Medical Policy

BlueCross BlueShield Kansas
An Independent licensee of the Blue Cross Blue Shield Association

Title: Genetic and Protein Biomarkers for the Diagnosis and Cancer Risk Assessment of Prostate Cancer

Related Policies:	▪ *Gene Expression Profiling and Protein Biomarkers for Prostate Cancer Management*

Professional / Institutional
Original Effective Date: February 11, 2011
Latest Review Date: January 13, 2026
Current Effective Date: January 13, 2026

State and Federal mandates and health plan member contract language, including specific provisions/exclusions, take precedence over Medical Policy and must be considered first in determining eligibility for coverage. To verify a member's benefits, contact Blue Cross and Blue Shield of Kansas Customer Service.

The BCBSKS Medical Policies contained herein are for informational purposes and apply only to members who have health insurance through BCBSKS or who are covered by a self-insured group plan administered by BCBSKS. Medical Policy for FEP members is subject to FEP medical policy which may differ from BCBSKS Medical Policy.

The medical policies do not constitute medical advice or medical care. Treating health care providers are independent contractors and are neither employees nor agents of Blue Cross and Blue Shield of Kansas and are solely responsible for diagnosis, treatment and medical advice.

If your patient is covered under a different Blue Cross and Blue Shield plan, please refer to the Medical Policies of that plan.

Populations	Interventions	Comparators	Outcomes
Individuals: • Who are being considered for initial prostate biopsy	Interventions of interest are: • Testing for genetic and protein biomarkers of prostate cancer	Comparators of interest are: • Standard clinical examination including measurement of percent free prostate-specific antigen	Relevant outcomes include: • Overall survival • Disease-specific survival • Test validity • Resource utilization • Quality of life
Individuals: • Who are being considered for repeat prostate biopsy	Interventions of interest are: • Testing for genetic and protein biomarkers of prostate cancer	Comparators of interest are: • Standard clinical examination including measurement of percent free prostate-specific antigen	Relevant outcomes include: • Overall survival • Disease-specific survival • Test validity • Resource utilization • Quality of life

DESCRIPTION

Various genetic and protein biomarkers are associated with prostate cancer. These tests have the potential to improve the accuracy of differentiating between which men should undergo prostate biopsy and which rebiopsy after a prior negative biopsy. This evidence review addresses these types of tests for cancer risk assessment.

OBJECTIVE

The objective of this evidence review is to determine whether testing for genetic and protein prostate biomarkers improves the net health outcome in men for whom an initial prostate biopsy or a repeat prostate biopsy is being considered.

BACKGROUND

Prostate Cancer

Prostate cancer is the most common cancer, and the second most common cause of cancer death in men. Prostate cancer is a complex, heterogeneous disease, ranging from microscopic tumors unlikely to be life-threatening to aggressive tumors that can metastasize, leading to morbidity or death. Early localized disease can usually be treated with surgery and radiotherapy, although active surveillance may be adopted in men whose cancer is unlikely to cause major health problems during their lifespan or for whom the treatment might be dangerous. In patients with inoperable or metastatic disease, treatment consists of hormonal therapy and possibly chemotherapy. The lifetime risk of being diagnosed with prostate cancer for men in the U.S. is approximately 16%, while the risk of dying of prostate cancer is 3%. [1,] African American men have the highest prostate cancer risk in the U.S.; the incidence of prostate cancer is about 60% higher and the mortality rate is more than 2 to 3 times greater than that of White men.[2,] Autopsy results have suggested that about 30% of men over the age of 55 and 60% of men over the age of 80 who die of other causes have incidental prostate cancer [3,], indicating that many cases of cancer are unlikely to pose a threat during a man's life expectancy.

Grading

The most widely used grading scheme for prostate cancer is the Gleason system. [4,] It is an architectural grading system ranging from 1 (well-differentiated) to 5 (undifferentiated); the score is the sum of the primary and secondary patterns. A Gleason score of 6 or less is low-grade prostate cancer that usually grows slowly; 7 is an intermediate grade; 8 to 10 is high-grade cancer that grows more quickly. A revised prostate cancer grading system has been adopted by the National Cancer Institute and the World Health Organization.[5,] A cross-walk of these grading systems is shown in Table 1.

Table 1. Prostate Cancer Grading Systems

Grade Group	Gleason Score (Primary and Secondary Pattern)	Cells
1	6 or less	Well-differentiated (low grade)
2	7 (3 + 4)	Moderately differentiated (moderate grade)
3	7 (4 + 3)	Poorly differentiated (high grade)
4	8	Undifferentiated (high grade)
5	9 to 10	Undifferentiated (high grade)

Numerous genetic alterations associated with the development or progression of prostate cancer have been described, with the potential for the use of these molecular markers to improve the selection process of men who should undergo prostate biopsy or rebiopsy after an initial negative biopsy.

REGULATORY STATUS

Clinical laboratories may develop and validate tests in-house and market them as a laboratory service; laboratory-developed tests must meet the general regulatory standards of the Clinical Laboratory Improvement Amendments (CLIA). Laboratories that offer laboratory-developed tests must be licensed under the CLIA for high-complexity testing. The following laboratories are certified under the CLIA: BioReference Laboratories and GenPath Diagnostics (subsidiaries of OPKO Health; 4Kscore®), ARUP Laboratories, Mayo Medical Laboratories, LabCorp, BioVantra, others (PCA3 assay), Clinical Research Laboratory (Prostate Core Mitomic Test™), MDx Health (SelectMDx, ConfirMDx), Innovative Diagnostics (PHI™), MiCheck® Prostate (Minomic Inc.), and ExoDx® Prostate (Exosome Diagnostics). To date, the U.S. Food and Drug Administration (FDA) has chosen not to require any regulatory review of these tests.

In February 2012, the Progensa® PCA3 Assay (Gen-Probe; now Hologic) was approved by the FDA through the premarket approval process. The Progensa PCA3 Assay has been approved by the FDA to aid in the decision for repeat biopsy in men 50 years or older who have had 1 or more negative prostate biopsies and for whom a repeat biopsy would be recommended based on the current standard of care. The Progensa PCA3 Assay should not be used for men with atypical small acinar proliferation on their most recent biopsy. FDA product code: OYM.

In June 2012, proPSA, a blood test used to calculate the Prostate Health Index (PHI ; Beckman Coulter) was approved by the FDA through the premarket approval process. The PHI test is indicated as an aid to distinguish prostate cancer from a benign prostatic condition in men ages 50 and older with prostate-specific antigen levels of 4 to 10 ng/mL and with digital rectal exam findings that are not suspicious. According to the manufacturer, the test reduces the number of prostate biopsies. FDA product code: OYA.

POLICY

A. The following genetic and protein biomarkers for the diagnosis of prostate cancer are considered **experimental / investigational**:
   1. Kallikrein markers (e.g., 4Kscore Test)
   2. Prostate Health Index (phi)
   3. HOXC6 and DLX1 testing (e.g., SelectMDx)
   4. PCA3, ERG, and SPDEF RNA expression in exosomes (e.g., ExoDx Prostate IntelliScore)
   5. Autoantibodies ARF 6, NKX3-1, 5-UTR-BMI1, CEP 164, 3-UTR-Ropporin, Desmocollin, AURKAIP-1, and CSNK2A2 (e.g., Apifiny)
   6. PCA3 testing (e.g., Progensa PCA3 Assay)
   7. TMPRSS: ERG fusion genes (e.g., MyProstate Score)
   8. Gene hypermethylation testing (e.g., ConfirmMDx)
   9. Mitochondrial DNA mutation testing (e.g., Prostate Core Mitomics Test)
   10. PanGIA Prostate
   11. MiCheck Prostate
   12. Candidate gene panels

B. Single-nucleotide variant testing for cancer risk assessment of prostate cancer is considered **experimental / investigational**.

Please refer to the member's contract benefits in effect at the time of service to determine coverage or non-coverage of these services as it applies to an individual member.

RATIONALE

This evidence review was created using a search of the PubMed database. The most recent literature update was performed through September 25 , 2025.

Evidence reviews assess whether a medical test is clinically useful. A useful test provides information to make a clinical management decision that improves the net health outcome. That is, the balance of benefits and harms is better when the test is used to manage the condition than when another test or no test is used to manage the condition.

The first step in assessing a medical test is to formulate the clinical context and purpose of the test. The test must be technically reliable, clinically valid, and clinically useful for that purpose. Evidence reviews assess the evidence on whether a test is clinically valid and clinically useful. Technical reliability is outside the scope of these reviews, and credible information on technical reliability is available from other sources.

Genetic and protein biomarker tests are best evaluated within the framework of a diagnostic or prognostic test because such frameworks provide diagnostic and prognostic information that assists in clinical management decisions. Because these tests are used as an adjunct to the usual diagnostic workup, it is important to evaluate whether the tests provide incremental information above the standard workup to determine whether the tests have utility in clinical practice.

BIOMARKER TESTING FOR SELECTION OF MEN FOR INITIAL PROSTATE BIOPSY

Clinical Context and Test Purpose

The purpose of genetic and protein biomarker testing for prostate cancer is to inform the selection of men who should undergo an initial biopsy. Conventional decision-making tools for identifying men for prostate biopsy include a digital rectal exam (DRE), serum prostate-specific antigen (PSA), and patient risk factors such as age, race, and family history of prostate cancer.

Digital rectal examination has a relatively low interrater agreement among urologists, with an estimated sensitivity, specificity, and positive predictive value (PPV) for diagnosis of prostate cancer of 59%, 94%, and 28%, respectively.[6,] Digital rectal examination might have a higher PPV in the setting of elevated PSA.[7,]

The risk of prostate cancer increases with increasing PSA levels; an estimated 15% of men with a PSA level of 4 ng/mL or less and a normal DRE, 30% to 35% of men with a PSA level between 4 ng/mL and 10 ng/mL, and more than 67% of men with a PSA level greater than 10 ng/mL will have biopsy-detectable prostate cancer.[8,9,] Use of PSA levels in screening has improved the detection of prostate cancer. The European Randomized Study of Screening for Prostate Cancer (ERSPC) trial and Göteborg Randomised Prostate Cancer Screening Trial demonstrated that biennial PSA screening reduces the risk of being diagnosed with metastatic prostate cancer.[10,11,12,13,14,] However, elevated PSA levels are not specific to prostate cancer; levels can be elevated due to infection, inflammation, trauma, or ejaculation. In addition, there are no clear cutoffs for cancer positivity with PSA. Using a common PSA level cutoff of 4.0 ng/mL, Wolf et al (2010), on behalf of the American Cancer Society, systematically reviewed the literature and calculated pooled estimates of elevated PSA sensitivity of 21% for detecting any prostate cancer and 5% for detecting high-grade cancers with an estimated specificity of 91%.[15,]

Existing screening tools have led to unnecessary prostate biopsies. More than 1 million prostate biopsies are performed annually in the U.S., with a resulting cancer diagnosis in 20% to 30% of men. About one-third of men who undergo prostate biopsy experience transient pain, fever, bleeding, and urinary difficulties. Serious biopsy risks (eg, bleeding or infection requiring hospitalization) have estimated rates ranging from less than 1% to 3%.[16,]

Given the risk, discomfort, burden of biopsy, and low diagnostic yield, there is a need for noninvasive tests that distinguish potentially aggressive tumors that should be referred for biopsy from clinically insignificant localized tumors or other prostatic conditions that do not need biopsy with the goal of avoiding low-yield biopsy.

The following PICO was used to select literature to inform this review.

Populations

The relevant population of interest are men for whom an initial prostate biopsy is being considered because of clinical symptoms (eg, difficulty with urination, elevated PSA).

The population for which these tests could be most informative is men in the indeterminate or "gray zone" range of PSA level on repeat testing with unsuspicious DRE findings. Repeat PSA testing is important because results initially reported being between 4 ng/mL and 10 ng/mL frequently revert to normal.[17,] The gray zone for PSA levels is usually between 3 or 4 ng/mL and

10 ng/mL, but PSA levels vary with age. Age-adjusted normal PSA ranges have been proposed but not standardized or validated.

Screening of men with a life expectancy of fewer than 10 years is unlikely to be useful because most prostate cancer progresses slowly. However, the age range for which screening is most useful is controversial. The ERSPC and Rotterdam trials observed benefits of screening only in men up to about 70 years old.

Interventions

For assessing future prostate cancer risk, numerous studies have demonstrated the association between many genetic and protein biomarker tests and prostate cancer. Commercially available tests for the selection of men for initial prostate biopsy include those described in Table 2.

Table 2. Commercially Available Tests to Determine Candidates for Initial Prostate Biopsy

Test	Manufacturer	Description
4Kscore	OPKO lab	Blood test that measures 4 prostate-specific kallikreins, which are combined into an algorithm to produce a risk score estimating the probability of finding high-grade prostate cancer (defined as a Gleason score ≥7) if a prostate biopsy were performed.
Prostate Health Index (phi)	Beckman Coulter	Blood assay that combines several components of PSA (total PSA, free PSA, [-2]proPSA) in an algorithm that includes patient age.
Mi-Prostate (MiPS) renamed MyProstate score 2021	University of Michigan MLabs LynxDx	Measures *TMPRSS2-ERG* gene fusion and calculates a probability score that incorporates serum PSA or the PCPT, and urine *TMPRSS2-ERG* and PCA3 scores
SelectMDx	MDxHealth	Clinical model that combines post-DRE urinary panel for *HOXC6* and *DLX1* gene expression with other risk factors
ExoDx Prostate IntelliScore (EPI)	Exosome Diagnostics	Urine panel for *PCA3, ERG*, and *SPDEF* RNA expression in exosomes
Apifiny	Armune BioScience (acquired by Exact Sciences in 2017)	Algorithm with detection of 8 autoantibodies (ARF 6, NKX3-1, 5' -UTR-BMI1, CEP 164, 3' -UTR-Ropporin, Desmocollin, AURKAIP-1, CSNK2A2) in serum
PCA3 score (eg Progensa)	• Hologic Gen-Probe • Many labs offer PCA3 tests (eg, ARUP Laboratories,	Measures *PCA3* mRNA in urine samples after prostate massage. *PCA3* mRNA may be normalized using PSA level to account for prostate cells.

Test	Manufacturer	Description
	Mayo Medical Laboratories, LabCorp)	
PanGIA Prostate	Genetics Institute of America	Analysis of a signature of small molecules, proteins, and cells with a proprietary machine learning algorithm.
MiCheck Prostate	Minomic Inc.	Algorithm based test that incorporates data from immunoassays for total prostate specific antigen (tPSA), free PSA (fPSA), and Human Epididymal Protein 4 (HE4) and the patient's age.

DRE: digital rectal exam; mRNA: messenger ribonucleic acid; PCPT: Prostate Cancer Prevention Trial; PSA: prostate-specific antigen.

Prostate-specific kallikreins (eg, 4Kscore) are a subgroup of enzymes that cleave peptide bonds in proteins. The intact PSA and human kallikrein 2 tests are immunoassays that employ distinct mouse monoclonal antibodies. The score combines the measurement of 4 prostate-specific kallikreins (total PSA, free PSA, intact PSA, human kallikrein), with an algorithm including patient age, DRE (nodules or no nodules), and a prior negative prostate biopsy. The 4K algorithm generates a risk score estimating the probability of finding high-grade prostate cancer (defined as a Gleason score ≥7) if a prostate biopsy were performed. The intended use of the test is to aid in a decision whether to proceed with a prostate biopsy. The test is not intended for patients with a previous diagnosis of prostate cancer, who have had a DRE in the previous 4 days, who have received 5α reductase inhibitor therapy in the previous 6 months, or who have undergone treatment for symptomatic benign prostatic hypertrophy in the previous 6 months.

The Prostate Health Index (phi; Beckman Coulter) is an assay that combines results of 3 blood serum immunoassays (total PSA, free PSA, [-2]proPSA [p2PSA]) numerically to produce a "PHI score." This score is calculated with the PHI algorithm using the following formula: ([-2]proPSA/free PSA) × √total PSA. The phi score is indicated for men 50 years and older with above-normal total PSA readings between 4.0 ng/mL and 10 ng/mL who have had a negative DRE in order to distinguish prostate cancer from benign prostatic conditions.

TMPRSS2 is an androgen-regulated transmembrane serine protease that is preferentially expressed in the normal prostate tissue. In prostate cancer, it may be fused to an E26 transformation-specific (ETS) family transcription factor (*ERG*, *ETV1*, *ETV4*, *ETV5*), which modulates transcription of target genes involved in cell growth, transformation, and apoptosis. The result of gene fusion with an ETS transcription gene (eg, MyProstate Score) is that the androgen-responsive promoter of *TMPRSS2* upregulates expression of the ETS gene, suggesting a mechanism for neoplastic transformation. Fusion genes may be detected in tissue, serum, or urine.

TMPRSS2-ERG gene rearrangements have been reported in 50% or more of primary prostate cancer samples.[18,] Although *ERG* appears to be the most common ETS family transcription factor involved in the development of fusion genes, not all are associated with *TMPRSS2*. About 6% of observed rearrangements are seen with *SLC45A3*, and about 5% appear to involve other types of rearrangement.[19,]

SelectMDx for prostate cancer uses a model that combines *HOXC6* and *DLX1* gene expression with traditional risk assessment models. *HOXC6* and *DLX1* mRNA is measured in post-DRE urine against kallikrein-related peptidase 3 as an internal reference.

ExoDx Prostate (IntelliScore), also called EPI, evaluates a urine-based 3-gene exosome expression assay using *PCA3* and *ERG* RNA in urine, normalized to *SPDEF*. Evidence on the association between the *PCA3* gene and prostate cancer aggressiveness is described in the next section on repeat biopsy. Measurement in exosomes, which are small double-lipid membrane vesicles that are secreted from cells, is novel. Exosomes encapsulate a portion of the parent cell cytoplasm and contain proteins and mRNA. They are shed into biofluids (eg, blood, urine). This test does not require DRE.

