 35 | 8 | 82 | 10 | (21) |

Table II. Characteristics of the studies included in the present systematic review and meta-analysis.

| Authors | Year of publication | No. of samples | Biomarker | Sample | Sens | Spec | No. of patients with disease | No. of patients without disease | TPs | FNs | TNs | FPs | (Refs.) |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Opoku Mensah *et al* | 2022 | 237 | PCA3 | Urine | 0,57 | 0,86 | 63 | 174 | 36 | 27 | 149 | 25 | (22) |
| Cheng *et al* | 2025 | 2,002 | FAM153C-RPL19 | Urine | 0,911 | 0,875 | 826 | 1.176 | 752 | 74 | 1.029 | 147 | (23) |

Sens, sensitivity; Spec, specificity; TPs, true positives; FNs, false negatives; TNs, true negatives; FPs, false positives.

bias, minimal applicability concerns, and a representative patient selection. Index tests of all studies revealed a low risk as the biomarker testing methods were well-reported, although some studies did not specify threshold values beforehand, leading to slight uncertainty (13-24). Reference standards generally had a low risk, supported by the use of appropriate standard diagnostic methods and no issues relevant to disease definition. Low risk was also demonstrated in the study flow and timing. Only a few studies exhibited a high risk of bias and almost no significant applicability issues.

*Meta-analysis of blood biopsy*

*Sensitivity of blood biopsy*. The forest plot depicted in Fig. 3 illustrates the comparison of the sensitivity of the three blood-based studies. There was a wide range of sensitivity values (~0.12 to 0.97), with a pooled sensitivity of 0.40 (95% CI, 0.25-0.57). This pooled sensitivity indicates that blood biomarkers generally have limited effectiveness in detecting prostate cancer, as they identify only ~40% of positive cases. A high heterogeneity ($I^2$=96.5%) was also found in the blood-based studies. The optimal diagnostic performance was observed in the multigene panel in the study by Constâncio *et al* (13) and the CTC+PSA markers identified in the study by Ried *et al* (15). By contrast, some individual biomarkers, including RASSF1A and SEPT9, demonstrated a significantly lower accuracy.

*Specificity of blood biopsy*. As regards specificity, the three blood-based studies reported very high values (~0.72 to 1.00), with a pooled specificity of 0.98 (95% CI, 0.93-0.99), as illustrated in Fig. 4. This indicates that blood biomarkers are highly effective at identifying individuals who do not have cancer, resulting in a low false-positive rate. Additionally, heterogeneity was high ($I^2$=94.6%); the effect direction remains very consistent, as almost studies demonstrated near-perfect specificity.

*Meta-analysis of urine biopsy*

*Sensitivity of urine biopsy*. The forest plot of the sensitivity urine biopsy-based studies (Fig. 5) revealed that this ranged from 0.67 to 0.95, with a pooled sensitivity of 0.83 (95% CI, 0.77-0.88). Both the gene panel in the study by Van Neste *et al* (18) and the EPI marker the study by McKiernan *et al* (17) demonstrated strong performance. The evaluation of heterogeneity remained high ($I^2$=91.8%), indicating significant biological and methodological variations between the studies.

*Specificity of urine biopsy*. The specificity of the urine-based biopsy studies varied significantly (Fig. 6); this was ~0.11 to 0.94 with a pooled specificity of only 0.62 (95% CI, 0.42-0.79). While two studies (19,22) exhibited a high performance, the majority of biomarkers reported in the study by Van Neste *et al* (18) exhibited low specificity values (<0.35). The very high heterogeneity ($I^2$=98.9%) indicates that a variety of structural factors, such as differences in molecular classification, detection thresholds and measurement technology, play a critical role.

*Meta-analysis specificity of blood biopsy and urine biopsy (random effects model)*. The specificity forest plot of blood biopsy vs. urine biopsy using the random effects model (Fig. 7) revealed a wide variation in diagnostic performance of 12

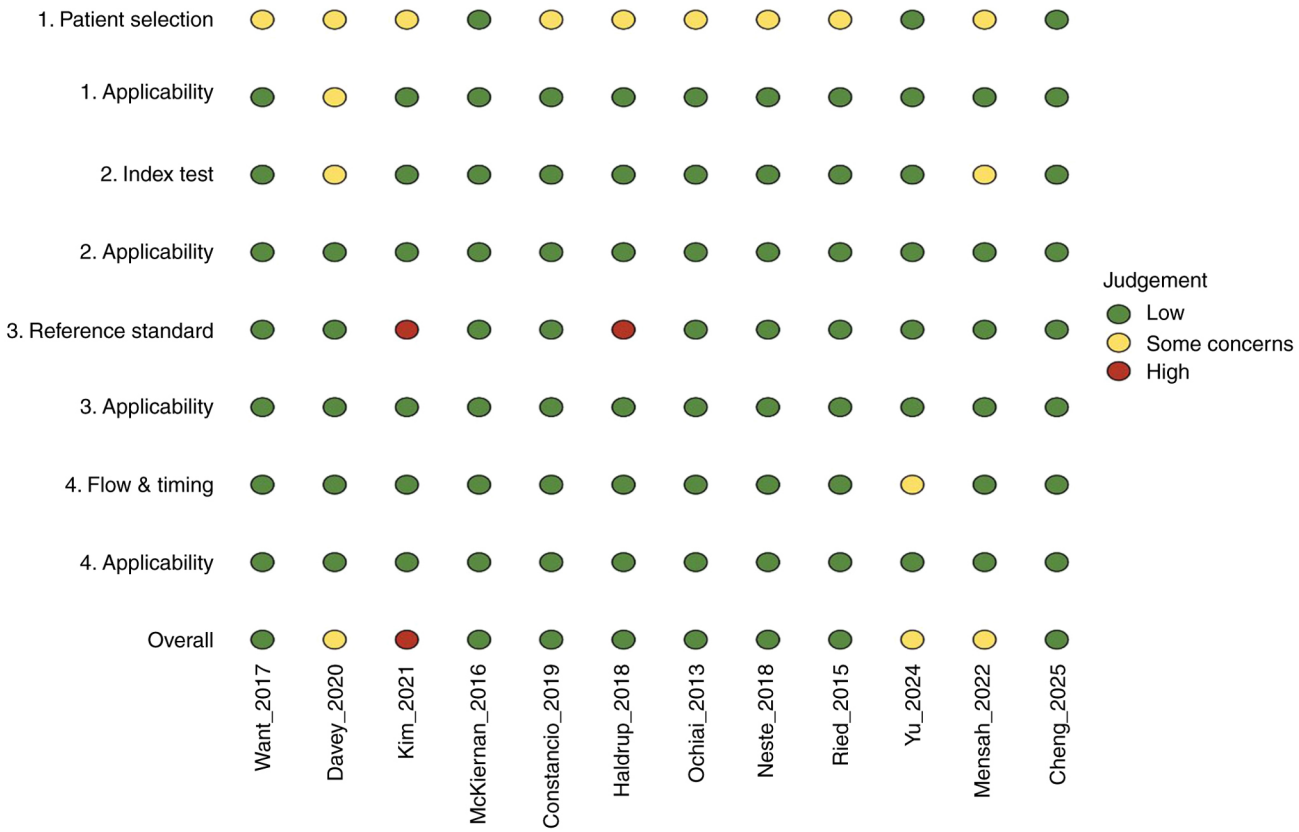


Figure 2. Summary of QUADAS-2 for assessing the risk of bias of the included studies. The studies included were the following: Constancio *et al* (13), Haldrup *et al* (14), Ried *et al* (15), Davey *et al* (24), Kim *et al* (16), McKiernan *et al* (17), Van Neste *et al* (18), Ochiai *et al* (19), Wang *et al* (20), Yu *et al* (21), Opoku Mensah *et al* (22) and Cheng *et al* (23).

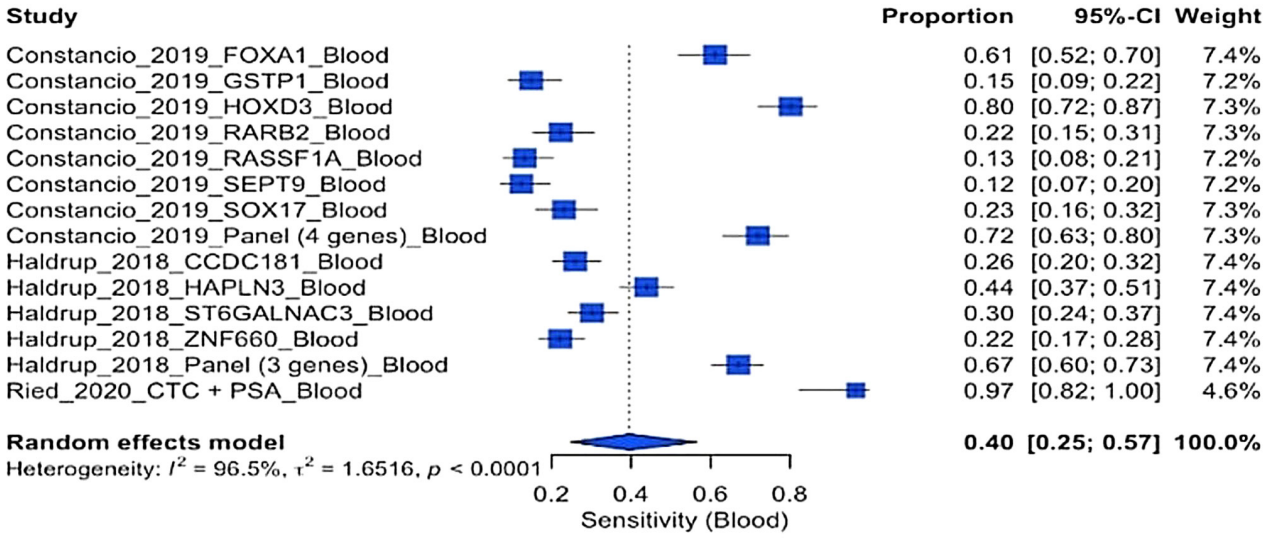


Figure 3. Forest plot of the sensitivity of the blood biopsy studies. The studies included were the following: Constancio *et al* (13), Haldrup *et al* (14) and Ried *et al* (15).

different studies. Blood-based biomarkers generally exhibited very high specificity, with the majority of values approaching 1.00 and relatively narrow confidence intervals, particularly in the study by Haldrup *et al* (14) and the gene panel in the study by Constancio *et al* (13).