Apifiny uses an algorithm to score the detection of 8 autoantibodies (ARF 6, NKX3-1, 5' -UTR-BMI1, CEP 164, 3' -UTR-Ropporin, Desmocollin, AURKAIP-1, CSNK2A2) in serum. The identified biomarkers play a role in processes such as androgen response regulation and cellular structural integrity and are proteins that are thought to play a role in prostate tumorigenesis.

PanGIA Prostate is a urine test that uses a device with binding pockets for small molecules, proteins, and cells. Results are uploaded to the cloud and a machine learning algorithm compares the results with a signature from patients who have had a positive biopsy and patients who have had a negative prostate biopsy. The report includes a diagnosis with the level of confidence in the diagnosis.

MiCheck® Prostate is an algorithm that combines the testing results of three Abbott ARCHITECT™ serum immunoassays (total prostate specific antigen [tPSA], free PSA [fPSA] and Human Epididymal Protein 4 [HE4]) and one clinical factor (i.e. the patient's Age). The MiCheck Prostate algorithm combines these results to calculate a Percentage Risk Score, which provides an indication of the likelihood of the presence of clinically significant Prostate Cancer (csPCa) (Gleason score >3+4) and is called the MiCheck %Risk of csPCa.

Comparators

Standard clinical examination for determining who requires a biopsy might include DRE, review of the history of PSA levels, along with consideration of risk factors such as age, race, and family history. The ratio of free (or unbound) PSA to total PSA (percent free PSA) is lower in men who have prostate cancer than in those who do not. A percent free PSA cutoff of 25% has been shown to have a sensitivity and specificity of 95% and 20%, respectively, for men with total PSA levels between 4.0 ng/mL and 10.0 ng/mL.[20,]

The best way to combine all risk information to determine who should go to biopsy is not standardized. Risk algorithms have been developed that incorporate clinical risk factors into a risk score or probability. Two examples are the Prostate Cancer Prevention Trial (PCPT) predictive model[21,] and the Rotterdam Prostate Cancer risk calculator (also known as the ERSPC-Risk Calculator 4 [ERSPC-RC]).[22,] The American Urological Association and the Society of Abdominal Radiology (2016) recommend that high-quality prostate magnetic resonance imaging, if available, should be strongly considered in any patient with a prior negative biopsy who has persistent clinical suspicion for prostate cancer and who is under evaluation for a possible repeat biopsy.[23,]

Outcomes
The beneficial outcome of the test is to avoid a negative biopsy for prostate cancer. A harmful outcome is a failure to undergo a biopsy that would be positive for prostate cancer, especially when the disease is advanced or aggressive. Thus the relevant measures of clinical validity are the sensitivity and negative predictive value (NPV). The appropriate reference standard is a biopsy, though prostate biopsy is an imperfect diagnostic tool. Biopsies can miss cancers and repeat biopsies are sometimes needed to confirm the diagnosis. Detection rates vary by biopsy method and patient characteristics.

The timeframe of interest for calculating performance characteristics is time to biopsy results. Men who forgo biopsy based on test results could miss or delay the diagnosis of cancer. Longer follow-up would be necessary to determine the effects on overall survival (OS).

Study Selection Criteria
For the evaluation of clinical validity, studies that meet the following eligibility criteria were considered:
- Reported on the accuracy of the marketed version of the technology (including any algorithms used to calculate scores)
- Included a suitable reference standard
- Patient/sample clinical characteristics were described
- Patient/sample selection criteria were described.

Studies were excluded from the evaluation of the clinical validity of the test because they did not use the marketed version of the test, did not include information needed to calculate performance characteristics, did not use an appropriate reference standard or the reference standard was unclear, did not adequately describe the patient characteristics, or did not adequately describe patient selection criteria.

KALLIKREINS BIOMARKERS AND 4KSCORE TEST

Clinically Valid
A test must detect the presence or absence of a condition, the risk of developing a condition in the future, or treatment response (beneficial or adverse).

REVIEW OF EVIDENCE

Systematic Reviews
Russo et al (2017) performed a systematic review of studies that evaluated the diagnostic accuracy of the 4Kscore test in patients undergoing biopsy with a PSA level between 2 ng/mL and 20 ng/mL (Table 3). Results of the DRE were not described. The NPV to exclude any type of cancer ranged from 28% to 64% (Table 4). The NPV of the 4Kscore test to exclude high-grade (Gleason score ≥7) cancer ranged from 95% to 99%.

Mi et al (2021) performed a systematic review and meta-analysis of studies reporting the diagnostic accuracy of the 4Kscore test to detect high-grade prostate cancer using cutoff values of 7.5% to 10%.[24,] Pooled analyses found acceptable diagnostic accuracy (see Table 4). However, significant heterogeneity among the included studies lowered confidence in the results.

Kawada et al (2024) performed a systematic review and meta-analysis of 49 studies reporting on the diagnostic accuracy of liquid biomarkers for the detection of clinically significant prostate cancer; investigators did not explicitly define "clinically significant" prostate cancer.[25,] Ten prospective studies (n=11,586) specifically evaluated the 4Kscore. Pooled analyses found acceptable diagnostic accuracy (see Table 4). Additionally, in subgroup analyses that included 6 studies in the biopsy-naive setting (n=9283), the sensitivity, specificity, PPV, and NPV were 87% (95% CI, 81 to 91), 57% (95% CI, 50 to 63), 29% (95% CI, 19 to 41), and 96% (95% CI, 92 to 98), respectively.

Table 3. Characteristics of Systematic Reviews Assessing the Clinical Validity of the 4Kscore for Diagnosing Prostate Cancer

Study	Studies, Design	Dates	Key Inclusion Criteria	Reference Studies Included
Russo et al (2017)[26,]	10 Observational cohort	2010-2015	Blood samples were collected before biopsy; indication for biopsy was independent of 4K results	Biopsy for prostate cancer detection (overall or high grade with Gleason score ≥7)
Mi et al (2021)[24,]	Observational cohort 7 retrospective, 2 prospective	Searches through December 2019	Cohort or case-control studies of the diagnostic accuracy of the 4Kscore using biopsy as the gold standard and providing data to calculate test characteristics. Studies not using cutoff values of 7.5% to 10% were excluded.	Biopsy for detection of high-grade prostate cancer (Gleason score ≥7)
Kawada et al (2024)[25,]	49 total; 10 prospective cohorts evaluating the 4Kscore	Searches through March 2023	Studies on the diagnostic accuracy of the 4Kscore using biopsy as the gold standard and providing data to calculate test characteristics; patients in included studies were suspected of harboring prostate cancer or any reason	Systematic biopsy and image-targeted biopsy

Table 4. Results of Systematic Reviews Assessing the Clinical Validity of 4Kscore for Diagnosing Prostate Cancer

Study	Studies Included	N	Outcomes	Sens (95% CI), %	Spec (95% CI), %	PPV Range %	NPV Range %	OR (95% CI)	AUC (95% CI)
Russo et al (2017)[26,]	10	NR	Diagnostic performance for any prostate cancer	74 (73 to 76)	60 (59 to 61)	59 to 92	28 to 64	4.6 (3.5 to 6.1)	NR
Russo et al (2017)[26,](subgroup analysis)	10	NR	Diagnostic performance for high-grade prostate cancer	87 (85 to 89)	61 (60 to 62)	8 to 43	95 to 99	10.2 (8.1 to 12.8)	NR
Mi et al (2021)[24,]	7 retrospective, 2 prospective	9847	Diagnostic performance for high-grade prostate cancer	90 (86 to 92)	44 (36 to 52)	NR	NR	7 (5 to 8)	0.81 (0.77 to 0.84)
Kawada et al (2024)[25,]	10 prospective cohorts	11,586	Diagnostic performance for prostate cancer	87 (83 to 91)	58 (49 to 66)	19 to 40	92 to 98	8.84 (6.17 to 12.68)	0.83 (NR)

AUC: area under the curve; CI: confidence interval; NPV: negative predictive value; NR: not reported; OR: odds ratio; PPV: positive predictive value; Sens: sensitivity; Spec: specificity.

Prospective Studies

Additional prospective validation study of the 4Kscore test conducted in different populations has been published (Tables 5 and 6).

Bhattu et al (2021) conducted a retrospective exploratory analysis using data from the 2 previously published validation studies, to determine test performance with a cut-off of 7.5% as the indication to proceed with biopsy. [27,]

Tables 7 and 8 summarize the relevance and design and conduct limitations. A major limitation was the inclusion of patients outside the indeterminate range of PSA. Although Bhattu reported test characteristics in the subgroup of patients with PSA between 3 and 10, this study was limited by its retrospective design.

Longer-term data on the incidence of prostate cancer in men who do not have a biopsy following testing with the marketed version of 4Kscore are not available. However, a case-control study by Stattin et al (2015), which was a nested cohort study of more than 17,000 Swedish men, estimated that, for men age 60 with PSA levels of 3 or higher and a kallikrein-related peptidase 3

risk score less than 10%, the risk of metastasis at 20 years was 1.95% (95% confidence interval [CI], 0.64% to 4.66%).[28,]

Table 5. Characteristics of Clinical Validity Studies Assessing the 4Kscore Test

Study	Study Population	Design	Reference Standard	Timing of Reference and Index Tests	Blinding of Assessors	Comment
Bhattu et al (2021)[27,]	Combined analysis of patients from the above 2 studies, evaluating the test at a cut off of 7.5% as the indication to proceed with biopsy.	Retrospective exploratory analysis of data from the above 2 studies	Prostate biopsy with ≥10 cores	Blood sample taken prior to biopsy	Yes	

DRE: digital rectal exam; PSA: prostate-specific antigen.

Table 6. Results of Clinical Validity Studies Assessing the 4Kscore Test

Study	Initial N	Final N	Performance Characteristics (95% CI)			
			4Kscore	*Comparators*		
Bhattu et al (2021)[27,]			Sens (%)	Spec (%)	PPV (%)	NPV (%)
• All patients (N = 1378) • African Americans (n = 290) • non-African Americans (n = 1088) • Patients ages 45 to 75 years with PSA 3 to 10 (n = 920)	• 1378	• 1378 • 290 • 1088 • 920	• 94 • 95 • 94 • 92	• 42 • 39 • 42 • 35	• 37 • 49 • 33 • 31	• 95 • 93 • 96 • 94

AUC: area under the curve; CI: confidence interval; hK2: human kallikrein 2 (kallikreins are a subgroup of enzymes that cleave peptide bonds in proteins); NPV: negative predictive value; NR: not reported; PCPT: Prostate Cancer Prevention Trial; PPV: positive predictive value; PSA: prostate-specific antigen; Sens: sensitivity; Spec: specificity.

Table 7. Study Relevance Limitations

Study	Population[a]	Intervention[b]	Comparator[c]	Outcomes[d]	Duration of Follow-Up[e]
Bhattu et al (2021)[27,]					

The study limitations stated in this table are those notable in the current review; this is not a comprehensive gaps assessment.
PSA: prostate-specific antigen.
[a] Population key: 1. Intended use population unclear; 2. Clinical context is unclear; 3. Study population is unclear; 4. Study population not representative of intended use.
[b] Intervention key: 1. Classification thresholds not defined; 2. Version used unclear; 3. Not intervention of interest.
[c] Comparator key: 1. Classification thresholds not defined; 2. Not compared to credible reference standard; 3. Not compared to other tests in use for same purpose.
[d] Outcomes key: 1. Study does not directly assess a key health outcome; 2. Evidence chain or decision model not explicated; 3. Key clinical validity outcomes not reported (sensitivity, specificity, and predictive values); 4. Reclassification of diagnostic or risk categories not reported; 5. Adverse events of the test not described (excluding minor discomforts and inconvenience of venipuncture or noninvasive tests).
[e] Follow-Up key: 1. Follow-up duration not sufficient with respect to natural history of disease (true-positives, true-negatives, false-positives, false-negatives cannot be determined).

Table 8. Study Design and Conduct Limitations

Study	Selection[a]	Blinding[b]	Delivery of Test[c]	Selective Reporting[d]	Data Completeness[e]	Statistical[f]
Bhattu et al (2021)[27,]				Retrospective, exploratory analysis		1. Confidence intervals for test characteristics not reported.

The study limitations stated in this table are those notable in the current review; this is not a comprehensive gaps assessment.
[a] Selection key: 1. Selection not described; 2. Selection not random or consecutive (ie, convenience).
[b] Blinding key: 1. Not blinded to results of reference or other comparator tests.
[c] Test Delivery key: 1. Timing of delivery of index or reference test not described; 2. Timing of index and comparator tests not same; 3. Procedure for interpreting tests not described; 4. Expertise of evaluators not described.
[d] Selective Reporting key: 1. Not registered; 2. Evidence of selective reporting; 3. Evidence of selective publication.
[e] Data Completeness key: 1. Inadequate description of indeterminate and missing samples; 2. High number of samples excluded; 3. High loss to follow-up or missing data.
[f] Statistical key: 1. Confidence intervals and/or p values not reported; 2. Comparison with other tests not reported.

Retrospective Studies

Verbeek et al (2019) conducted a retrospective comparison of the discriminatory ability of the 4Kscore test compared to the Rotterdam Prostate Cancer Risk Calculator.[29,] The cohort included 2872 men with a PSA >3.0 from the European Randomized Study of Screening for Prostate Cancer Rotterdam. The 4K panel was measured in frozen serum samples. The areas under the curve (AUCs) were similar, with an AUC of 0.88 for the 4K score and 0.87 for the Rotterdam Prostate Cancer Risk Calculator (p=.41). Addition of the 4K score to the Rotterdam Prostate Cancer Risk Calculator had a modest, though statistically significant improvement in discriminatory ability with an AUC of 0.89. A limitation of this study is that men were included who had a PSA outside of the levels of interest, which would be between 3 and 10 ng/ml.

Clinically Useful
A test is clinically useful if the use of the results informs management decisions that improve the net health outcome of care. The net health outcome can be improved if patients receive correct therapy, more effective therapy, or avoid unnecessary therapy, or avoid unnecessary testing.

Direct Evidence
Direct evidence of clinical utility is provided by studies that have compared health outcomes for patients managed with and without the test. Because these are intervention studies, the preferred evidence would be from randomized controlled trials (RCTs).

No RCTs reporting direct evidence of utility for clinical outcomes were identified.

Chain of Evidence
Indirect evidence on clinical utility rests on clinical validity. If the evidence is insufficient to demonstrate test performance, no inferences can be made about clinical utility.

Various cutoffs for the kallikrein-related peptidase 3 probability score were used in decision-curve analyses to estimate the number of biopsies versus cancers missed. Parekh et al (2015) estimated that 307 biopsies could have been avoided and 24 cancer diagnoses would have been delayed with a 9% 4Kscore cutoff for biopsy, and 591 biopsies would have been avoided with 48 diagnoses delayed with a 15% cutoff.[30,] However, inferences on clinical utility cannot be made due to deficiencies in estimating the clinical validity that is described in the previous section.

Konety et al (2015) reported on the results of a survey of 35 U.S. urologists identified through the 4Kscore database at OPKO Lab as belonging to practices that were large users of the test.[31,] All 611 patients of participating urologists to whom men were referred for an abnormal PSA level or DRE and had a 4Kscore test were included. Urologists, who received the 4Kscore as a continuous risk percentage, were retrospectively asked about their plans for biopsy before and after receiving the test results and whether the 4Kscore test results influenced their decisions. The physicians reported that the 4Kscore results influenced decisions in 89% of men and led to a 64.6% reduction in prostate biopsies. The 4Kscore risk categories (low-risk: <7.5%, intermediate risk: 7.5% to 19.9%, high-risk: ≥20%) correlated highly (p<.001) with biopsy outcomes in 171 men with biopsy results.

Subsection Summary: Kallikreins Biomarkers and 4Kscore Test
There is uncertainty regarding clinical performance characteristics such as sensitivity, specificity, and predictive value due to the following factors: a lack of standardization of cutoffs to recommend biopsy, study populations including men with low (<4 ng/mL) and high (>10 ng/mL) baseline PSA levels, positive DRE results likely outside the intended use population, and lack of comparison with models using information from a standard clinical examination. Very few data are available on longer-term clinical outcomes of men who are not biopsied based on 4Kscore results. The evidence needed to conclude the test has clinical validity is insufficient.

Absent direct evidence of clinical utility, a chain of evidence might be constructed. The 4Kscore test is associated with a diagnosis of aggressive prostate cancer. The incremental value of the 4Kscore concerning clinical examination and risk calculators in the intended use population is unknown due to deficiencies in estimating clinical validity. There is no prospective evidence that the use of 4Kscore changes management decisions. Given that the test manufacturer's website

states the test is for men with inconclusive results, the inclusion of men with PSA levels greater than 10 ng/mL and a positive DRE in the validation studies are likely not reflective of the intended use population. The chain of evidence is incomplete.

PROPSA AND PROSTATE HEALTH INDEX

Clinically Valid

A test must detect the presence or absence of a condition, the risk of developing a condition in the future, or treatment response (beneficial or adverse).