By contrast, urine biomarkers demonstrated a much wider range of specificities. Some urine markers, such as the HOXC4, HOXC6, DLX1, PCA3 and TDRD1 panels (19), exhibited low to moderate specificity, indicating a higher rate of false positives in certain groups. However, other urine markers, similar with the Flotillin2 + PARK7 and WWP1/RAB5B panels (18), exhibited high specificity. This variation highlights that the specificity of urine biomarkers is highly dependent on the type of biomarker and the analytical methods used.

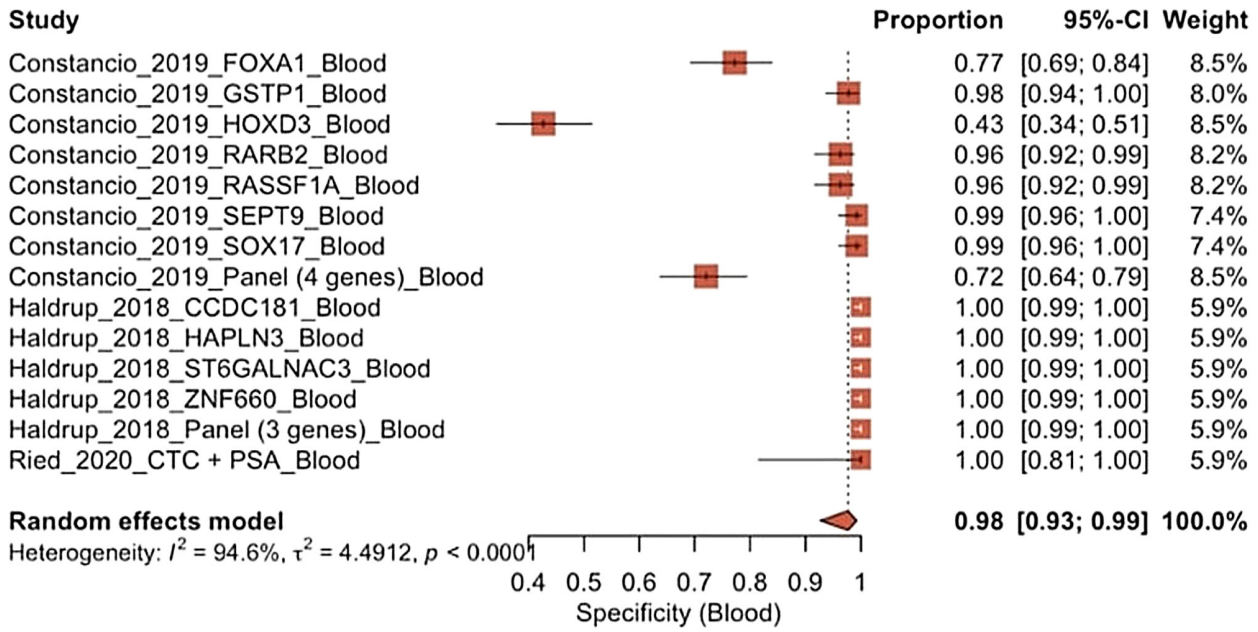


Figure 4. Forest plot of the specificity of the blood biopsy studies. The studies included were the following: Constancio *et al* (13), Haldrup *et al* (14) and Ried *et al* (15).