REVIEW OF EVIDENCE

Systematic Reviews

Several systematic reviews and meta-analyses have evaluated the clinical validity of p2PSA (proPSA) and PHI tests. The characteristics of the most relevant and comprehensive reviews are shown in Table 9. All primary studies were observational and most were retrospective. Reviews included studies of men with a positive, negative, or inconclusive DRE; Pecoraro et al (2016)[32,] restricted eligibility to studies including PSA levels between 2 ng/mL and 10 ng/mL, while Russo et al (2017)[26,] restricted eligibility to studies including PSA levels between 2 ng/mL and 20 ng/mL. Anyango and Kawada (previously introduced) included studies in men of any age with any range of PSA levels and Anyango reported results according to different cutoffs.[33,25,]

Pecoraro et al (2016) rated most of the 17 primary studies as low quality due to the design (most were retrospective), lack of blinding of outcome assessors to reference standard results, lack of clear cutoffs for diagnosis, and lack of explicit diagnostic question.[32,] Russo et al (2017) included 23 studies that were mostly prospective and rated as moderate quality.[26,]Kawada et al (2024) included 22 studies that were mostly prospective cohorts; 14 studies included patients in the initial biopsy setting and 8 included patients in both initial and repeat biopsy settings.[25,] Overall, there was high heterogeneity across studies but pooled estimates showed generally low NPV (5% to 91%) and low specificity (25% to 52%) when sensitivity was 85% to 93% (Table 10). In a subgroup analysis, Kawada et al (2024) pooled data from 6 studies comprising patients in the biopsy-naïve setting; the pooled sensitivity, specificity, PPV, and NPV, were 87% (95% CI, 81 to 91), 57% (95% CI, 50 to 63), 36% (95% CI, 27 to 46), and 92% (95% CI, 87 to 95), respectively.[25,]

Table 9. Characteristics of Systematic Reviews Assessing the Clinical Validity of the PHI Test for Diagnosing Prostate Cancer

Study	Studies Included	Dates	Key Inclusion Criteria[a]	Design	Reference Studies Included
Pecoraro et al (2016)[32,]	17	2003 to 2014	PSA level 2 to 10 ng/mL	Prospective, retrospective, and mixed prospective/ retrospective, observational	
Russo et al (2017)[26,]	23	2010 to 2015	Blood samples were collected before biopsy; PSA level 2 to 20 ng/mL; indication for biopsy was independent of PHI results	Mostly retrospective , observational	Biopsy for prostate cancer detection (overall or high grade with Gleason score ≥7)
Anyango et al (2021)[33,]	12	2015 to 2018	Studies that enrolled men of any age who had a diagnosis of aggressive PCa as determined from biopsy specimens, and with any range of PSA levels	Observational cross-sectional, cohort, or case-control designs in which the index and reference tests were interpreted in the same group of participants.	Biopsy Gleason score
Kawada et al (2024)[25,]	49 total; 22 evaluating the PHI test	Searches through March 2023	Studies on the diagnostic accuracy of PHI using biopsy as the gold standard and providing data to calculate test characteristics; patients in included studies were suspected of harboring prostate cancer or any reason	Prospective, retrospective	Systematic biopsy and image-targeted biopsy

PCa: prostate cancer; PHI: Prostate Health Index; PSA: prostate-specific antigen.
[a] Results from all studies were with or without digital rectal exam.

Table 10. Results of Systematic Reviews on the Clinical Validity of the PHI Test for Diagnosing Prostate Cancer

Study	Studies/N (Range)	Outcomes	Sens (95% CI), %	Spec (95% CI), %	PPV Range, %	NPV Range, %	OR (95% CI), %
Pecoraro et al (2016)[32,]	17/6912 (63 to 1091)	Diagnostic performance for any prostate cancer	Set at 90	Phi: 31 (29 to 33) Total PSA: 25 (23 to 27)			
Russo et al (2017)[26,]	23	Diagnostic performance for any prostate cancer	89 (88 to 90)	34 (32 to 35)	76 to 98	15 to 63	4.4 (3.3 to 5.8)
Russo et al (2017)[26,26,](subset)	7	Diagnostic performance for high-grade prostate cancer	93 (90 to 95)	26 (25 to 28)	88 to 99	5 to 31	3.5 (2.5 to 5.0)
Anyango et al (2021)[33,]	Total 12/8462 PHI <25: 3/3222 PHI 26 to 35: 6/6030 PHI >36: 5/1476	Diagnostic accuracy in determining the aggressiveness of prostate cancer	PHI <25: 97 (95 to 98) PHI 26 to 35: 87 (8 to 91) PHI >36: 72 (64 to 79)	PHI <25: 10 (6 to 16) PHI 26 to 35: 45 (39 to 50) PHI >36: 74 (68 to 80)			
Kawada et al (2024)[25,]	22/8652 (77 to 1538)	Diagnostic performance for clinically significant prostate cancer	85 (80 to 89)	52 (43 to 60)	38 (31 to 45)	91 (87 to 93)	6.28 (4.79 to 8.24)

CI: confidence interval; NPV: negative predictive value: OR: odds ratio; PHI: Prostate Health Index; PPV: positive predictive value; PSA: prostate-specific antigen; Sens: sensitivity; Spec: specificity.

Retrospective Studies

Loeb et al (2017) conducted a modeling study to compare established risk calculators with and without the PHI test.[29,] The population for this retrospective analysis included 728 men from the prospective multicenter clinical trial of PHI (Catalona et al, 2011).[34,] The probability of aggressive prostate cancer was evaluated at each value of PHI from 1 to 100. The addition of PHI to the PCPT 2.0 risk calculator increased the AUC for the discrimination of aggressive prostate cancer from 0.575 to 0.696 (p<.001), while the addition of phi to the ERSPC 4 plus DRE risk calculator increased the AUC from 0.650 to 0.711 (p=.014).

Clinically Useful
A test is clinically useful if the use of the results informs management decisions that improve the net health outcome of care. The net health outcome can be improved if patients receive correct therapy, more effective therapy, or avoid unnecessary therapy, or avoid unnecessary testing.

Direct Evidence
Direct evidence of clinical utility is provided by studies that have compared health outcomes for patients managed with and without the test. Because these are intervention studies, the preferred evidence would be from RCTs.

No RCTs directly measuring the effect of the PHI test on clinical outcomes were found.

Chain of Evidence
Indirect evidence on clinical utility rests on clinical validity. If the evidence is insufficient to demonstrate test performance, no inferences can be made about clinical utility.

A chain of evidence might be used to demonstrate clinical utility if each link in the chain is intact. Two observational studies have shown a reduction or delay in biopsy procedures for men with PSA levels in the 4 to 10 ng/mL range, nonsuspicious DRE findings, and a low PHI score. Tosoian et al (2017) found a 9% reduction in the rate of biopsy among 345 men who underwent PHI testing compared with 1318 men who did not.[35,] There was an associated 8% reduction in the incidence of negative biopsies in men who had PHI testing, but the interpretation of results is limited because the use of the PHI test was based solely on provider discretion. A prospective multicenter study by White et al (2018) evaluated physician recommendations for biopsy before and after receiving the PHI test result.[36,] The PHI score affected the physician's management plan in 73% of cases, with biopsy deferrals when the PHI score was low and the decision to perform biopsies when the PHI score was 36 or more. A chain of evidence requires evidence that the test could be used to affect health outcomes, and that the test is clinically valid. Due to questions about the clinical validity of the test, a chain of evidence cannot be constructed.

Subsection Summary: proPSA and Prostate Health Index
Many studies and systematic reviews of these studies have reported on the clinical validity of PHI. Primary studies included men with positive, negative, and inconclusive DRE and men with PSA levels outside of the 4- to 10-ng/mL range. There is no standardization of cutoffs used in a clinical setting for diagnosis. With sensitivity around 90% for the detection of any prostate cancer, specificity ranged from 25% to 52% and NPV, which would indicate an absence of disease and allow patients to forego biopsy, ranged from 5% to 91%. For high-grade disease, the sensitivity of the PHI test was 93%, with a NPV ranging from 5% to 31%.

The PHI test is associated with a diagnosis of prostate cancer. Although observational studies have shown a reduction or delay in a biopsy with PHI testing, a chain of evidence cannot be constructed about an improvement in health outcomes due to limitations in clinical validity. The chain of evidence is incomplete.

TMPRSS FUSION GENES AND MYPROSTATE SCORE

Clinically Valid
A test must detect the presence or absence of a condition, the risk of developing a condition in the future, or treatment response (beneficial or adverse).

Review of Evidence
Validation studies on the combined 2-gene test (*TMPRSS2-ERG* and *PCA3*) are shown in Table 11. Sanda et al (2017), from the National Cancer Institute Early Detection Research Network, reported separate developmental and validation cohorts for high-grade prostate cancer in men undergoing initial prostate biopsy.[37,] For the validation cohort, any of the following was considered a positive result: PSA level greater than 10 ng/mL, urine *TMPRSS2-ERG* score greater than 8, or urine *PCA3* score greater than 20. Performance characteristics of this algorithm, compared with the individual markers, are shown in Table 12. Analysis showed that specificity could be increased from 17% to 33% compared with PSA alone, without loss of sensitivity. The difference in specificity was statistically significant, with a prespecified 1-sided p-value of.04 (lower bound of 1-sided 95% CI, 0.73%).

In the study by Tomlins et al (2016), 80% of the 1244 patients were undergoing initial biopsy due to elevated PSA levels (Table 11).[38,] Thresholds were not defined and the AUCs for predicting any cancer using PSA alone, PCPT risk calculator alone, or the Mi-Prostate Score (MiPS) alone are shown in Table 12. The AUC for MiPS was significantly improved compared with the PCPT risk calculator ($p<.001$). However, a study by Ankerst et al (2019) found that adding *TMPRSS2-ERG* to a PCPT risk calculator plus *PCA3* did not improve the AUC.[39,] The online PCPT risk calculator now includes both the *PCA3* and *TMPRSS2-ERG* scores, which will be used for further validation.

Tosoian et al (2021) reported on a study to establish and validate a threshold for the MyProstateScore test (previously named MiPS) to rule out Gleason Group > 2 prostate cancer.[40,] A threshold of <10 was identified in a training cohort and validated using a combined dataset that included 977 biopsy naive men from the validation study previously reported in Tomlins et al (2016) and 548 biopsy naive men prospectively enrolled as part of an Early Detection Research Network study that did not evaluate the MyProstateScore. In the overall cohort, sensitivity was 97.0%, specificity was 32.6%, NPV was 97.5%, and PPV was 29.1%. Results were similar in the subgroup of men with PSA between 3 and 10 or with PSA <3 with suspicious DRE. The study authors are co-founders and have equity in LynDx, which has licensed the urine biomarkers evaluated in the study.

The multiinstitutional Canary Prostate Surveillance Study (PASS) was reported by Newcomb et al (2019).[41,] The study included 782 men under active surveillance (2,069 urine samples) to examine the association of urinary PCA3 and TMPRSS2:ERG with biopsy-based reclassification. TMPRSS2:ERG was not associated with short-term reclassification at the first surveillance biopsy.

Table 11. Characteristics of Studies Assessing the Clinical Validity of the Combined *TMPRSS2-ERG* and *PCA3* Score

Study; Trial	Study Population	Design	Reference Standard	Threshold for Positive Index Test	Timing of Reference and Index Tests	Blinding of Assessors	Comment
Sanda et al (2017)[37,]	561 men who had initial prostate biopsy	4-center PRoBE criteria	HG (Gleason score ≥7) prostate cancer on biopsy	Algorithm with PSA level >10 ng/mL; *T2:ERG* score >8; or *PCA3* score >20	Samples collected after DRE and prior to biopsy	Yes	A separate developmental cohort of 516 men is reported
Tomlins et al (2016)[38,]	1244 men who had initial (80%) or repeat biopsy due to elevated PSA	7-center prospective	Any cancer or HG cancer (Gleason score ≥7)		Samples collected after DRE and prior to biopsy	Yes	A MiPS score threshold was not provided, so sensitivity and NPV were not calculated

DRE: digital rectal exam; HG; high-grade; MiPS: Mi-Prostate Score; NPV: negative predictive value; PRoBE: prospective-specimen-collection, retrospective-blinded-evaluation; PSA: prostate-specific antigen; *T2:ERG: TMPRSS2-ERG*.

Table 12. Results of Studies Assessing the Clinical Validity of the Combined *TMPRSS2-ERG* and *PCA3* Score

Study	Initial N	Final N	Threshold	Sens (95% CI)	Spec (95% CI)	PPV (95% CI)	NPV (95% CI)	p[a]
Sanda et al (2017)[37,]	561	561						
PSA level, ng/mL			3	91.2 (86.6 to 95.8)	16.7 (13.1 to 20.3)	28.2 (28.9 to 29.5)	84.1 (75.1 to 90.3)	
PCA3			7	96.6 (93.7 to 99.5)	18.4 (14.7 to 22.1)	29.8 (28.6 to 30.9)	93.8 (86.2 to 97.3)	
PCA3, T2:ERG			20, 8	90.5 (85.8 to 95.2)	35.4 (30.8 to 40.0)	33.4 (31.5 to 35.4)	91.2 (86.1 to 94.6)	
PSA level >10 ng/mL; *T2:ERG* score >8; or *PCA3* score >20				92.6 (88.4 to 96.8)	33.4 (28.8 to 37.9)	33.2 (31.4 to 35.1)	92.6 (87.5 to 95.8)	
				AUC (95% CI not reported)				

Study	Initial N	Final N	Threshold	Sens (95% CI)	Spec (95% CI)	PPV (95% CI)	NPV (95% CI)	p[a]
			Excluded Samples	PSA Alone	PCPT Risk Calculator	PSA Plus *PCA3*	MiPS	
Tomlins et al (2016)[38,]	1244	1225	19 with insufficient samples for analysis					
Any cancer				0.59	0.64	0.73	0.75	<.001
High-grade cancer				0.65	0.71	0.75	0.77	<.001

AUC: area under the curve; CI: confidence interval; MiPS: Mi-Prostate Score; NPV: negative predictive value; PCPT: Prostate Cancer Prevention Trial; PPV: positive predictive value;PSA: prostate-specific antigen; Sens: sensitivity; Spec: specificity; *T2:ERG*: *TMPRSS2-ERG*.
[a] P-value for MiPS vs PCPT risk calculator.

Tables 13 and 14 summarize relevance and design and conduct limitations for each study.

Table 13. Study Relevance Limitations

Study	Population[a]	Intervention[b]	Comparator[c]	Outcomes[d]	Duration of Follow-Up[e]
Sanda et al (2017)[37,]	4. Some patients were 70 y, 16% had an abnormal DRE; median PSA level was 4.8 ng/mL				
Tomlins et al (2016)[38,]	4. 25% were >70 y, 23% had an abnormal DRE; median PSA level was 4.7 ng/mL		3. Not compared with most current (v2) PCPT risk calculator		

DRE: digital rectal exam; PCPT: Prostate Cancer Prevention Trial; PSA: prostate-specific antigen.
The study limitations stated in this table are those notable in the current review; this is not a comprehensive gaps assessment.
[a] Population key: 1. Intended use population unclear; 2. Clinical context is unclear; 3. Study population is unclear; 4. Study population not representative of intended use.
[b] Intervention key: 1. Classification thresholds not defined; 2. Version used unclear; 3. Not intervention of interest.
[c] Comparator key: 1. Classification thresholds not defined; 2. Not compared to credible reference standard; 3. Not compared to other tests in use for same purpose.
[d] Outcomes key: 1. Study does not directly assess a key health outcome; 2. Evidence chain or decision model not explicated; 3. Key clinical validity outcomes not reported (sensitivity, specificity, and predictive values); 4. Reclassification of diagnostic or risk categories not reported; 5. Adverse events of the test not described (excluding minor discomforts and inconvenience of venipuncture or noninvasive tests).
[e] Follow-Up key: 1. Follow-up duration not sufficient with respect to natural history of disease (true-positives, true-negatives, false-positives, false-negatives cannot be determined).

Table 14. Study Design and Conduct Limitations

Study	Selection[a]	Blinding[b]	Delivery of Test[c]	Selective Reporting[d]	Data Completeness[e]	Statistical[f]
Sanda et al (2017)[37,]						
Tomlins et al (2016)[38,]						1. Confidence intervals not reported

The study limitations stated in this table are those notable in the current review; this is not a comprehensive gaps assessment.
[a] Selection key: 1. Selection not described; 2. Selection not random or consecutive (ie, convenience).
[b] Blinding key: 1. Not blinded to results of reference or other comparator tests.
[c] Test Delivery key: 1. Timing of delivery of index or reference test not described; 2. Timing of index and comparator tests not same; 3. Procedure for interpreting tests not described; 4. Expertise of evaluators not described.
[d] Selective Reporting key: 1. Not registered; 2. Evidence of selective reporting; 3. Evidence of selective publication.
[e] Data Completeness key: 1. Inadequate description of indeterminate and missing samples; 2. High number of samples excluded; 3. High loss to follow-up or missing data.
[f] Statistical key: 1. Confidence intervals and/or p values not reported; 2. Comparison with other tests not reported.

Clinically Useful

A test is clinically useful if the use of the results informs management decisions that improve the net health outcome of care. The net health outcome can be improved if patients receive correct therapy, more effective therapy, or avoid unnecessary therapy or testing.

Direct Evidence

Direct evidence of clinical utility is provided by studies that have compared health outcomes for patients managed with and without the test. Because these are intervention studies, the preferred evidence would be from RCTs.

Sanda et al (2017) calculated that restricting biopsy to participants with positive findings on *TMPRSS2-ERG* score*, PCA3* score, or PSA level at thresholds of 8, 20, and 10, respectively, would have avoided 42% of unnecessary biopsies (true negative) and 12% of low-grade cancers.[37,] It was estimated that 7% of cancers would be missed using the combined threshold, compared with 21% using a *PCA3* threshold of 7.

Tomlins et al (2016) also used decision-curve analysis to estimate the number of biopsies that would have been performed and cancers that would have been missed using a MiPS risk cutoff for biopsy in their cohort.[38,] Compared with a biopsy-all strategy, using a MiPS cutoff for aggressive cancer of 15% would have avoided 36% of biopsies while missing 7 % of any prostate cancer and 1.6% of high-grade prostate cancer diagnoses. Using the PCPT risk calculator cutoff of 15% for aggressive cancer would have avoided 68% of biopsies while missing 25% of any cancer and 8% of high-grade cancer.

Chain of Evidence

Indirect evidence on clinical utility rests on clinical validity. If the evidence is insufficient to demonstrate test performance, no inferences can be made about clinical utility.

No studies were found that directly show the effects of using MiPS results on clinical outcomes. Given the lack of direct evidence of utility, a chain of evidence would be needed to demonstrate clinical utility. The MiPS test is associated with a diagnosis of prostate cancer and aggressive prostate cancer. The clinical validity study of the MiPS test included men with relevant PSA levels but also included men with a positive DRE who would not likely forego biopsy.

Subsection Summary: *TMPRSS* Fusion Genes and MyProstate Score
Concomitant detection of *TMPRSS2-ERG* and *PCA3* in addition to the multivariate PCPT risk calculator may more accurately identify men with prostate cancer than with PSA level alone or the PCPT risk calculator alone. However, adding *TMPRSS2-ERG* score to PSA level plus *PCA3* score only resulted in a 0.02 difference in the AUC compared with the combination of PSA plus *PCA3*, with a maximum AUC of 0.77 for the detection of high-grade cancer. In a study from the National Cancer Institute Early Detection Research Network, using either/or thresholds of *TMPRSS2-ERG* plus *PCA3* score or PSA level improved specificity compared with PSA alone, without a loss in sensitivity. It does not appear from this study that an algorithm that combines *TMPRSS2-ERG,PCA3,* or PSA level has any incremental improvement in NPV of 92.6% (95% CI, 87.5% to 95.8%) over *PCA3* score alone 93.8% (95% CI, 86.2% to 97.3%).

Current evidence on the *TMPRSS2-ERG* and *PCA3* scores is insufficient to support its use. The MiPS test has data suggesting an improved AUC compared with the PCPT risk calculator in a validation study, and improved specificity compared with PSA level in another study, but improvement in diagnostic accuracy compared to individual components of the algorithm at similar thresholds has not been reported. Data on clinical utility are lacking. No prospective data are available on using the MiPS score for decision making. The chain of evidence is incomplete.

SELECTMDX FOR PROSTATE CANCER

Clinically Valid
A test must detect the presence or absence of a condition, the risk of developing a condition in the future, or treatment response (beneficial or adverse).

REVIEW OF EVIDENCE

Systematic Reviews
The previously introduced systematic review by Kawada et al (2024) included 9 studies evaluating the clinical validity of SelectMDX in patients with clinically significant prostate cancer.[25,] The characteristics of the review and results are provided in Tables 15 and 16, respectively. In subgroup analyses that included 7 studies in the biopsy-naïve setting, the pooled sensitivity, specificity, PPV, and NPV were 85% (95% CI, 72 to 92), 54% (95% CI, 38 to 69), 39% (95% CI, 29 to 50), and 90% (95% CI, 82 to 95), respectively.

Table 15. Characteristics of Systematic Reviews Assessing the Clinical Validity of SelectMDX for Diagnosing Prostate Cancer

Study	Studies Included	Dates	Key Inclusion Criteria[a]	Design	Reference Studies Included
Kawada et al (2024)[25,]	49 total; 9 evaluating SelectMDX	Searches through March 2023	Studies on the diagnostic accuracy of SelectMDX using biopsy as the gold standard and providing data to calculate test characteristics; patients in included studies were suspected of harboring prostate cancer or any reason	Prospective, retrospective	Systematic biopsy and image-targeted biopsy

Table 16. Results of Systematic Reviews on the Clinical Validity of SelectMDX for Diagnosing Prostate Cancer

Study	Studies/N (Range)	Outcomes	Sens (95% CI), %	Spec (95% CI), %	PPV Range, %	NPV Range, %	OR (95% CI), %
Kawada et al (2024)[25,]	9/2609	Diagnostic performance for prostate cancer	82% (69 to 91)	56% (41 to 70)	39% (30% to 49%)	90% (82% to 95%)	6.16 (2.62 to 14.49)

CI: confidence interval; NPV: negative predictive value: OR: odds ratio; PPV: positive predictive value; PSA: prostate-specific antigen; Sens: sensitivity; Spec: specificity.

Prospective Studies

Van Neste et al (2016) evaluated a risk calculator that added *HOXC6* and *DLX1* expression to a clinical risk model that included DRE, PSA density, and previous cancer negative biopsies (Table 17).[42,] A training set in 519 men and an independent validation set in 386 men were assessed. When evaluating the risk model in men who were in the "gray zone" of PSA level between 3 ng/mL and 10 ng/mL, the AUC was significantly higher than a clinical risk model alone, Prostate Cancer Prevention Trial Risk Calculator (PCPTRC) for detection of any cancer or for detection of high-grade cancer (Table 18). Limitations of this study is the inclusion of men with an abnormal DRE (Tables 19 and 20), which was the strongest predictor of prostate cancer in the training set (odds ratio [OR]=5.53; 95% CI, 2.89 to 10.56) and inclusion of men who were scheduled for either initial or repeat biopsy. The OR for *HOXC6* and *DLX1* expression in this model was 1.68 (95% CI, 1.38 to 2.05; p<.003).

Table 17. Characteristics of Clinical Validity Studies Assessing SelectMDx for Prostate Cancer

Study	Study Population	Design	Reference Standard	Threshold for Positive Index Test	Timing of Reference and Index Tests	Blinding of Assessors
Van Neste et al (2016)[42,]	386 men with PSA level >3 ng/mL scheduled for initial (89%) or repeat biopsy	Prospective	Prostate cancer on biopsy	NR	Urine sample taken after DRE and prior to biopsy	NR

DRE: digital rectal exam; NR: not reported; PSA: prostate-specific antigen.

Table 18. Results of Clinical Validity Studies Assessing SelectMDx for Prostate Cancer

Study	Total N	N With PSA Level <10 ng/mL	N with PSA Level <10 ng/mL and Normal DRE Results	AUC for the Risk Score in Patients With PSA Level <10 ng/mL (95% CI)				
				Any Cancer	HG Cancer	PCPTRC	p	
Van Neste et al (2016)[42,]	386	264		0.90 (0.85 to 0.96)	0.78 (0.68 to 0.88)	0.66 (0.57 to 0.75)	.001	

AUC: area under the curve; CI: confidence interval; DRE: digital rectal exam; HG; high-grade; IQR: interquartile range; MRI: magnetic resonance imaging; NPV: negative predictive value; NR: not reported; PCPTRC: Prostate Cancer Prevention Trial Risk Calculator; PPV: positive predictive value; PSA: prostate-specific antigen; Sens: sensitivity; Spec: specificity.

Table 19. Study Relevance Limitations

Study	Population[a]	Intervention[b]	Comparator[c]	Outcomes[d]	Duration of Follow-Up[e]
Van Neste et al (2016)[42,]	4. 31% of men had abnormal DRE and men were undergoing either initial or repeat biopsy. The study was conducted in Europe and not representative of the U.S. population	3. The clinical risk model was changed for the Haese et al (2019) publication			

DRE: digital rectal exam; PCPTR: Prostate Cancer Prevention Trial Risk; PSA: prostate specific antigen; %fPSA: percent free PSA.
The study limitations stated in this table are those notable in the current review; this is not a comprehensive gaps assessment.
[a] Population key: 1. Intended use population unclear; 2. Clinical context is unclear; 3. Study population is unclear; 4. Study population not representative of intended use.
[b] Intervention key: 1. Classification thresholds not defined; 2. Version used unclear; 3. Not intervention of interest.
[c] Comparator key: 1. Classification thresholds not defined; 2. Not compared to credible reference standard; 3. Not compared to other tests in use for same purpose.
[d] Outcomes key: 1. Study does not directly assess a key health outcome; 2. Evidence chain or decision model not explicated; 3. Key clinical validity outcomes not reported (sensitivity, specificity, and predictive values); 4. Reclassification of diagnostic or risk categories not reported; 5. Adverse events of the test not described (excluding minor discomforts and inconvenience of venipuncture or noninvasive tests).
[e] Follow-Up key: 1. Follow-up duration not sufficient with respect to natural history of disease (true-positives, true-negatives, false-positives, false-negatives cannot be determined).

Table 20. Study Design and Conduct Limitations

Study	Selection[a]	Blinding[b]	Delivery of Test[c]	Selective Reporting[d]	Data Completeness[e]	Statistical[f]
Van Neste et al (2016)[42,]		1. Blinding not reported			1. Inadequate description of indeterminate samples	

The study limitations stated in this table are those notable in the current review; this is not a comprehensive gaps assessment.
[a] Selection key: 1. Selection not described; 2. Selection not random or consecutive (ie, convenience).
[b] Blinding key: 1. Not blinded to results of reference or other comparator tests.
[c] Test Delivery key: 1. Timing of delivery of index or reference test not described; 2. Timing of index and comparator tests not same; 3. Procedure for interpreting tests not described; 4. Expertise of evaluators not described.
[d] Selective Reporting key: 1. Not registered; 2. Evidence of selective reporting; 3. Evidence of selective publication.
[e] Data Completeness key: 1. Inadequate description of indeterminate and missing samples; 2. High number of samples excluded; 3. High loss to follow-up or missing data.
[f] Statistical key: 1. Confidence intervals and/or p values not reported; 2. Comparison with other tests not reported.

Clinically Useful

A test is clinically useful if the use of the results informs management decisions that improve the net health outcome of care. The net health outcome can be improved if patients receive correct therapy, more effective therapy, or avoid unnecessary therapy, or avoid unnecessary testing.

Direct Evidence

Direct evidence of clinical utility is provided by studies that have compared health outcomes for patients managed with and without the test. Because these are intervention studies, the preferred evidence would be from RCTs.

No trials were identified that compared health outcomes for patients managed with and without the test.

Van Neste et al (2016) estimated that when using a cutoff of 98% NPV for high-grade (Gleason ≥7) prostate cancer, there would be a total reduction in biopsies by 42% and a decrease in unnecessary biopsies by 53%.[42,]

Chain of Evidence
Indirect evidence on clinical utility rests on clinical validity. If the evidence is insufficient to demonstrate test performance, no inferences can be made about clinical utility. Current evidence on clinical validity is insufficient.

Because the clinical validity of SelectMDx for Prostate Cancer has not been established, a chain of evidence supporting the clinical utility of this test cannot be constructed.

Subsection Summary: SelectMDx for Prostate Cancer
A systematic review evaluating SelectMDX amongst other liquid biomarkers in patients suspected of harboring prostate cancer or any reason reported a sensitivity and specificity of 82% and 56%, respectively. One validation from 2019 reported that a risk model that added an expression of *HOX6* and *DLX1* to a newly revised clinical risk model (patient age, DRE, and PSA density) increased the AUC for the detection of high-grade cancer. However, men who are in the "gray zone" who have a PSA level between 3 ng/mL and 10 ng/mL and normal DRE are the patients who would most likely be considered for this test. Comparison with the PCPTR was not reported for this population of interest, limiting the interpretation of this study. It is also not known whether SelectMDx would provide additional specificity when compared to percent free PSA (%fPSA). An additional limitation is that the study was conducted in a European population, which is primarily Caucasian and would not be representative of the U.S. population. A more recent study from 2021 found that use of the SelectMDx test in biopsy-naive men resulted in a 38% reduction of biopsy procedures, a 35% reduction of overdetection of low-grade prostate cancer and could save 38% of MRIs, at the cost of missing 10% of high-grade prostate cancers compared to biopsy for all patients. However, the use of MRI alone in all patients to select for prostate biopsy had the highest net benefit as a prebiopsy stratification tool.

No trials identified have compared health outcomes for patients managed with and without the SelectMDx for Prostate Cancer. A chain of evidence depends on clinical validity. Current evidence on adding *HOXC6* and *DLX1* expression to a clinical risk model is insufficient to support its use. Data on SelectMDx have suggested an improved AUC (0.78) compared with the PCPTRC (0.66) in 1 validation study that included men with PSA levels in the indeterminate range. Sensitivity and specificity rates have not been reported. No prospective data are available on using SelectMDx for decision making. Present studies on clinical validity are insufficient to establish a chain of evidence. The chain of evidence is incomplete.

EXODX PROSTATE (INTELLISCORE)

Clinically Valid
A test must detect the presence or absence of a condition, the risk of developing a condition in the future, or treatment response (beneficial or adverse).

Review of Evidence
McKiernan et al (2016) conducted a multicenter validation study of urine exosome *PCA3*, *ERG*, and *SPDEF* RNA expression to predict high-grade (Gleason score ≥7) prostate cancer (Table 21).[43,] The threshold for a positive test was derived from a training set separate from the validation set. The assay improved on the standard of care alone, with an AUC of 0.73 compared with 0.63 for the standard of care (p<.001) and 0.62 for the PCPTRC (Table 22). Diagnostic performance is shown in Table 22, with a sensitivity of 97% and NPV of 96%.

Table 21. Characteristics of Clinical Validity Studies Assessing ExoDx Prostate (IntelliScore)

Study	Study Population	Design	Reference Standard	Threshold for Positive Index Test	Timing of Reference and Index Tests	Blinding of Assessors
McKiernan et al (2016)[43,]	1064 men ≥50 y with PSA level 2 to 10 ng/mL and scheduled for initial biopsy	Multicenter prospective	Gleason score ≥7 prostate cancer on biopsy	15.6 derived from a separate training set	Urine collection prior to biopsy	Yes

PSA: prostate-specific antigen.

Table 22. Results of Clinical Validity Studies Assessing ExoDx Prostate (IntelliScore)

Study	Initial N	Final N	Excluded Samples	Area Under the Curve (95% CI)			
				ExoDx + SOC	SOC Alone	PCPTRC	p
McKiernan et al (2016)[43,]	1064	519 in intended use population	Technical reasons or failure to meet study criteria	0.73 (0.68 to 0.77)	0.63 (0.58 to 0.68)	0.62 (0.57 to 0.67)	<.001
				Diagnostic Performance (95% CI), %			
				Sensitivity	Specificity	PPV	NPV
				97.44 (93.93 to 100)	27.68 (21.09 to 34.28)	37.25 (30.62 to 43.89)	96.08 (90.75 to 100)

CI: confidence interval; NPV: negative predictive value; PCPTRC: Prostate Cancer Prevention Trial Risk Calculator; PPV: positive predictive value; PSA: prostate-specific antigen; SOC: standard of care.

Tables 23 and 24 summarize relevance and design and conduct limitations in each study.

Table 23. Study Relevance Limitations

Study	Population[a]	Intervention[b]	Comparator[c]	Outcomes[d]	Duration of Follow-Up[e]
McKiernan et al (2016)[43,]	4. Study population included patients with suspicious DRE		3. Standard of care did not include DRE or free PSA results		

DRE: digital rectal exam; PSA: prostate-specific antigen.
The study limitations stated in this table are those notable in the current review; this is not a comprehensive gaps assessment.
[a] Population key: 1. Intended use population unclear; 2. Clinical context is unclear; 3. Study population is unclear; 4. Study population not representative of intended use.
[b] Intervention key: 1. Classification thresholds not defined; 2. Version used unclear; 3. Not intervention of interest.

[c] Comparator key: 1. Classification thresholds not defined; 2. Not compared to credible reference standard; 3. Not compared to other tests in use for same purpose.
[d] Outcomes key: 1. Study does not directly assess a key health outcome; 2. Evidence chain or decision model not explicated; 3. Key clinical validity outcomes not reported (sensitivity, specificity, and predictive values); 4. Reclassification of diagnostic or risk categories not reported; 5. Adverse events of the test not described (excluding minor discomforts and inconvenience of venipuncture or noninvasive tests).
[e] Follow-Up key: 1. Follow-up duration not sufficient with respect to natural history of disease (true-positives, true-negatives, false-positives, false-negatives cannot be determined).

Table 24. Study Design and Conduct Limitations

Study	Selection[a]	Blinding[b]	Delivery of Test[c]	Selective Reporting[d]	Data Completeness[e]	Statistical[f]
McKiernan et al (2016)[43,]			1. The timing of urine sampling was not described			

The study limitations stated in this table are those notable in the current review; this is not a comprehensive gaps assessment.
[a] Selection key: 1. Selection not described; 2. Selection not random or consecutive (ie, convenience).
[b] Blinding key: 1. Not blinded to results of reference or other comparator tests.
[c] Test Delivery key: 1. Timing of delivery of index or reference test not described; 2. Timing of index and comparator tests not same; 3. Procedure for interpreting tests not described; 4. Expertise of evaluators not described.
[d] Selective Reporting key: 1. Not registered; 2. Evidence of selective reporting; 3. Evidence of selective publication.
[e] Data Completeness key: 1. Inadequate description of indeterminate and missing samples; 2. High number of samples excluded; 3. High loss to follow-up or missing data.
[f] Statistical key: 1. Confidence intervals and/or p values not reported; 2. Comparison with other tests not reported.

Clinically Useful

A test is clinically useful if the use of the results informs management decisions that improve the net health outcome of care. The net health outcome can be improved if patients receive correct therapy, more effective therapy, or avoid unnecessary therapy, or avoid unnecessary testing.

Tutrone et al (2020) reported a trial that evaluated the effect of ExoDx Prostate on the decision to biopsy (Tables 25 through 28)[44,]. This multicenter, prospective, blinded RCT was conducted in partnership with CareFirst BlueCross/BlueShield of Maryland and included 1094 men with a PSA 2 to 10 ng/ml who were considered for prostate biopsy based on clinical criteria. All patients had the test, but only patients randomized to the ExoDx Prostate arm received the test results. The primary outcome of the study was to determine if ExoDX Prostate could reduce initial biopsies. The secondary endpoint was the successful diagnosis of high-grade prostate cancer. A total of 942 patients (86.1%) had complete data and usable samples. In the ExoDx Prostate arm, 93 patients received low-risk test results and 106 patients (23%) received recommendations to defer biopsy. High-risk ExoDx Prostate scores led to a recommendation for biopsy in 87% of the 365 ExoDx Prostate-positive patients. Compliance with a recommendation for biopsy was 72% in the ExoDx Prostate arm compared to about 40% in the control arm, leading to increased biopsy rates in the ExoDx Prostate arm (58%) compared to controls (39%). In African-American patients, who represented 23% of the patient population, 91% had high-risk scores. The study did not meet its primary endpoint. The main effect of the test was to increase biopsies with an increase in the number of at least Grade Group 2 cancers, but there was also an increase in the number of men biopsied who had no cancer or low-grade cancer compared to the control arm. Additional limitations of the study are the inclusion of men with very low PSA (2 ng/ml) and the lack of information on what screening had preceded the referral for biopsy. It is unclear if the standard of care for repeat PSA and %fPSA were assessed prior to the decision to biopsy, if controls

received this standard of care, or if the test was intended as a replacement for repeat PSA and %fPSA.