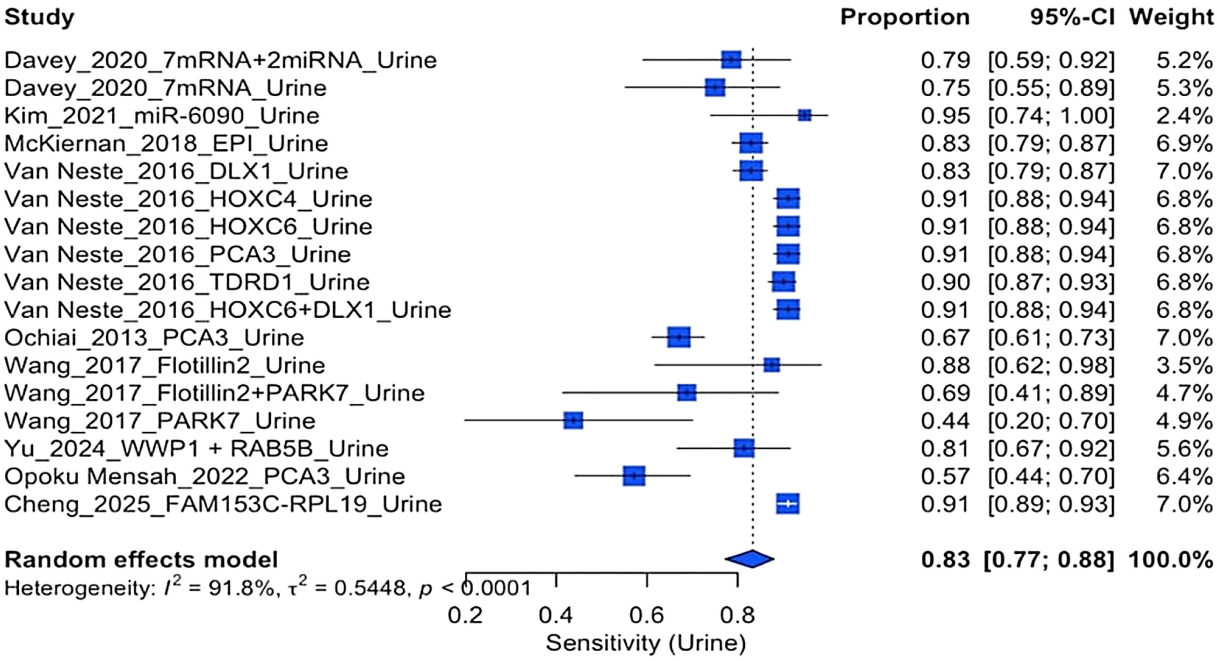


Figure 5. Forest plot of the sensitivity of the urine biopsy studies. The studies included were the following: Constancio *et al* (13), Haldrup *et al* (14) and Ried *et al* (15). Davey *et al* (24), Kim *et al* (16), McKiernan *et al* (17), Van Neste *et al* (18) Ochiai *et al* (19) Wang *et al* (20), Yu *et al* (21), Opoku Mensah *et al* (22) and Cheng *et al* (23).

Overall, the random effects model yielded a pooled specificity of 0.87 (95%, CI 0.74-0.94), indicating a good diagnostic ability to identify subjects without prostate cancer. However, the significant dispersion of studies along the horizontal axis, corresponding to $I^2$=98.4%, indicates extreme heterogeneity and substantial variation between studies (Fig. 7).

*Meta-analysis sensitivity of blood biopsy and urine biopsy (random effects model).* The forest plot of sensitivity of blood biopsy and urine biopsy using random model effect (Fig. 8) revealed significant variations in diagnostic performance between the studies. Generally, the blood-based studies exhibited lower and more widely dispersed sensitivity values (ranging from 0.12 to 0.80), particularly for single markers. By contrast, the urine-based studies exhibited relatively high and consistent sensitivity values (~0.67 to 0.97). This distinction is noticeable in the distribution of confidence intervals at the top of the graph, where several urine biomarkers, including

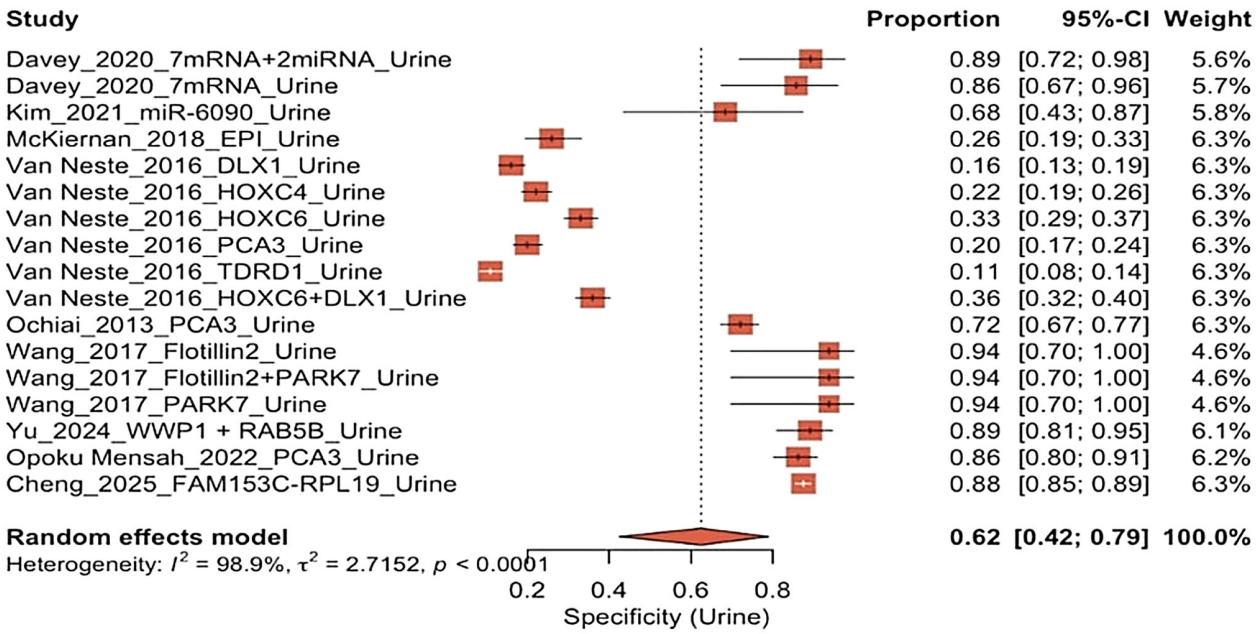


Figure 6. Forest plot of the specificity of urine biopsy studies. The studies included were the following: Constancio *et al* (13), Haldrup *et al* (14) and Ried *et al* (15). Davey *et al* (24), Kim *et al* (16), McKiernan *et al* (17), Van Neste *et al* (18), Ochiai *et al* (19), Wang *et al* (20), Yu *et al* (21), Opoku Mensah *et al* (22) and Cheng *et al* (23).

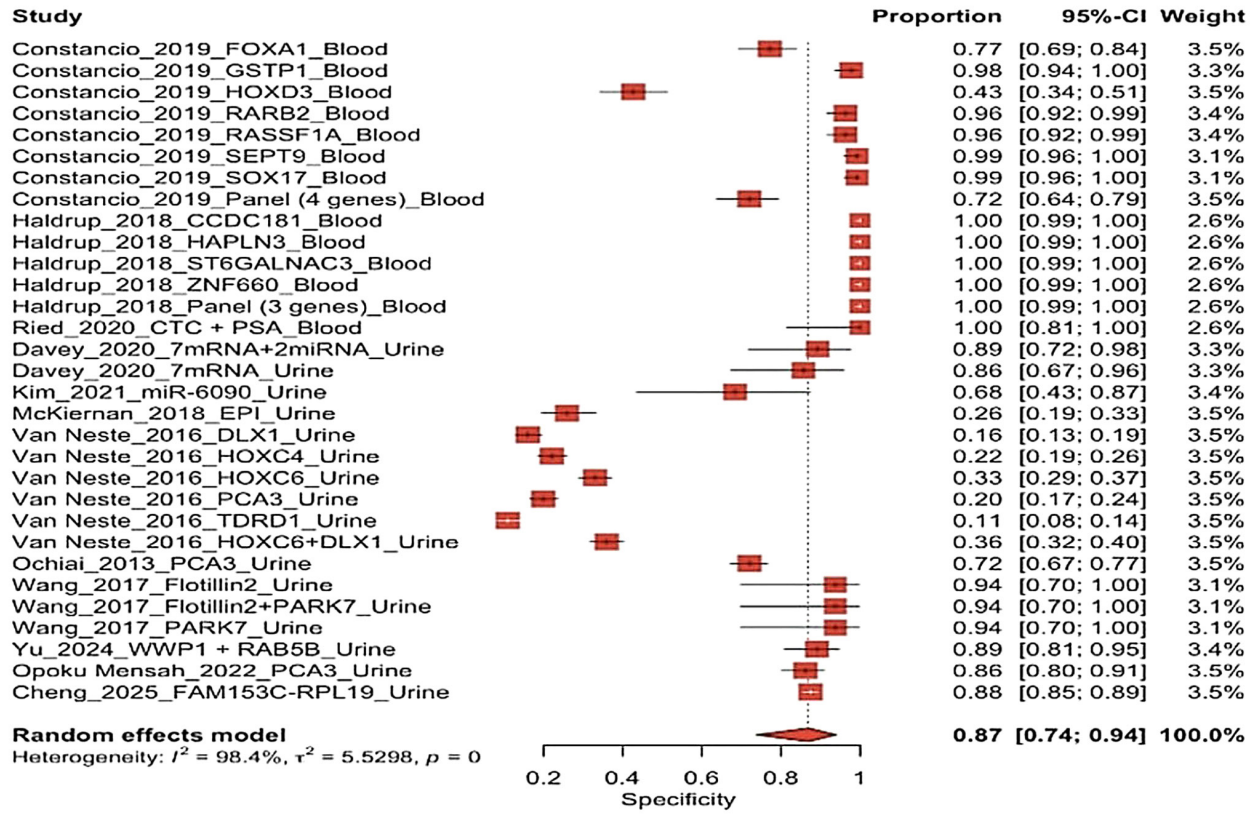


Figure 7. Specificity forest plot comparing blood biopsy and urine biopsy studies using a random effects model. The studies included were the following: Constancio *et al* (13), Haldrup *et al* (14), Ried *et al* (15), Davey *et al* (24), Kim *et al* (16), McKiernan *et al* (17), Van Neste *et al* (18), Ochiai *et al* (19), Wang *et al* (20), Yu *et al* (19), Opoku Mensah *et al* (22) and Cheng *et al* (23).

HOXC6, HOXC4 and PCA3, and the multigene panel from the study Van Neste *et al* (18) cluster in areas indicating high sensitivity with relatively narrow confidence intervals. This suggests that these estimates are more stable. By contrast, the majority of blood-based biomarkers (such as RASSF1A, SEPT9, RARB2 and SOX17) exhibited a low to intermediate sensitivity with wider confidence intervals, reflecting greater variability in estimates and smaller sample sizes (13).