Tutrone et al (2023) reported on a retrospective outcome analysis follow-up study of the initial 2020 study reported above.[45,] Of the original 1094 cohort, 833 patients had complete follow-up data at 2.5 years. In this analysis, patients returned to routine standard of care after enrollment in the clinical utility trial, and a retrospective outcome analysis was conducted. The average time from ExoDX Prostate testing to the first biopsy was significantly longer in the low-risk ExoDX Prostate arm (216 days) compared to high-risk ExoDX Prostate arm (68.7 days; p <.001) and when compared to low-risk ExoDX Prostate patients in the standard of care arm (79.4 days; p <.001). In the ExoDx Prostate arm, low-risk patients had significantly fewer biopsies than high-risk patients (44.6% vs 79.0%, p<.001); in the standard of care arm the decision to defer was independent of ExoDx Prostate score and, as a result, did not differ between low-risk and high-risk scores. Patients in both arms with low-risk ExoDx Prostate scores had lower rates of high-grade prostate cancer at 2.5 years than high-risk ExoDx Prostate score patients(7.9% vs. 26.8%; p<.001), and the ExoDx Prostate arm discovered 21.8% (106 vs 87) more high-grade prostate cancer than the standard of care arm. Limitations of this interim analysis mimic limitations that were described in the above study; the study was also retrospective in nature.

Table 25. Summary of Key RCT Characteristics

Study; Trial	Countries	Sites	Dates	Participants	Interventions	
					Active	***Comparator***
Tutrone et al (2020)[44,]	U.S.	24	2017-2018	1094 men aged > 50 with PSA 2 to 10 ng/ml who were considered for biopsy based on clinical criteria	458 patients received EPI results	484 patients had the test but did not receive the test results

EPI: ExoDx Prostate (Inteliscore); PSA: prostate specific antigen; RCT: randomized controlled trial; NR: not reported

Table 26. Summary of Key RCT Results

Study	Biopsy Rate n(%)	No Cancer Rate n(%)	Grade 1 Cancer Rate n(%)	GG2 to GG4 Cancer Rate n(%)
Tutrone et al (2020)[44,]				
EPI	264 (57.5%)	113 (42.8%)	73 (27.7%)	78 (29.5%)
Control	190 (39.3%)	83 (43.7%)	47 (24.7%)	60 (31.6%)
Tutrone et al (2023)				

EPI: ExoDx Prostate (Inteliscore); GG: Grade Group; RCT: randomized controlled trial.

Table 27. Study Relevance Limitations

Study	Population[a]	Intervention[b]	Comparator[c]	Outcomes[d]	Follow-Up[e]
Tutrone et al (2020)[44,]	4. Included men with very low PSA levels (eg, 2 ng/ml)		1. Standard of care was not defined.	1. The primary outcome was not achieved. The study found an increase in compliance without a decrease in the rate of no cancers or GG1 cancers	

GG: Grade Group; PSA: prostate specific antigen.
The study limitations stated in this table are those notable in the current review; this is not a comprehensive gaps assessment.
[a] Population key: 1. Intended use population unclear; 2. Clinical context is unclear; 3. Study population is unclear; 4. Study population not representative of intended use.
[b] Intervention key: 1. Not clearly defined; 2. Version used unclear; 3. Delivery not similar intensity as comparator; 4.Not the intervention of interest.
[c] Comparator key: 1. Not clearly defined; 2. Not standard or optimal; 3. Delivery not similar intensity as intervention; 4. Not delivered effectively.
[d] Outcomes key: 1. Key health outcomes not addressed; 2. Physiologic measures, not validated surrogates; 3. No CONSORT reporting of harms; 4. Not establish and validated measurements; 5. Clinical significant difference not prespecified; 6. Clinical significant difference not supported.
[e] Follow-Up key: 1. Not sufficient duration for benefit; 2. Not sufficient duration for harms

Table 28. Study Design and Conduct Limitations

Study	Allocation[a]	Blinding[b]	Selective Reporting[c]	Data Completeness[d]	Power[e]	Statistical[f]
Tutrone et al (2020)[44,]	3. Randomization procedures were not described	1. Pathologists were blinded, but patients and clinicians were not blinded to treatment assignment when test results were revealed.	2. The high number of false positives in patients with no cancer or low grade cancer was not discussed.			

The study limitations stated in this table are those notable in the current review; this is not a comprehensive gaps assessment.
[a] Allocation key: 1. Participants not randomly allocated; 2. Allocation not concealed; 3. Allocation concealment unclear; 4. Inadequate control for selection bias.
[b] Blinding key: 1. Not blinded to treatment assignment; 2. Not blinded outcome assessment; 3. Outcome assessed by treating physician.
[c] Selective Reporting key: 1. Not registered; 2. Evidence of selective reporting; 3. Evidence of selective publication.
[d] Data Completeness key: 1. High loss to follow-up or missing data; 2. Inadequate handling of missing data; 3. High number of crossovers; 4. Inadequate handling of crossovers; 5. Inappropriate exclusions; 6. Not intent to treat analysis (per protocol for noninferiority trials).

[e] Power key: 1. Power calculations not reported; 2. Power not calculated for primary outcome; 3. Power not based on clinically important difference.
[f] Statistical key: 1. Analysis is not appropriate for outcome type: (a) continuous; (b) binary; (c) time to event; 2. Analysis is not appropriate for multiple observations per patient; 3. Confidence intervals and/or p values not reported; 4. Comparative treatment effects not calculated.

Subsection Summary: ExoDx Prostate (IntelliScore)
The ExoDx Prostate (IntelliScore) assay showed a sensitivity of 97% and NPV of 96% for high-grade prostate cancer in men over 50 years of age who had PSA levels between 2 ng/mL and 10 ng/mL. The primary limitation of the study was that patients with a suspicious DRE were enrolled in the study, but DRE or free PSA were not included in the comparison prediction.

One RCT was identified on ExoDx Prostate. It is unclear from this report whether the test is intended to be used in addition to repeat PSA and %fPSA, or if the test is intended to be used as a replacement for the current standard of care. In either event, the study did not meet its primary endpoint of decreasing unnecessary biopsies. The main impact of the test was to increase biopsies overall, without decreasing the percentage of no cancer or low-grade cancer identified on biopsy. Because of the increase in biopsy rates, there is a potential for this test to lead to overtreatment of slow-growing prostate cancer.

Apifiny
Schipper et al (2015) identified 8 autoantibodies associated with prostate cancer in a case-control study of men 40 to 70 years old with prostate cancer and PSA levels between 2.5 ng/mL and 20 ng/mL, compared to healthy men 25 to 40 years of age with PSA levels less than 1.0 ng/mL.[46,] When the algorithm was applied to an independent validation set, the AUC was 0.69 (95% CI, 0.62 to 0.75).

Subsection Summary: Apifiny
Evidence on Apifiny is preliminary. In a validation set, the AUC was 0.69. The threshold for a positive test has not been determined and the sensitivity, specificity, PPV, and NPV rates compared with established tests have not been reported. Studies validating the diagnostic performance of Apifiny are needed.

PanGIA Prostate
No studies were identified on PanGia Prostate

MICHECK PROSTATE

Clinically Valid
A test must detect the presence or absence of a condition, the risk of developing a condition in the future, or treatment response (beneficial or adverse).

Review of Evidence
Evidence on the MiCheck Prostate test is preliminary.

Nonrandomized Clinical Trials
Serum samples from 332 U.S. patients scheduled for a biopsy due to an elevated age-adjusted PSA from the MiCheck-01 prospective study were used to develop and train the algorithm for the MiCheck prostate test to accurately predict the presence of aggressive prostate cancer (Gleason

Score [GS] ≥3+4) versus nonaggressive prostate cancer. [47,] A Monte Carlo cross-validation was applied to the algorithm to avoid over fitting, in which, the data was randomly split in two-third for the training set and one-third for the performance test set (repeated 2000 times). MiCheck prostate test reported an area under the curve (AUC) of 0.83 (95% CI: 0.76 to 0.89), specificity of 50%, and sensitivity of 95% with an odds ratio of 227.76 (95% CI: 41.30 to 1255.90; p<0001). Furthermore, the test classified 224 of 320 evaluable PSA patients as positive and 96 as negative for aggressive prostate cancer with a positive predictive value (PPV) of 49% and a negative predicative value (NPV) of 92% for cancers with GS ≥3+4 resulting in up to 30% of biopsies having the potential to be avoided with a delaying diagnosis in only 6.8% of patients.

In a recent study from 2023 (MP17-07) MiCheck Prostate test performance was assessed in was validation set (N=192), the AUC was 0.79 and was significantly higher than PSA (0.58; p<.0001) [48,]

Clinically Useful
A test is clinically useful if the use of the results informs management decisions that improve the net health outcome of care. The net health outcome can be improved if patients receive correct therapy, more effective therapy, or avoid unnecessary therapy, or avoid unnecessary testing.

Direct Evidence
Direct evidence of clinical utility is provided by studies that have compared health outcomes for patients managed with and without the test. Because these are intervention studies, the preferred evidence would be from RCTs.

No RCTs directly measuring the effect of the MiCheck Prostate test on clinical outcomes were found.

Subsection Summary: Micheck Prostate
The MiCheck Prostate test showed a sensitivity of 95% and NPV of 92% for aggressive prostate cancer in men over 40 years of age irrespective of PSA levels. The primary limitations of the study were the nonrandomized retrospective study design and the small sample size comprised of an exclusive contemporary U.S. population.

No RCTs were identified using the MiCheck Prostate test. It is unclear from the nonrandomized trial whether the test is intended to be used in addition to repeat PSA and %fPSA, or if the test is intended to be used as a replacement for the current standard of care. The main impact of the test was to decrease the overall number of biopsies without delaying diagnosis for prostate cancer patients.

COMPARATIVE STUDIES

4Kscore and SelectMDx
Wysock et al (2020) compared the performance of 4Kscore and SelectMDx to inform decisions of whether to perform a prostate biopsy.[49,] New referrals (N=128) with elevated PSA were advised to undergo both 4K score and SelectMDX; 114 men underwent both tests. There was poor concordance between the 2 tests, with discordant guidance in 45.6% of the population. Since biomarker results were used to determine which patients should undergo biopsy (ie the reference

test was not obtained for all patients), it cannot be determined which of the tests was more accurate.

INITIAL OR REPEAT BIOPSY

PCA3 Score (eg, Progensa PCA3 Assay)

Some studies have assessed men who are scheduled for an initial biopsy, although the U.S. Food and Drug Administration (FDA) approved indication for the Progensa PCA3 Assay is to aid in the decision for repeat biopsy in men 50 years or older who have had 1 or more negative prostate biopsies and for whom a repeat biopsy would be recommended based on current standard of care. Evaluation of the PCA3 score is relevant to both initial and repeat prostate biopsy.

Clinically Valid

A test must detect the presence or absence of a condition, the risk of developing a condition in the future, or treatment response (beneficial or adverse).

REVIEW OF EVIDENCE

Systematic Reviews

Several systematic reviews and meta-analyses have described the clinical validity of the PCA3 Assay. The characteristics of the reviews are described in Table 29. All primary studies were observational, with 1 study using the placebo arm from an RCT. Reviewers selected studies of men with positive, negative, or inconclusive DRE without restrictions on PSA levels. Kawada et al (2024) and Cui et al (2016) both reported on the results of a systematic review of case-control or cohort studies.[50,25,]In both reviews, studies assessed both initial and repeat biopsy. Studies in the review by Cui et al (2016) had a quality rating of moderate to high, whereas the quality of included studies in the review by Kawada et al (2024) was not reported. Rodriguez et al (2020) conducted a systematic review of PCA3 in men who had not yet undergone biopsy.[51,] Nine studies in men without prior biopsy were identified, and 5 studies that used a cutoff of 35 were included in the meta-analysis. The assessment by Nicholson et al (2015) for the National Institute for Health and Care Excellence included 11 cohorts of men for whom initial prostate biopsy results were negative or equivocal.[52,]

Results from the systematic reviews are shown in Table 30. In the meta-analysis by Cui et al (2016), the most common PCA3 assay cutoff for categorizing low- and high-risk was 35 (25 of 46 studies).[50,] The estimates of AUC were lower for studies that included men having repeated (0.68) versus initial (0.80) biopsies. A PCA3 assay cutoff was not provided by Kawada et al (2024), and the pooled sensitivity and specificity were 85% and 37%, respectively; the majority of data were from patients in the initial biopsy setting.[25,]

Rodriguez et al (2020) found a pooled sensitivity of 69% and specificity of 65% in the 5 studies that used a cutoff of 35 in men without prior biopsy.[51,] The studies were all prospective cohorts and rated as having a low risk of bias, except for uncertainty in flow and timing.

Nicholson et al (2015) included 13 reports describing 11 cohorts, including 1 from the placebo arm of an RCT.[52,] Referral criteria for repeat biopsy, were varied, often unclear, and differed based on whether normal or abnormal DREs were included. The mean or median PSA, when reported, ranged from 4.9 to 11.0 ng/mL and the prevalence of cancer on repeat biopsy varied

from 11.4% to 68.3%. Meta-analyses were not performed due to heterogeneity. The addition of PCA3 to clinical assessment, as a continuous or categorical variable, generally led to an improvement in AUC, but studies that fixed sensitivity and derived specificity and those that reported decision-curve analysis had mixed results.

Table 29. Characteristics of Systematic Reviews Assessing the Clinical Validity of Progensa PCA3 Assay for Diagnosing Prostate Cancer

Study	Studies	Dates	Key Inclusion Criteria	Design	Reference Studies Included
Kawada et al (2024)[25,]	49 total; 7 evaluating PCA3	Searches through March 2023	Studies on the diagnostic accuracy of PCA3 using biopsy as the gold standard and providing data to calculate test characteristics; patients in included studies were suspected of harboring prostate cancer or any reason	Prospective and retrospective cohort	Biopsy as reference standard
Rodriguez et al 2020[51,]	5	2007-2014	PCA3 cutoff of 35 in men without prior biopsy	Prospective cohort	Biopsy as reference standard
Cui et al (2016)[50,]	46	Up to 2014		Prospective, retrospective (case-control or cohort) OBS	Biopsy as reference standard
Nicholson et al (2015)[52,]	11	2000-2014	Initial prostate biopsy negative or equivocal, 6+ cores in initial biopsy, with or without DRE	Prospective and mixed (prospective/retrospective) OBS (1 included a cohort from a RCT)	Biopsy as reference standard

DRE: digital rectal exam; OBS: observational; RCT: randomized controlled trial.

Table 30. Results of Systematic Reviews Assessing the Clinical Validity of Progensa PCA3 Assay for Diagnosing Prostate Cancer

Study	Studies	N (Range)	Outcomes	Sens (95% CI), %	Spec (95% CI), %	AUC (95% CI) or Range
Kawada et al (2024)[25,]	7	2833 (128 to 692)	Any prostate cancer on initial or repeat biopsy	85% (74% to 92%)	37% (21% to 57%)	0.73 (NR)
Rodriguez et al 2020[51,]	5	2,083 (80 to 692)	Any prostate cancer on initial biopsy	Pooled 69% (61% to 75%)	65% (55% to 73%)	0.73 (0.67 to 0.80)
Cui et al (2016)[50,]	46	12,295 (NR)	Any prostate cancer on initial or repeat biopsy	Pooled: 65% (63% to 66%) Range: 47% to 95%	• Pooled: 73% (72% to 74%) • Range: 22%-100%	0.75 (0.74 to 0.77)
Nicholson et al (2015)[52,]	11	3336 (41 to 1072)	Any prostate cancer on repeat biopsy	CA alone range, 44% to 48% CA plus PCA3 range, 39% to 46%	Fixed at 80%	• CA alone: 0.55 to 0.75 • CA plus PCA3: 0.61 to 0.76

AUC: area under the curve; CA: clinical assessment; CI: confidence interval; NR: not reported; Sens: sensitivity; Spec: specificity.

Prospective Studies

Not included in the systematic reviews was a prospective trial from the National Cancer Institute on the clinical validity of the PCA3 assay to complement PSA-based detection of prostate cancer (Tables 31 and 32).[53,] The trial was designed to evaluate whether PCA3 greater than 60 could improve the PPV of an initial biopsy and whether PCA3 less than 20 could improve the NPV of a repeat biopsy. Of the 859 men in the study, 562 were presenting for their initial prostate biopsy and 297 were presenting for repeat biopsy. For the detection of high-grade cancer, the performance of the PCPT risk calculator was modestly improved by adding PCA3 assay results to the risk calculator factors, with an AUC improvement from 0.74 to 0.78 for initial biopsy and 0.74 to 0.79 on repeat biopsy ($p \leq .003$). The PPV of the PCA3 assay at a threshold of 60 ng/mL to detect prostate cancer in an initial biopsy was 80% (95% CI, 72% to 86%), while the NPV of the PCA3 assay at a threshold of 20 ng/mL for prostate cancer in men undergoing repeat biopsy was 88% (95% CI, 81% to 93%). Estimates of biopsies avoided and cancer missed at this threshold is described in the section on clinical utility.