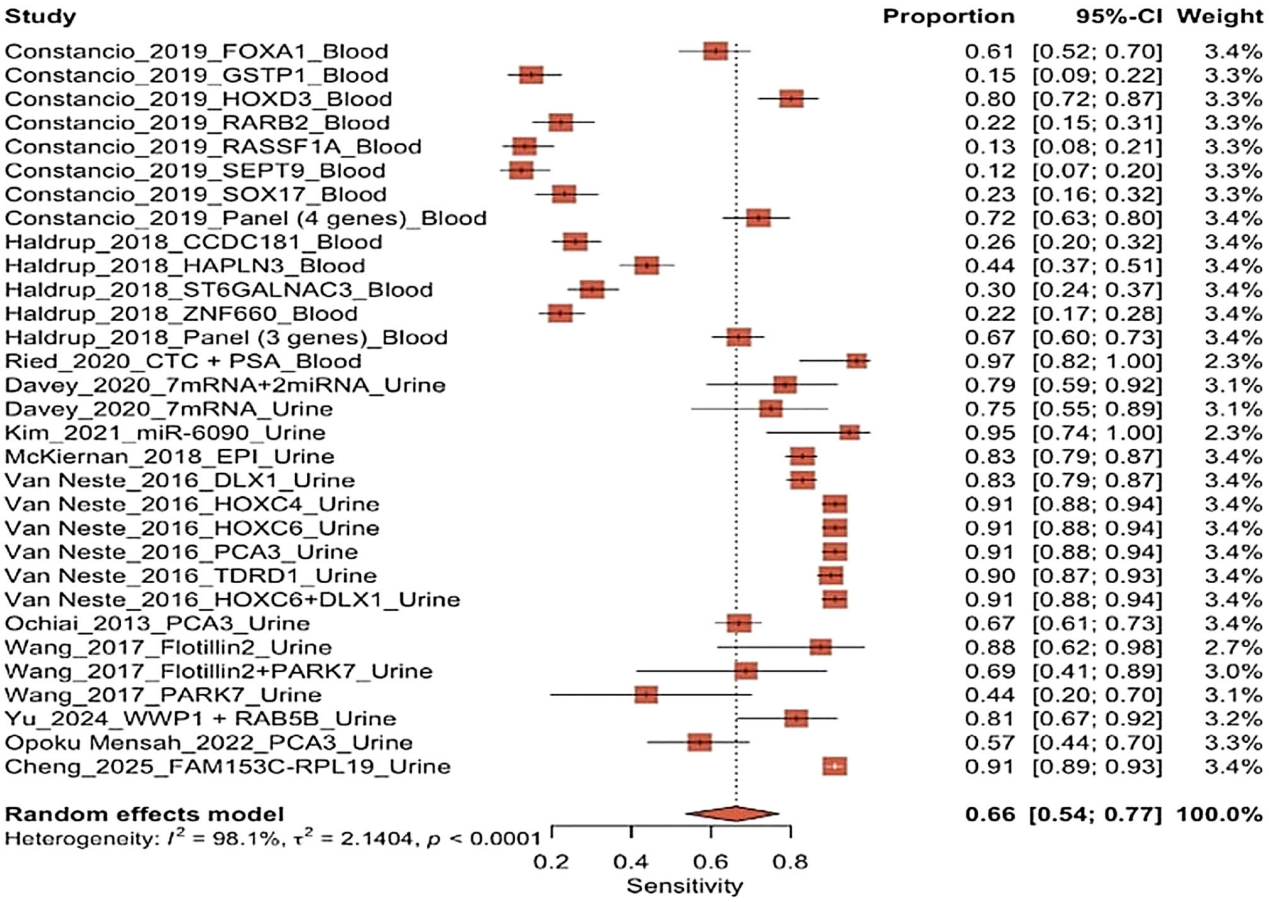


Figure 8. The sensitivity forest plot comparing blood biopsy and urine biopsy studies using a random effect model. The studies included were the following: Constancio *et al* (13), Haldrup *et al* (14), Ried *et al* (15), Davey *et al* (24), Kim *et al* (16), McKiernan *et al* (17), Van Neste *et al* (18), Ochiai *et al* (19), Wang *et al* (20), Yu *et al* (21), Opoku Mensah *et al* (22) and Cheng *et al* (23).

The random effects model indicated a pooled sensitivity of 0.66 (95% CI, 0.54-0.77), suggesting that the average sensitivity of all the diagnostic tests included in this meta-analysis is at a moderate level. However, the wide range of study effect points and the significant variation in study values highlight a high degree of heterogeneity among the studies, which aligns with the previously reported $I^2$ values. This analysis also revealed that no single study or biomarker predominantly influenced the results of the meta-analysis, as the weights assigned to the studies are fairly evenly distributed (~3.4%). This lack of dominance by any one study increases confidence that the pooled results are not being overly influenced by a single source.

The forest sensitivity plot indicated that while some biomarkers demonstrated high diagnostic performance on their own, their collective sensitivity accuracy was only moderate and can vary significantly across different studies. This highlights the necessity for additional subgroup analyses and bivariate methods to gain a comprehensive understanding of the relationship between sensitivity and specificity.

*Risk of bias assessment for specificity (funnel plot and Egger's test).* Egger's test was used for linear regression method in the meta-analysis to detect potentials publication bias by measuring the asymmetry of the funnel plot. The bias assessment for specificity funnel plot analysis of the present meta-analysis (Fig. 9) revealed a clear asymmetrical distribution of study points. Studies with a high specificity are clustered in the upper right-hand corner of the plot, while those with lower specificity are less common and unevenly distributed toward the left-hand corner. This imbalanced distribution correlates with the significant result from Egger's test (t=2.60; P=0.014), which indicates funnel plot asymmetry and suggests the possibility of bias in specificity estimates.

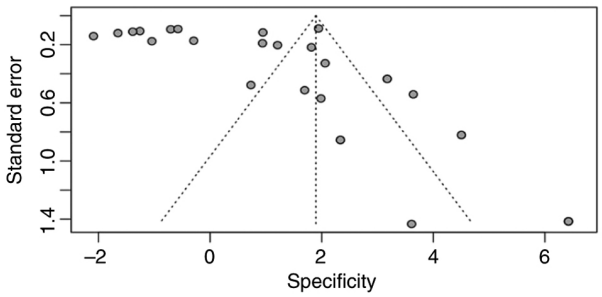


Figure 9. Funnel plot for specificity.

*Risk of bias assessment for sensitivity (funnel plot and Egger's test).* The bias assessment for sensitivity funnel plot analysis (Fig. 10) revealed a relatively symmetrical distribution of study points around the combined effect line, exhibiting no evidence

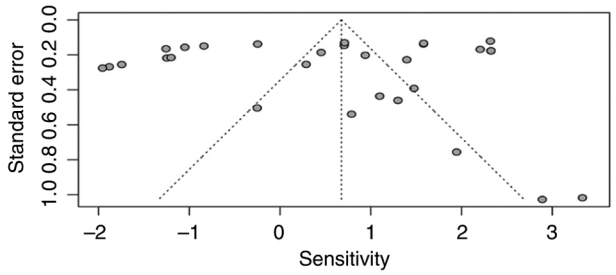


Figure 10. Funnel plot for sensitivity.

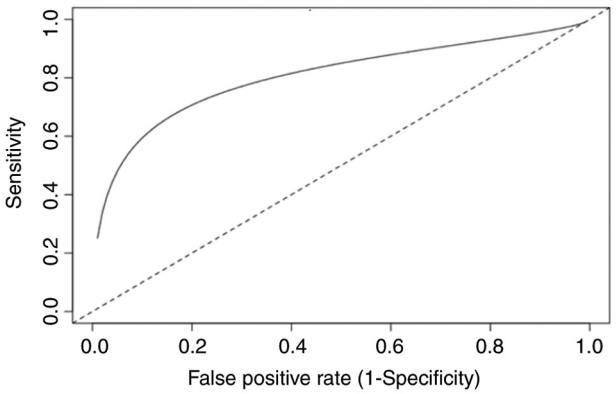


Figure 11. Summary receiver operating characteristic curve - bivariate Reitsma model for included studies.

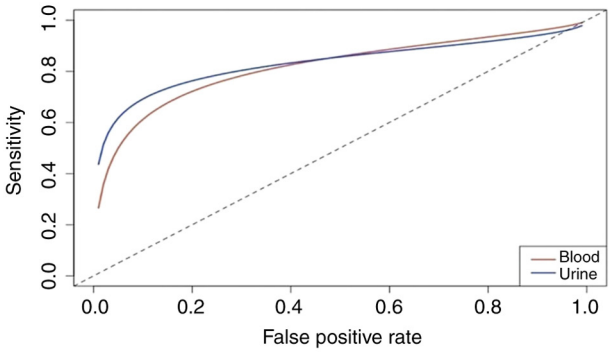


Figure 12. Comparison of summary receiver operating characteristic curve curves for blood biopsy and urine biopsy studies.

of missing small studies on either side. This plot analysis aligns with the non-significant result of Egger's test (t=-0.64; P=0.53); a small, non-significant bias estimate (-2.05; SE=3.23) supports this finding. However, the funnel plot reveals a wide vertical distribution of studies, suggesting high variation in standard errors among them. This observation is consistent with very high residual heterogeneity ($tau^2$=53.24), which complicates interpretation as Egger's test is generally less sensitive to datasets characterized by extreme heterogeneity. The highly varied data ($tau^2$=53.7), influenced by significant differences in precision levels between studies also observed in this test.

*Diagnostic performance with bivariate random effects (Reitsma model) for included studies*. The analysis of the Reitsma bivariate random effects model for the included studies (Fig. 11) demonstrated that prostate biomarkers within the overall dataset exhibited solid diagnostic performance, exhibiting moderate sensitivity and high specificity. The logit intercept for sensitivity [the logit-transformed sensitivity (tsens)=0.62; P=0.015] and the false-positive rate [the logit-transformed false-positive rate (tpfr)=-1.80; P<0.001] were both statistically significant. This resulted in a pooled sensitivity estimate of 0.65 (95% CI, 0.53-0.76) and a false-positive rate of 0.14 (95% CI, 0.07-0.27) following probability transformation. The likelihood ratio for a positive result (LR+) of 4.83 significantly enhances the odds of having cancer, whereas the likelihood ratio for a negative result (LR-) of 0.41 suggests limited ability to exclude the disease. The DOR of 11.77 indicates a good overall discriminatory capacity. The SROC curve, with an AUC of 0.80, indicates a strong sensitivity-false-positive association that significantly exceeds the random line, indicating good discriminatory ability. The random effects model revealed significant heterogeneity, with considerable between-study standard deviations for sensitivity (1.39), the false-positive rate (2.26) and strong positive correlation between sensitivity and the false-positive rate (ρ=0.81).

Heterogeneity from the Zhou-Dendukuri approach ($I^2$=33.8%) indicated moderate variability after accounting for the bivariate structure of the model. By contrast, heterogenicity based on the Holling approach yielded higher values (ranging from 84 to 94%), suggesting that factors, such as sample size and study design significantly influence the variability of the results (8,9).