A similar validation study was published by Ankerst et al (2018) in 854 men who underwent a diagnostic biopsy.[39,] The addition of PCA3 to the PCPTRC increased the AUC (95% CI) from 70% (66.0% to 74.0%) to 76.4% (72.8% to 80.0%). The AUC with *TMPRSS2:ERG* added to both was 77.1% (73.6% to 80.6%). These have been added to the online risk tool for further validation. Investigators have also been assessing the effect of age on PCA3 values, finding that age adjusted values improve the diagnostic performance of the test.[54,]

The prospective multi-institutional Canary PASS was reported by Newcomb et al (2019)[41,] The study included 782 men under active surveillance (2,069 urine samples) to examine the association of urinary PCA3 and *TMPRSS2:ERG* with biopsy-based reclassification. Under the PASS protocol, PSA is measured every 3 months and ultrasound-guided biopsies are performed 12 and 24 months after diagnosis, then every 2 years. Post-DRE urine samples were collected every 6 months. Modeling showed minimal benefit of adding PCA3 to a model with clinical variables, improving the AUC from 0.743 to 0.753.

Table 31. Characteristics of Clinical Validity Studies Assessing the Progensa PCA3 Assay

Study	Study Population	Design	Reference Standard	Threshold for Positive Index Test	Timing of Reference and Index Tests	Blinding of Assessors
Wei et al (2014)[53,]	910 men scheduled for a diagnostic prostate biopsy (initial or repeat)	Prospective	Any prostate cancer on biopsy or HG prostate cancer (Gleason score >6)	Determined a priori at thresholds of <20 and >60	Urine samples collected following DRE and prior to biopsy	Yes

DRE: digital rectal exam; HG: high-grade.

Table 32. Results of Clinical Validity Studies Assessing the Progensa PCA3 Assay

Study	Initial N	Final N	Excluded Samples	Clinical Validity (95% Confidence Interval), %			
				Sens	Spec	PPV	NPV
Wei et al (2014)[55,]							
	910	859	27				
Initial biopsy PCA3 >60		562		42 (36 to 48)	91 (87 to 94)	80 (72 to 86)	
Repeat biopsy PCA3 <20		297		76 (64 to 86)	52 (45 to 58)		88 (81 to 93)

NPV: negative predictive value; PPV: positive predictive value; Sens: sensitivity; Spec: specificity.
No notable limitations were identified for study relevance or design and conduct.

Clinically Useful

A test is clinically useful if the use of the results informs management decisions that improve the net health outcome of care. The net health outcome can be improved if patients receive correct therapy, more effective therapy, or avoid unnecessary therapy , or avoid unnecessary testing.

Direct Evidence

Direct evidence of clinical utility is provided by studies that have compared health outcomes for patients managed with and without the test. Because these are intervention studies, the preferred evidence would be from RCTs.

Clinical utility studies using assay results for decision making for an initial biopsy, repeat biopsy, or treatment have not been reported, nor have studies of the effects of using assay results on clinical outcomes.

Chain of Evidence
Indirect evidence on clinical utility rests on clinical validity. If the evidence is insufficient to demonstrate test performance, no inferences can be made about clinical utility.

Several studies using decision analysis to estimate the cost-benefit tradeoff between a reduction in unnecessary biopsies and missed prostate cancers have been published. One group reported potential reductions in unnecessary biopsies of 48% to 52%, with attendant increases in missed prostate cancers of 6% to 15% using either a PCA3-based nomogram[56,] or PCA3 level corrected for prostate volume (PCA3 density).[57,] Merdan et al (2015) used decision analysis to simulate long-term outcomes associated with the use of the PCA3 score to trigger repeat biopsy compared with the PCPT risk calculator in men with at least 1 previous negative biopsy and elevated PSA levels.[58,] They estimated that incorporating the PCA3 score of 25 (biopsy threshold) into the decision to recommend repeat biopsy could avoid 55.4% of repeat biopsies, with a 0.93% reduction in the 10-year survival rate. Wei et al (2014) calculated that for men with a PCA3 score less than 20 and PSA less than 4 ng/mL, 8% of men would have avoided a repeat biopsy with 9% of low-grade cancers missed and no high-grade cancers missed.[53,] If only PCA3 scores less than 20 were taken into account, 46% of men would have avoided rebiopsy but 12% would have undiagnosed cancer and 3% would have undiagnosed high-grade cancer. For patients undergoing an initial biopsy, 13% of aggressive cancers would have been underdiagnosed.

Subsection Summary: PCA3 Score (eg, Progensa PCA3 Assay)
At least 47 studies have evaluated the clinical validity of PCA3 mRNA to facilitate decision making for initial or repeat prostate biopsy, and there are systematic reviews of those studies. Studies of the PCA3 score as a diagnostic test for prostate cancer have reported sensitivities and specificities mostly in the moderate range (eg, 76% sensitivity, 52% specificity). One systematic review that focused on studies of repeat biopsy found mixed results regarding whether the PCA3 assay could improve diagnostic accuracy over clinical assessment alone. Other systematic reviews found an AUC of 0.73 in men having an initial biopsy compared to 0.68 for the PCA3 assay in men having repeat biopsies. Other recent studies have reported minimal benefit of adding PCA3 to a model with clinical variables.

Given the lack of direct evidence of utility, a chain of evidence would be needed to demonstrate clinical utility. Studies of the PCA3 score as a diagnostic test for prostate cancer have reported sensitivities and specificities in the moderate range. Consideration of rebiopsy based only on PCA3 scores was estimated to miss 3% of aggressive cancers. One estimate suggested that adding a PCA3 score to PSA level would reduce rebiopsy rates by 8%, while another analysis suggested that over half of rebiopsies could be avoided by adding the PCA3 score to the PCPT risk calculator. No prospective studies were found describing differences in management based on PCA3 risk assessment. The clinical utility of the PCA3 test is uncertain because it is not clear whether its use can change management in ways that improve patient outcomes. The chain of evidence is incomplete.

BIOMARKER TESTING FOR SELECTION OF MEN FOR REPEAT PROSTATE BIOPSY

Clinical Context and Test Purpose

The purpose of genetic and protein biomarker testing for prostate cancer is to inform the selection of men who should undergo repeat biopsy. The conventional decision-making tools for identifying men for prostate biopsy include DRE, serum PSA, and patient risk factors such as age, race, and family history of prostate cancer and are described in the previous section on selecting men for initial prostate biopsy.

Given the risk, discomfort, burden of biopsy, and the low diagnostic yield, there is a need for noninvasive tests that distinguish potentially aggressive tumors that should be referred for rebiopsy from clinically insignificant localized tumors or other prostatic conditions that do not need rebiopsy, with the goal of avoiding low-yield biopsy.

The following PICO was used to select literature that provides evidence relevant to this review.

Populations

The relevant population of interest are men for whom a rebiopsy is being considered because the results of an initial prostate biopsy were negative or equivocal and other clinical symptoms remain suspicious.

Interventions

For assessing future prostate cancer risk, numerous studies have demonstrated the association between many genetic and protein biomarker tests and prostate cancer. Commercially available tests for selection of men for repeat prostate biopsy include those described in Table 33.

Table 33. Commercially Available Tests to Determine Candidates for Repeat Prostate Biopsy

Test	Manufacturer	Description
PCA3 Score (eg, Progensa PCA3 Assay)	• Hologic Gen-Probe • Many labs offer PCA3 tests (eg, ARUP Laboratories, Mayo Medical Laboratories, LabCorp)	Measures PCA3 mRNA in urine samples after prostate massage. PCA3 mRNA may be normalized using PSA to account for prostate cells.
ConfirmMDx	MDxHealth	Measures methylation of the genes *GSTP1, APC*, and *RASSF1* in tissue sample
Prostate Core Mitomics Test (PCMT)	Mitomics (formerly Genesis Genomics)	Measures deletions in mitochondrial DNA by polymerase chain reaction in tissue sample
Gene panel testing	Many labs offer SNV testing,	Panel tests for prostate cancer risk are offered as laboratory-developed tests

Test	Manufacturer	Description
	such as Life Technologies, LabCorp (23andme), and ARUP Laboratories (deCODE)	
MyProstate Score 2.0	LynxDx	Urine test designed to predict the presence of clinically significant prostate cancer (Grade Group ≥2 or Gleason score ≥7) by analyzing a comprehensive array of 18 unique gene transcripts

mRNA: messenger ribonucleic acid; PCA3: prostate cancer antigen 3; PSA: prostate-specific antigen; SNV: single nucleotide variant.

PCA3 is a noncoding long-chain RNA that is highly overexpressed in prostate cancer compared with noncancerous prostate tissue and is detectable in urine. The Progensa PCA3 Assay is approved by the FDA to facilitate decision making among men with prior negative prostate biopsies.

Epigenetic changes-chromatin protein modifications that do not involve changes to the underlying DNA sequence but can change gene expression-have been identified in specific genes. An extensive literature has reported significant associations between epigenetic DNA modifications and prostate cancer. ConfirmMDx (MDxHealth) is a commercially available test for gene methylation intended to distinguish true- from false-negative prostate biopsies to avoid the need for repeat biopsy.

The Prostate Core Mitomics Test (PCMT; Mitomics; formerly Genesis Genomics) is a proprietary test intended to determine whether a patient has prostate cancer, despite a negative prostate biopsy, by assessing a 3.4-kilobases deletion in mitochondrial DNA by polymerase chain reaction to detect "tumor field effect." The test is performed on the initial negative prostate biopsy tissue and is being evaluated in men who have had an initial negative biopsy. A negative PCMT result is intended to confirm the result of the negative biopsy so that the patient can avoid a second biopsy, while a positive PCMT is intended to indicate that the patient is at high-risk of undiagnosed prostate cancer.

Single nucleotide variants (SNVs) occur when a single nucleotide is replaced with another, and are the most common type of genetic variation in humans. They occur normally throughout the genome and can act as biologic markers for disease association. Genome-wide association studies have identified correlations between prostate cancer risk and specific SNVs. However, it is widely accepted that, individually, SNV-associated disease risk is low and of no value in screening, although multiple SNVs in combination may account for a higher proportion of prostate cancer. Investigators have begun to explore the use of algorithms incorporating information from multiple SNVs to increase the clinical value of testing.

Comparators

Standard clinical examination for determining who requires a biopsy might include DRE, a review of the history of PSA values, and consideration of risk factors such as age, race, and family history. The ratio of free (unbound) PSA to total PSA is lower in men who have prostate cancer

than in those who do not. A percent free PSA cutoff of 25% has been shown to have a sensitivity and specificity of 95% and 20%, respectively, for men with total PSA levels between 4.0 ng/mL and 10.0 ng/mL.[20,]

The best way to combine all of the risk information to determine who should go to biopsy is not standardized. Risk algorithms have been developed that incorporate clinical risk factors into a risk score or probability. Two examples are the PCPT predictive model[21,] and the ERSPC-RC.[22,] The American Urological Association and the Society of Abdominal Radiology recently recommended that high-quality prostate magnetic resonance imaging, if available, should be strongly considered in any patient with a prior negative biopsy who has persistent clinical suspicion for prostate cancer and who is under evaluation for a possible repeat biopsy.[23,]

Outcomes

The beneficial outcome of the test is to avoid a negative biopsy for prostate cancer. A harmful outcome is a failure to undergo a biopsy that would be positive for prostate cancer, especially when the disease is advanced or aggressive. Thus, the relevant measures of clinical validity are sensitivity and NPV. The appropriate reference standard is a biopsy, though prostate biopsy is an imperfect diagnostic tool. Biopsies can miss cancers and repeat biopsies are sometimes needed to confirm the diagnosis. Detection rates vary by biopsy method and patient characteristics, with published estimates between 10% and 28% for a second biopsy and 5% and 10% for a third biopsy.[59,60,]. The timeframe of interest for calculating performance characteristics is time to biopsy results. Men who forego biopsy based on test results could miss or delay the diagnosis of cancer. Longer follow-up would be necessary to determine the effects on OS.

Study Selection Criteria

For the evaluation of clinical validity, studies that meet the following eligibility criteria were considered:

- Reported on the accuracy of the marketed version of the technology (including any algorithms used to calculate scores)
- Included a suitable reference standard
- Patient/sample clinical characteristics were described
- Patient/sample selection criteria were described.

Studies were excluded from the evaluation of the clinical validity of the test because they did not use the marketed version of the test, did not include information needed to calculate performance characteristics, did not use an appropriate reference standard or the reference standard was unclear, did not adequately describe the patient characteristics, or did not adequately describe patient selection criteria.

GENE HYPERMETHYLATION AND CONFIRMMDX

Clinically Valid

A test must detect the presence or absence of a condition, the risk of developing a condition in the future, or treatment response (beneficial or adverse).

Review of Evidence

Three blinded multicenter validation studies of the ConfirmMDx test have been performed, 1 of which was conducted in African American men (Tables 34 and 35).[61,62,63,] For the cases that had

a positive second biopsy after an initial negative biopsy, sensitivity ranged from 62% to 74%, with an NPV for a negative second biopsy ranging from 79% to 90%. Multivariate analysis of potential predictors of cancer on repeat biopsy, corrected for age, PSA, DRE, and first biopsy histopathology characteristics, showed that the ConfirmMDx test was the most significant independent predictor of patient outcome in both the Detection of Cancer Using Methylated Events in Negative Tissue (DOCUMENT) (OR=2.69; 95% CI, 1.60 to 4.51) and Methylation Analysis to Locate Occult Cancer (MATLOC) (OR=3.17; 95% CI, 1.81 to 5.53) studies.

Van Neste et al (2016) and Partin et al (2016) reported on results of combined data from the DOCUMENT and MATLOC studies for patients with high-grade (Gleason score, ≥7) prostate cancer.[64,65,] DNA methylation was the most significant and important predictor of high-grade cancer, with an NPV of 96% (precision not reported) and an OR of 9.80 (95% CI, 2.12 to 45.23).

Table 34. Characteristics of Clinical Validity Studies Assessing ConfirmMDx

Study	Study Population	Design	Reference Standard	Threshold for Positive Index Test	Timing of Reference and Index Tests	Blinding of Assessors	Comment
Waterhouse et al (2018)[63,]	Archived, cancer-negative prostate biopsy core tissue samples from 211 African American men from 7 U.S. urology centers	Retrospective, ConfirmMDx performed on first biopsy	Repeat biopsy	NR	<30 mo	Yes	55% of men had a normal DRE; median PSA level was 6.2 ng/mL
Partin et al (2014)[62,62,]DOCUMENT	Archived, cancer-negative prostate biopsy core tissue samples from 350 men from 5 U.S. urology centers	Retrospective, case-control with assay performed on archived samples	Repeat biopsy	NR	<24 mo	Yes	60% of men had a normal DRE; median PSA level was 5.3 ng/mL
Stewart et al (2013)[61,61,]MATLOC	Archived cancer-negative	Retrospective ConfirmMDx	Repeat biopsy	NR	<30 mo	Yes	73% of men had benign

Study	Study Population	Design	Reference Standard	Threshold for Positive Index Test	Timing of Reference and Index Tests	Blinding of Assessors	Comment
	prostate biopsy core tissue samples from 498 men from the U.K. and Belgium	performed on first biopsy					DRE; median PSA level was 5.9 ng/mL

DOCUMENT: Detection of Cancer Using Methylated Events in Negative Tissue study; DRE: digital rectal exam; MATLOC: Methylation Analysis to Locate Occult Cancer study; NR: not reported; PSA: prostate-specific antigen.

Table 35. Results of Clinical Validity Studies Assessing ConfirmMDx

Study; Trial	Initial N	Final N	Excluded Samples	Prevalence of Condition	Clinical Validity (95% CI), %			
					Sens	Spec	PPV	NPV
Waterhouse et al (2018)[63,]	NR	211	NR	81 had positive second biopsy (cases), 130 had negative second biopsy (controls)	74 (63 to 83)	60 (51 to 69)	54 (47 to 60)	79 (72 to 85)
Partin et al (2014)[62,]; DOCUMENT	350	320	30	92 had positive second biopsy (cases), 228 had negative second biopsy (controls)	62 (51 to 72)	64 (57 to 70)		88 (85 to 91)
Stewart et al (2013)[61,]; MATLOC	498	483	15	87 had positive second biopsy, 396 had negative second biopsy (controls)	68 (57 to 77)	64 (59 to 69)		90 (87 to 93)
Summary					51 to 83	51 to 70	54	72 to 93

CI: confidence interval; DOCUMENT: Detection of Cancer Using Methylated Events in Negative Tissue study; MATLOC: Methylation Analysis to Locate Occult Cancer study; NPV: negative predictive value; NR: not reported; PPV: positive predictive value; Sens: sensitivity; Spec: specificity.

Tables 36 and 37 summarize the relevance and design and conduct limitations in each study.

Table 36. Study Relevance Limitations

Study; Trial	Population[a]	Intervention[b]	Comparator[c]	Outcomes[d]	Duration of Follow-Up[e]
Waterhouse et al (2018)[63,]		1. Classification thresholds not described (proprietary)			
Partin et al (2014)[62,]; DOCUMENT		1. Classification thresholds not described (proprietary)			
Stewart et al (2013)[61,]; MATLOC		1. Classification thresholds not defined. Training set with a stepwise approach to maximize NPV			

DOCUMENT: Detection of Cancer Using Methylated Events in Negative Tissue study; MATLOC: Methylation Analysis to Locate Occult Cancer study; NPV: negative predictive value.
The study limitations stated in this table are those notable in the current review; this is not a comprehensive gaps assessment.
[a] Population key: 1. Intended use population unclear; 2. Clinical context is unclear; 3. Study population is unclear; 4. Study population not representative of intended use.
[b] Intervention key: 1. Classification thresholds not defined; 2. Version used unclear; 3. Not intervention of interest.
[c] Comparator key: 1. Classification thresholds not defined; 2. Not compared to credible reference standard; 3. Not compared to other tests in use for same purpose.
[d] Outcomes key: 1. Study does not directly assess a key health outcome; 2. Evidence chain or decision model not explicated; 3. Key clinical validity outcomes not reported (sensitivity, specificity, and predictive values); 4. Reclassification of diagnostic or risk categories not reported; 5. Adverse events of the test not described (excluding minor discomforts and inconvenience of venipuncture or noninvasive tests).
[e] Follow-Up key: 1. Follow-up duration not sufficient with respect to natural history of disease (true-positives, true-negatives, false-positives, false-negatives cannot be determined).