*SROC curve between blood biopsy vs. urine biopsy*. The comparison of SROC curves between blood biopsy vs. urine biopsy (Fig. 12) revealed a significant difference in diagnostic performance between blood and urine biomarkers. In the low to medium range of false-positive rates, the curve for urine biomarkers is consistently higher than that for blood biomarkers. The blood curve is higher in the section of the graph that show a very low false-positive rate, indicating stronger specificity. Both curves are positioned far from the random diagonal line, which confirms that both urine and blood tests have significant diagnostic accuracy that surpasses chance-based predictions. The alignment of the two curves in the upper right-hand corner of the graph suggests that at very high false-positive rates, when diagnostic discrimination is minimal, the sensitivity of blood and urine tests becomes similar.

## Discussion

The present meta-analysis of 12 studies (8-19) that investigated blood biopsy and urine biopsy with different biomarkers as a diagnostic tool for PCa found that accuracy was significantly affected by biomarker type, analytical approach and sample characteristics. The effectiveness of PCa biomarkers depends on the type of biopsy (blood or urine) and the type of biomarker. Accuracy was significantly affected by the variation in molecular targets or biomarker type, RNA/miRNA/methylation platforms, laboratory protocols and population characteristics. Clinically, the high sensitivity and accuracy of urine biomarker tests support this biopsy in diagnosing of PCa, rendering urine biomarkers suitable for ruling out the disease compared with blood biomarkers.

Blood-based biomarkers generally exhibit very high specificity, indicating that these biomarker effectively exclude non-cancer individuals with a low risk of false positives. By contrast, urine biomarkers display a wider range of specificities, from low to moderate, which can lead to a higher rate of false positives in certain groups. However, some urine biomarkers, such as the Flotillin2 + PARK7 (15) or WWP1/RAB5B panels (19) demonstrate a high specificity. This difference underscores that the specificity of urine biomarkers is highly dependent on the type of biomarker and the analytical platform used. The observed heterogeneity also reveals significant variation across different studies.

The forest plot of specificity indicated that blood biomarkers are more consistent and superior for rule-in diagnosis, whereas urine biomarkers exhibit greater variability in their specificity performance. To better understand the diagnostic performance of both types of biomarkers, these combined results should be analyzed using subgroup and bivariate models for a more comprehensive assessment. Blood biopsy provides the significant advantage of enabling repeated collection, which aids in the early detection of resistance mechanisms that can influence treatment strategies. This approach allows for the more effective implementation of combination therapies. By utilizing genomic, transcriptomic and epigenomic analyses on blood samples, the prediction of patient phenotypes can be enhanced and liquid biopsies can be optimized in clinical settings. An effective PCa blood assay should guide essential clinical decisions, including diagnosis, molecular characterization, early risk stratification, timely relapse detection and informed treatment choices regarding intensification or cessation (25). Collaboration among clinicians, researchers and bioinformaticians is essential to address this challenge to identify who should be molecularly profiled, improve biomarker-driven clinical trial designs, overcome technical obstacles, and successfully integrate liquid biopsies into standard clinical practice, ultimately enhancing the outcomes of patients with PCa.

The liquid biopsy is revolutionizing cancer care as an effective non-invasive approach for early diagnosis, tailored biopsy selection, ongoing surveillance of low-risk cancer, and monitoring for recurrence after treatment. By harnessing this advanced technology, highly sensitive and specific biomarkers can be identified, enabling earlier detection and superior risk stratification. This paves the way for personalized treatment plans that cater to the unique needs of each patient with PCa, ensuring improved outcomes and quality of life (26-28).

Further meta-analyses are required to assess the diagnostic performance of each biomarker from blood and urine biopsy as a single or combined biomarker with different prevalence, thresholds and laboratory approaches (genomic, transcriptomic and epigenomic analyses) for clinical settings. Another key aspect of these biomarkers is their cost effectiveness. The main challenges are ensuring that tests are reliable, affordable, and clinically useful to prevent both overdiagnosis and undertreatment.

In conclusion, according to the present meta-analysis, blood biopsy has potential for use as a tool for confirming diagnoses (rule-in), although this biopsy may have a low to moderate sensitivity. By contrast, urine biopsy provides a significant advantage with the higher and more reliable sensitivity, although with greater variability in specificity. This unique profile renders urine biopsy an optimal choice for initial screenings (rule-out), helping to identify potential issues early and effectively. Selecting the right type of biomarker both from blood or urine biopsy can enhance diagnostic accuracy and improve patient care.

**Acknowledgements**

Not applicable.

**Funding**

No funding was received.

**Availability of data and materials**

The data generated in the present study may be requested from the corresponding author.

**Author's contributions**

All authors (FES, FR, CAM and ARAHH) conducted a significant portion of the literature search and drafted the manuscript, had full access to all data in the study and take responsibility for the integrity of the data and the accuracy of the data analysis, provided critical review and feedback on the manuscript. All authors have read and approved the final version of the manuscript. FES and FR confirm the authenticity of all the raw data.

**Ethics approval and consent to participate**

Not applicable.

**Patient consent for publication**

Not applicable.

**Competing interests**

The authors declare that they have no competing interests.

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