Table 37. Study Design and Conduct Limitations

Study; Trial	Selection[a]	Blinding[b]	Delivery of Test[c]	Selective Reporting[d]	Data Completeness[e]	Statistical[f]
Waterhouse et al (2018)[32,]	1. Selection not described				1. Inadequate description of indeterminate and missing samples	
Partin et al (2014)[52,]; DOCUMENT						
Stewart et al (2013)[66,]: MATLOC						

DOCUMENT: Detection of Cancer Using Methylated Events in Negative Tissue study; MATLOC: Methylation Analysis to Locate Occult Cancer study;
The study limitations stated in this table are those notable in the current review; this is not a comprehensive gaps assessment.
[a] Selection key: 1. Selection not described; 2. Selection not random or consecutive (ie, convenience).
[b] Blinding key: 1. Not blinded to results of reference or other comparator tests.
[c] Test Delivery key: 1. Timing of delivery of index or reference test not described; 2. Timing of index and comparator

tests not same; 3. Procedure for interpreting tests not described; 4. Expertise of evaluators not described.
[d] Selective Reporting key: 1. Not registered; 2. Evidence of selective reporting; 3. Evidence of selective publication.
[e] Data Completeness key: 1. Inadequate description of indeterminate and missing samples; 2. High number of samples excluded; 3. High loss to follow-up or missing data.
[f] Statistical key: 1. Confidence intervals and/or p values not reported; 2. Comparison with other tests not reported.

Clinically Useful
A test is clinically useful if the use of the results informs management decisions that improve the net health outcome of care. The net health outcome can be improved if patients receive correct therapy, more effective therapy, or avoid unnecessary therapy, or avoid unnecessary testing.

Direct Evidence
Direct evidence of clinical utility is provided by studies that have compared health outcomes for patients managed with and without the test. Because these are intervention studies, the preferred evidence would be from RCTs.

Aubry et al (2013) estimated the reduction in biopsies associated with ConfirmMDx use.[67,] Using the performance characteristics from MATLOC, the authors estimated that 1106 biopsies per 1 million people would be avoided. The study did not include a decision analysis comparing the tradeoff in a reduction in biopsies and missed cancers.

Chain of Evidence
Indirect evidence on clinical utility rests on clinical validity. If the evidence is insufficient to demonstrate test performance, no inferences can be made about clinical utility.

Because the clinical validity of ConfirmMDx has not been established, a chain of evidence supporting the clinical utility of this test cannot be constructed.

Subsection Summary: Gene Hypermethylation and ConfirmMDx
Three retrospective clinical validation studies have reported on the ConfirmMDx score in men who have undergone repeat biopsy. The studies did not provide estimates of validity compared with other risk prediction models. ConfirmMDx was shown to be the most significant predictor of patient outcome in a multivariate model that included age, PSA level, DRE, and first biopsy histopathology characteristics. Sensitivity ranged from 62% to 74% and NPV from 79% to 90%. In a subsequent analysis of ConfirmMDx in men with high-grade prostate cancer on rebiopsy, the NPV was 96%, but the precision of the estimate was not reported.

No studies were found that directly show the effects of using ConfirmMDx test results on clinical outcomes. Given the lack of direct evidence of utility, a chain of evidence would be needed to demonstrate clinical utility. The ConfirmMDx test is associated with a diagnosis of prostate cancer and aggressive prostate cancer, but studies did not compare performance characteristics with standard risk prediction models. No data are currently available on the longer-term clinical outcomes of the men who did not have biopsy based on ConfirmMDx results. The chain of evidence is incomplete.

PROSTATE CORE MITOMICS TEST

Clinically Valid
A test must detect the presence or absence of a condition, the risk of developing a condition in the future, or treatment response (beneficial or adverse).

Review of Evidence
Robinson et al (2010) assessed the clinical value of a 3.4-kilobase mitochondrial deletion in predicting rebiopsy outcomes.[68,] Levels of the deletion were measured by a quantitative polymerase chain reaction in prostate biopsies negative for cancer from 101 men who underwent repeat biopsy within 1 year and had known outcomes. The clinical performance of the deletion was calculated with the use of an empirically established cycle threshold cutoff, the lowest cycle threshold as diagnostic of prostate cancer, and the histopathologic diagnosis on the second biopsy. Final data were based on 94 patients, who on the second biopsy had 20 malignant and 74 benign diagnoses. The cycle cutoff gave a sensitivity and specificity of 84% and 54%, respectively, with an area under the receiving operating curve of 0.75. The NPV was 91%.

Clinically Useful
A test is clinically useful if the use of the results informs management decisions that improve the net health outcome of care. The net health outcome can be improved if patients receive correct therapy, more effective therapy, or avoid unnecessary therapy, or avoid unnecessary testing.

Direct Evidence
Direct evidence of clinical utility is provided by studies that have compared health outcomes for patients managed with and without the test. Because these are intervention studies, the preferred evidence would be from RCTs.

Legisi et al (2016) queried a pathology services database to identify (1) men who had a negative initial prostate biopsy and a negative PCMT (n=644), and (2) men who had a negative initial prostate biopsy and a repeat biopsy (n=823). Of the 644 patients with a negative PCMT, 35 had a repeat biopsy and 5 (14.2%) were false-negatives who were found to have cancer on rebiopsy. The number of false-negatives of the patients who did not have a repeat biopsy cannot be determined from this study.[69,] Of the second group of 823 men who had a repeat biopsy, 132 had a PCMT. Changes in physician decision-making led to earlier detection of prostate cancer by 2.5 months and an increase in cancer detection rates, but this was only observed when men with atypical small acinar proliferation on index biopsy were not included. Interpretation of these results is limited because testing was not random or consecutive.

Chain of Evidence
Indirect evidence on clinical utility rests on clinical validity. If the evidence is insufficient to demonstrate test performance, no inferences can be made about clinical utility.

Because the clinical validity of PCMT has not been established, a chain of evidence supporting the clinical utility of this test cannot be constructed.

Subsection Summary: Prostate Core Mitomics Test
The PCMT has preliminary data on its performance characteristics in a small validation study, showing a sensitivity of 84%, specificity of 91%, and NPV of 91%.

No studies were found that directly show the effects of using PCMT results on clinical outcomes. Given the lack of direct evidence of utility, a chain of evidence would be needed to demonstrate clinical utility. The PCMT has preliminary data on performance characteristics in a small validation study, but independent confirmation of clinical validity is needed. The studies did not provide estimates of validity compared with clinical examination and standard risk scores. Changes in physician decision-making led to earlier detection of prostate cancer and an increase in cancer detection rates, but the interpretation of these results is limited by potential selection bias. No data are available on long-term clinical outcomes. Data on clinical utility are lacking.

CANDIDATE GENE PANELS

Clinically Valid

A test must detect the presence or absence of a condition, the risk of developing a condition in the future, or treatment response (beneficial or adverse).

Review of Evidence

A 3-gene panel (*HOXC6*, *TDRD1*, *DLX1*) developed by Leyten et al (2015) is now commercially available as SelectMDx (see above).[70,] Xiao et al (2016) reported the development of an 8-gene panel (*PMP22*, *HPN*, *LMTK2*, *FN1*, *EZH2*, *GOLM1*, *PCA3*, *GSTP1*) that distinguished high-grade prostate cancer from indolent prostate cancer with a sensitivity of 93% and NPV of 61% (Tables 38 and 39).[71,] Validation of this panel is needed.

Table 38. Characteristics of Clinical Validity Studies Assessing Candidate Gene Panels

Study	Study Population	Design	Reference Standard
Xiao et al (2016)[71,]	Specimens from 158 men	Retrospective	High-grade prostate cancer on biopsy

Table 39. Results of Clinical Validity Studies Assessing Candidate Gene Panels

Study	N	Clinical Validity (95% CI), %			
		Sens	Spec	PPV	NPV
Xiao et al (2016)[71,]; 8-gene panel	158	93 (88 to 97)	70 (36 to 104)	98 (95 to 100)	61 (25 to 97)

CI: confidence interval; NPV: negative predictive value; PPV: positive predictive value; Sens: sensitivity; Spec: specificity.

Clinically Useful

A test is clinically useful if the use of the results informs management decisions that improve the net health outcome of care. The net health outcome can be improved if patients receive correct therapy, more effective therapy, or avoid unnecessary therapy, or avoid unnecessary testing.

Direct Evidence

Direct evidence of clinical utility is provided by studies that have compared health outcomes for patients managed with and without the test. Because these are intervention studies, the preferred evidence would be from RCTs.

Chain of Evidence
Indirect evidence on clinical utility rests on clinical validity. If the evidence is insufficient to demonstrate test performance, no inferences can be made about clinical utility.

Because the clinical validity of these multigene tests has not been established, a chain of evidence supporting the clinical utility of these tests cannot be constructed.

Subsection Summary: Candidate Gene Panels
Numerous studies have demonstrated the association between SNVs and prostate cancer. Gene panels that evaluate the likelihood of prostate cancer on biopsy are in development.

MYPROSTATE SCORE

Clinically Valid
A test must detect the presence or absence of a condition, the risk of developing a condition in the future, or treatment response (beneficial or adverse).

Review of Evidence
Tosoian et al (2023) evaluated the MyProstate Score test in men with persistent risk of Grade Group ≥2 cancer after a negative biopsy who are being considered for repeat biopsy.[72,] A total of 422 men underwent repeat biopsy in the primary study cohort; the validation cohort consisted of 268 men. Thresholds of 15 and 40 met pre-defined performance criteria in the primary cohort (median PSA 6.4; IQR, 4.3 to 9.1); upon biopsy, 58 men (14%) were found to have Grade Group ≥ 2 cancer, and 25 men (5.9%) had Grade Group ≥ 3 cancer. In the validation cohort, repeat biopsy was negative in 205 men (76%), and revealed Grade Group 1 cancer in 38 men (14%); it also demonstrated Grade Group ≥ 2 cancer in 25 men (9.3%).The rule-out threshold of 15 provided 100% NPV and 100% sensitivity for Grade Group ≥ 2 cancer. Using the upper threshold of 40 to rule-in biopsies for only men at highest risk would have avoided 179 biopsies (67%) maintained a 95% NPV value.

Clinically Useful
A test is clinically useful if the use of the results informs management decisions that improve the net health outcome of care. The net health outcome can be improved if patients receive correct therapy, more effective therapy, or avoid unnecessary therapy, or avoid unnecessary testing.

Direct Evidence
Direct evidence of clinical utility is provided by studies that have compared health outcomes for patients managed with and without the test. Because these are intervention studies, the preferred evidence would be from RCTs.

Clinical utility studies using MyProstate Score results for decision making for repeat biopsy or treatment have not been reported,

Chain of Evidence
Indirect evidence on clinical utility rests on clinical validity. If the evidence is insufficient to demonstrate test performance, no inferences can be made about clinical utility.

Because the clinical validity of MyProstate Score has not been established, a chain of evidence supporting the clinical utility of this test cannot be constructed.

Subsection Summary: MyProstate Score
One recent prospective study reports sensitivity and specificity data for the MyProstate Score test. No studies were found that directly show the effects of using MyProstate test results on clinical outcomes.

SUPPLEMENTAL INFORMATION
The purpose of the following information is to provide reference material. Inclusion does not imply endorsement or alignment with the evidence review conclusions.

Practice Guidelines and Position Statements
Guidelines or position statements will be considered for inclusion in 'Supplemental Information' if they were issued by, or jointly by, a US professional society, an international society with US representation, or National Institute for Health and Care Excellence (NICE). Priority will be given to guidelines that are informed by a systematic review, include strength of evidence ratings, and include a description of management of conflict of interest.

American Urological Association et al
In 2023, the American Urological Association (AUA) and the Society of Urologic Oncology (SUO) published updated guidelines on the early detection of prostate cancer. Specific guidance related to diagnosis, risk assessment, and utilization of biomarkers are stated in Table 40 below. [73,]

Table 40. Relevant AUA/SUO Guideline Statements on Prostate Cancer Screening and Biopsy

Guideline Statement	Evidence Grade and Strength
When screening for prostate cancer, clinicians should use PSA as the first screening test	Strong Recommendation; Evidence Level: Grade A
For people with a newly elevated PSA, clinicians should repeat the PSA prior to a secondary biomarker, imaging, or biopsy	Expert Opinion
Clinicians may use digital rectal exam (DRE) alongside PSA to establish risk of clinically significant prostate cancer	Conditional Recommendation; Evidence Level: Grade C
For people undergoing prostate cancer screening, clinicians should not use PSA velocity as the sole indication for a secondary biomarker, imaging, or biopsy	Strong Recommendation; Evidence Level: Grade B
Clinicians may use adjunctive urine or serum markers when further risk stratification would influence the decision regarding whether to proceed with biopsy.	Conditional Recommendation; Evidence Level: Grade C
After a negative biopsy, clinicians should not solely use a PSA threshold to decide whether to repeat the biopsy	Strong Recommendation; Evidence Level: Grade B
After a negative biopsy, clinicians may use blood-, urine-, or tissue-based biomarkers selectively for further risk stratification if results are likely to influence the decision	Conditional Recommendation; Evidence Level: Grade C

Guideline Statement	Evidence Grade and Strength
regarding repeat biopsy or otherwise substantively change the patient's management	
In patients with multifocal HGPIN [high-grade prostatic intraepithelial neoplasia], clinicians may proceed with additional risk evaluation, guided by PSA/DRE and mpMRI findings	Expert Opinion

DRE: digital rectal exam; PSA: prostate-specific antigen; mpMRI: multi-parametric magnetic resonance imaging

National Comprehensive Cancer Network

The National Comprehensive Cancer Network (NCCN) guidelines for prostate cancer early detection (v.2.2024) recommend that any man with a PSA level greater than 3 ng/mL undergo workup for benign disease, repeat PSA, and DRE (category 2A evidence).[74,]

The NCCN guidelines state that "biomarkers that improve the specificity of detection are not, as yet, mandated as first-line screening tests in conjunction with serum PSA. However, there may be some patients who meet PSA standards for consideration of prostate biopsy, but for whom the patient and/or the physician wish to further define risk". The guidelines recommend that the probability of high-grade cancer (Gleason score ≥3+4, Grade Group 2 or higher) may be further defined utilizing biomarkers that improve the specificity of screening that includes percent free PSA, with consideration of the Prostate Health Index (PHI), SelectMDx, 4K score, ExoDx Prostate Test , MyProstate Score (MPS), and IsoPSA. NCCN also noted that the extent of validation of these tests across diverse populations is variable and is not yet known how these tests could be applied in optimal combination with magnetic resonance imaging (MRI).

For men who had a negative biopsy but are thought to be at higher risk, NCCN recommends to consider biomarkers that improve the specificity of screening (category 2A evidence). Tests that should be considered in the post-biopsy setting include percent-free PSA, 4Kscore, PHI, PCA3, ConfirmMDx, ExoDx Prostate Test, MPS, and IsoPSA.

National Institute for Health and Care Excellence

In 2019 and in 2021, when guidelines were updated, the NICE guidelines did not recommend the Progensa PCA3 Assay or the PHI test for use in men with suspicion of prostate cancer who had a negative or inconclusive prostate biopsy. [75,]

U.S. Preventive Services Task Force Recommendations

The U.S. Preventive Services Task Force (2018) updated recommendations for prostate cancer screening. Genetic and protein biomarkers addressed in this evidence review, including *PCA3*, were not mentioned. [76,]

The U.S. Preventive Services Task Force advises individualized decision making about screening for prostate cancer after discussion with a clinician for men ages 55 to 69 (C recommendation) and recommends against PSA-based screening in men 70 and older (D recommendation). An update of these recommendations is pending.

Ongoing and Unpublished Clinical Trials

Some currently ongoing and unpublished trials that might influence this review are listed in Table 41.

Table 41. Summary of Key Trials

NCT No.	Trial Name	Planned Enrollment	Completion Date
Ongoing			
NCT04100811[a]	Validating the miR Scientific Sentinel™ Platform (Sentinel PCC4 Assay) in Men Undergoing Core Needle Biopsy Due to Suspicion of Prostate Cancer for Distinguishing Between no Cancer, Low-, Intermediate- and High-Risk Prostate Cancer	4000	Dec 2024
NCT04079699	Predicting Prostate Cancer Using a Panel of Plasma and Urine Biomarkers Combined in an Algorithm in Elderly Men Above 70 Years	700	Oct 2039
NCT05050084	Parallel Phase III Randomized Trials of Genomic-Risk Stratified Unfavorable Intermediate Risk Prostate Cancer: De-Intensification and Intensification Clinical Trial Evaluation (GUIDANCE)	2050	Apr 2037

NCT: national clinical trial.
[a] Denotes industry-sponsored or cosponsored trial.

CODING

The following codes for treatment and procedures applicable to this policy are included below for informational purposes. This may not be a comprehensive list of procedure codes applicable to this policy.

Inclusion or exclusion of a procedure, diagnosis or device code(s) does not constitute or imply member coverage or provider reimbursement. Please refer to the member's contract benefits in effect at the time of service to determine coverage or non-coverage of these services as it applies to an individual member.

The code(s) listed below are medically necessary ONLY if the procedure is performed according to the "Policy" section of this document.

CPT/HCPCS	
81313	PCA3/KLK3 (prostate cancer antigen 3 [non-protein coding]/kallikrein-related peptidase 3 [prostate specific antigen]) ratio (e.g., prostate cancer)
81539	Oncology (high-grade prostate cancer), biochemical assay of four proteins (Total PSA, Free PSA, Intact PSA, and human kallikrein-2 [hK2]), utilizing plasma or serum, prognostic algorithm reported as a probability score
81542	Oncology (prostate), MRNA, microarray gene expression profiling of 22 content genes, utilizing formalin-fixed paraffin-embedded tissue, algorithm reported as metastasis risk score
81551	Oncology (prostate), promoter methylation profiling by real-time PCR of 3 genes (GSTP1, APC, RASSF1), utilizing formalin-fixed paraffin-embedded tissue, algorithm reported as a likelihood of prostate cancer detection on repeat biopsy
86316	Immunoassay for tumor antigen, other antigen, quantitative (e.g., ca 50, 72-4, 549), each
0005U PLA for ExosomeDx® Prostate (IntelliScore) from Exosome Diagnostics, Inc	Oncology (prostate) gene expression profile by real-time RT-PCR of 3 genes (ERG, PCA3, and SPDEF), urine, algorithm reported as risk score
0021U PLA for Apifiny® by Armune Bioscience, Inc	Oncology (prostate), detection of 8 autoantibodies (ARF 6, NKX3-1, 5'-UTR-BMI1, CEP 164, 3'-UTR-Ropporin, Desmocollin, AURKAIP-1, CSNK2A2), multiplexed immunoassay and flow cytometry serum, algorithm reported as risk score
0047U PLA for Genomic Prostate Score® (GPS) Test, MDxHealth, Inc	Oncology (prostate), MRNA, gene expression profiling by real-time RT-PCR of 17 genes (12 content and 5 housekeeping), utilizing formalin-fixed paraffin-embedded tissue, algorithm reported as a risk score
0113U PLA for MyProstateScore, Lynx DX	Oncology (prostate), measurement of PCA3 and TMPRSS2-ERG in urine and PSA in serum following prostatic massage, by RNA amplification and fluorescence-based detection, algorithm reported as risk score
0228U PLA for PanGIA Prostate	Oncology (prostate), multianalyte molecular profile by photometric detection of macromolecules adsorbed on nanosponge array slides with machine

CPT/HCPCS	
	learning, utilizing first morning voided urine, algorithm reported as likelihood of prostate cancer
0339U PLA for SelectMDx for Prostate Cancer	Oncology (prostate), mRNA expression profiling of HOXC6 and DLX1, reverse transcription polymerase chain reaction (RT-PCR), first-void urine following digital rectal examination, algorithm reported as probability of high-grade cancer. SelectMDx for Prostate Cancer
0403U	Oncology (prostate), mRNA, gene expression profiling of 18 genes, first-catch urine, algorithm reported as percentage of likelihood of detecting clinically significant prostate cancer
0591U	Oncology (prostate cancer), biochemical analysis of 3 proteins (total PSA, free PSA, and HE4), plasma, serum, prognostic algorithm incorporating 3 proteins and digital rectal examination, results reported as a probability score for clinically significant prostate cancer

REVISIONS	
12-01-2011	Policy added to the bcbsks.com web site.
04-10-2012	In Coding section: Added HCPCS code: S3721 (effective 04-01-2012).
06-29-2012	Description section updated
	Rationale section updated
	References updated
01-01-2013	In Coding section: ▪ Added CPT code: 81479 (effective 01-01-2013) ▪ Removed CPT codes: 83890, 83891, 83892, 83893, 83894, 83896, 83897, 83898, 83900, 83901, 83902, 83903, 83904, 83905, 83906, 83907, 83908, 83909, 83912 (effective 12-31-2012)
08-20-2013	Description section reviewed with no changes made.
	Rationale section updated
	In Coding section: ▪ Coding instructions added.
	References updated
01-01-2015	Policy posted 01-16-2015
	In Coding section: ▪ Added CPT Codes: 81313 (Effective January 1, 2015)
01-01-2016	In Coding section: ▪ Removed HCPCS Code: S3721 (Effective January 1, 2016) ▪ Updated Coding notations.
01-20-2016	▪ Title revised to "Genetic and Protein Biomarkers for the Diagnosis and Cancer Risk Assessment of Prostate Cancer" from "Gene-Based Tests for Screening, Detection, and/or Management of Prostate Cancer" ▪ Added "See Also: Gene Expression Profiling and Protein Biomarkers for Prostate Cancer Management"
	Description section updated
	In Policy section: ▪ In Item A removed "Genetic tests for the screening, detection, and management" and "This includes, but is not limited to the following:" and added "The following genetic and protein biomarkers for the diagnosis" to read "The following genetic and protein

REVISIONS	
	biomarkers for the diagnosis of prostate cancer are considered experimental / investigational:" ▪ In Item A added the following E/I protein biomarkers: "1. Kallikrein markers (e.g., 4Kscore™ Test) 2. Metabolomic profiles (e.g., Prostarix™) 6. Mitochondrial DNA mutation testing (e.g., Prostate Core Mitomics Test™)" ▪ In Item A 3 added "testing" and removed "for disease diagnosis and prognosis" to read "PCA3 testing" ▪ In Item A 4 removed "for diagnosis and prognosis" to read "TMPRSS fusion genes" ▪ In Item A 5 added "Candidate" and removed "multiple gene tests" and "for prostate cancer diagnosis" to read "Candidate gene panels" ▪ In Item A 7 added "testing (e.g., ConfirmMDx®)" and removed "for diagnosis and prognosis" to read "Gene hypermethylation testing (e.g., ConfirmMDx®)" ▪ In Item A relocated "single-nucleotide polymorphisms (SNPs) for risk assessment" to stand-alone Item B to read "Single nucleotide polymorphisms (SNPs) testing for cancer risk assessment of prostate cancer is considered experimental / investigational."
	Rationale section updated
	In Coding section: ▪ Added CPT Codes: 81599, 0010M ▪ Updated Coding notations.
	References updated
12-20-2017	Description section updated
	In Policy section: ▪ In Item A added "Prostate Health Index (phi)" and removed "Metabolomic profiles (e.g., Prostarix™)" ▪ In Item B revised "polymorphisms (SNPs)" to "variant". ▪ Added Policy Guidelines – Information on Genetics Nomenclature Update and Genetic Counseling
	Rationale section updated
	In Coding section: ▪ Added CPT Codes: 81539 (Effective 01-01-2017), 81551 (Effective 01-01-2018) ▪ Removed CPT Code: 0010M (Terminated 01-01-2017) ▪ Coding notations updated
	References updated
07-01-2018	In Coding section: ▪ Added PLA Code: 0053U
02-27-2019	Description section updated
	In Policy section: ▪ In Item A added the following genetic and protein biomarkers "3. HOXC6 and DLX1 testing (e.g., SelectMDx) 4. PCA3, ERG, and SPDEF RNA expression in exosomes (e.g., ExoDx Prostate IntelliScore) 5. Autoantibodies ARF 6, NKX3-1, 5-UTR-BMI1, CEP 164, 3-UTR-Ropporin, Desmocollin, AURKAIP-1, and CSNK2A2 (e.g., Apifiny)" ▪ In Item A 6 added "(e.g., Progensa PCA3 Assay)" to read "PCA3 testing (e.g., Progensa PCA3 Assay)" ▪ In Item A 7 added "ERG" to read "TMPRSS: ERG fusion genes"
	Rationale section updated
	In Coding section ▪ Removed PLA Code: 0053U (This code does not apply to this policy) ▪ Add PLA Codes: 0005U, 0021U
	References updated

REVISIONS	
10-01-2019	In Coding section: ▪ Added PLA Code: 0113U
04-16-2021	Description section updated
	Rationale section updated
	In Coding section: Deleted CPT- 81479 Added CPT- 81542, 0047U, 0228U, and 86316
	References updated
05-07-2021	Description section updated
	In Policy section ▪ Added Item A.10
	Rationale section updated
	References updated
01-13-2022	Updated Description Section
	Updated Policy Section ▪ Section A7 Added (e.g., MyProstate Score)
	Updated Rationale Section
	Updated References Section
10-28-2022	Updated Coding Section ▪ Added Code 0339U (effective 10-01-2022)
01-30-2023	Updated Description Section
	Updated Rationale Section
	Update Coding Section ▪ Removed: 81599 ▪ Updated nomenclature for 0005U, 0021U, 0047U, 0113U, 0228U, and 0339U
	Update References Section
7-3-2023	Updated Coding Section ▪ 0113U updated nomenclature (eff. 7-1-2023) ▪ Removed ICD-10 Diagnoses box
10-02-2023	Updated Coding Section ▪ Added 0403U (eff. 10-01-2023)
01-05-2024	Updated Description Section
	Updated Policy Guidelines ▪ Removed Policy Guidelines "Genetic Counseling Experts recommend formal genetic counseling for individuals who are at risk for inherited disorders and who wish to undergo genetic testing. Interpreting the results of genetic tests and understanding risk factors can be difficult for some individuals; genetic counseling helps individuals understand the impact of genetic testing, including the possible effects the test results could have on the individual or their family members. It should be noted that genetic counseling may alter the utilization of genetic testing substantially and may reduce inappropriate testing; further, genetic counseling should be performed by an individual with experience and expertise in genetic medicine and genetic testing methods."
	Updated Rationale Section
	Updated References Section
07-01-2024	Updated Coding Section ▪ Updated nomenclature for 0047U (eff. 07-01-2024)
10-01-2024	Updated Coding Section ▪ Updated nomenclature for 0403U (eff. 10-01-2024)
12-23-2024	Updated Description Section

REVISIONS	
	Updated Rationale Section
	Updated References Section
01-13-2026	Updated Description
	Updated Policy Section ▪ Added Section A.11. MiCheck Prostate
	Updated Rationale Section
	Updated Coding Section ▪ Added: 0591U
	Updated Reference Section

REFERENCES

1. Howlader N, Noone AM, Krapcho M, et al. SEER Cancer Statistics Review, 1975-2014. Bethesda, MD: National Cancer Institute; 2017.
2. Odedina FT, Akinremi TO, Chinegwundoh F, et al. Prostate cancer disparities in Black men of African descent: a comparative literature review of prostate cancer burden among Black men in the United States, Caribbean, United Kingdom, and West Africa. Infect Agent Cancer. Feb 10 2009; 4 Suppl 1(Suppl 1): S2. PMID 19208207
3. Bell KJ, Del Mar C, Wright G, et al. Prevalence of incidental prostate cancer: A systematic review of autopsy studies. Int J Cancer. Oct 01 2015; 137(7): 1749-57. PMID 25821151
4. Gleason DF. Classification of prostatic carcinomas. Cancer Chemother Rep. Mar 1966; 50(3): 125-8. PMID 5948714
5. National Cancer Institute. SEER Database. https://seer.cancer.gov/seerinquiry/index.php?page=view&id=20170036&type=q. Accessed September 25, 2025.
6. Hoogendam A, Buntinx F, de Vet HC. The diagnostic value of digital rectal examination in primary care screening for prostate cancer: a meta-analysis. Fam Pract. Dec 1999; 16(6): 621-6. PMID 10625141
7. Gosselaar C, Roobol MJ, Roemeling S, et al. The role of the digital rectal examination in subsequent screening visits in the European randomized study of screening for prostate cancer (ERSPC), Rotterdam. Eur Urol. Sep 2008; 54(3): 581-8. PMID 18423977
8. Thompson IM, Pauler DK, Goodman PJ, et al. Prevalence of prostate cancer among men with a prostate-specific antigen level or =4.0 ng per milliliter. N Engl J Med. May 27 2004; 350(22): 2239-46. PMID 15163773
9. Catalona WJ, Smith DS, Ratliff TL, et al. Measurement of prostate-specific antigen in serum as a screening test for prostate cancer. N Engl J Med. Apr 25 1991; 324(17): 1156-61. PMID 1707140
10. Aus G, Bergdahl S, Lodding P, et al. Prostate cancer screening decreases the absolute risk of being diagnosed with advanced prostate cancer--results from a prospective, population-based randomized controlled trial. Eur Urol. Mar 2007; 51(3): 659-64. PMID 16934392
11. Buzzoni C, Auvinen A, Roobol MJ, et al. Metastatic Prostate Cancer Incidence and Prostate-specific Antigen Testing: New Insights from the European Randomized Study of Screening for Prostate Cancer. Eur Urol. Nov 2015; 68(5): 885-90. PMID 25791513
12. Arnsrud Godtman R, Holmberg E, Lilja H, et al. Opportunistic testing versus organized prostate-specific antigen screening: outcome after 18 years in the Göteborg randomized

population-based prostate cancer screening trial. Eur Urol. Sep 2015; 68(3): 354-60. PMID 25556937
13. Hugosson J, Carlsson S, Aus G, et al. Mortality results from the Göteborg randomised population-based prostate-cancer screening trial. Lancet Oncol. Aug 2010; 11(8): 725-32. PMID 20598634
14. Schröder FH, Hugosson J, Roobol MJ, et al. Screening and prostate-cancer mortality in a randomized European study. N Engl J Med. Mar 26 2009; 360(13): 1320-8. PMID 19297566
15. Wolf AM, Wender RC, Etzioni RB, et al. American Cancer Society guideline for the early detection of prostate cancer: update 2010. CA Cancer J Clin. 2010; 60(2): 70-98. PMID 20200110
16. Rosario DJ, Lane JA, Metcalfe C, et al. Short term outcomes of prostate biopsy in men tested for cancer by prostate specific antigen: prospective evaluation within ProtecT study. BMJ. Jan 09 2012; 344: d7894. PMID 22232535
17. Lavallée LT, Binette A, Witiuk K, et al. Reducing the Harm of Prostate Cancer Screening: Repeated Prostate-Specific Antigen Testing. Mayo Clin Proc. Jan 2016; 91(1): 17-22. PMID 26688045
18. Ruiz-Aragón J, Márquez-Peláez S. [Assessment of the PCA3 test for prostate cancer diagnosis: a systematic review and meta-analysis]. Actas Urol Esp. Apr 2010; 34(4): 346-55. PMID 20470697
19. Mackinnon AC, Yan BC, Joseph LJ, et al. Molecular biology underlying the clinical heterogeneity of prostate cancer: an update. Arch Pathol Lab Med. Jul 2009; 133(7): 1033-40. PMID 19642730
20. Partin AW, Brawer MK, Subong EN, et al. Prospective evaluation of percent free-PSA and complexed-PSA for early detection of prostate cancer. Prostate Cancer Prostatic Dis. Jun 1998; 1(4): 197-203. PMID 12496895
21. Thompson IM, Ankerst DP, Chi C, et al. Assessing prostate cancer risk: results from the Prostate Cancer Prevention Trial. J Natl Cancer Inst. Apr 19 2006; 98(8): 529-34. PMID 16622122
22. van Vugt HA, Roobol MJ, Kranse R, et al. Prediction of prostate cancer in unscreened men: external validation of a risk calculator. Eur J Cancer. Apr 2011; 47(6): 903-9. PMID 21163642
23. Rosenkrantz AB, Verma S, Choyke P, et al. Prostate Magnetic Resonance Imaging and Magnetic Resonance Imaging Targeted Biopsy in Patients with a Prior Negative Biopsy: A Consensus Statement by AUA and SAR. J Urol. Dec 2016; 196(6): 1613-1618. PMID 27320841
24. Mi C, Bai L, Yang Y, et al. 4Kscore diagnostic value in patients with high-grade prostate cancer using cutoff values of 7.5% to 10%: A meta-analysis. Urol Oncol. Jun 2021; 39(6): 366.e1-366.e10. PMID 33685800
25. Kawada T, Shim SR, Quhal F, et al. Diagnostic Accuracy of Liquid Biomarkers for Clinically Significant Prostate Cancer Detection: A Systematic Review and Diagnostic Meta-analysis of Multiple Thresholds. Eur Urol Oncol. Aug 2024; 7(4): 649-662. PMID 37981495
26. Russo GI, Regis F, Castelli T, et al. A Systematic Review and Meta-analysis of the Diagnostic Accuracy of Prostate Health Index and 4-Kallikrein Panel Score in Predicting Overall and High-grade Prostate Cancer. Clin Genitourin Cancer. Aug 2017; 15(4): 429-439.e1. PMID 28111174

27. Bhattu AS, Zappala SM, Parekh DJ, et al. A 4Kscore Cut-off of 7.5% for Prostate Biopsy Decisions Provides High Sensitivity and Negative Predictive Value for Significant Prostate Cancer. Urology. Feb 2021; 148: 53-58. PMID 33217456
28. Stattin P, Vickers AJ, Sjoberg DD, et al. Improving the Specificity of Screening for Lethal Prostate Cancer Using Prostate-specific Antigen and a Panel of Kallikrein Markers: A Nested Case-Control Study. Eur Urol. Aug 2015; 68(2): 207-13. PMID 25682340
29. Loeb S, Shin SS, Broyles DL, et al. Prostate Health Index improves multivariable risk prediction of aggressive prostate cancer. BJU Int. Jul 2017; 120(1): 61-68. PMID 27743489
30. Parekh DJ, Punnen S, Sjoberg DD, et al. A multi-institutional prospective trial in the USA confirms that the 4Kscore accurately identifies men with high-grade prostate cancer. Eur Urol. Sep 2015; 68(3): 464-70. PMID 25454615
31. Konety B, Zappala SM, Parekh DJ, et al. The 4Kscore® Test Reduces Prostate Biopsy Rates in Community and Academic Urology Practices. Rev Urol. 2015; 17(4): 231-40. PMID 26839521
32. Pecoraro V, Roli L, Plebani M, et al. Clinical utility of the (-2)proPSA and evaluation of the evidence: a systematic review. Clin Chem Lab Med. Jul 01 2016; 54(7): 1123-32. PMID 26609863
33. Anyango R, Ojwando J, Mwita C, et al. Diagnostic accuracy of [-2]proPSA versus Gleason score and Prostate Health Index versus Gleason score for the determination of aggressive prostate cancer: a systematic review. JBI Evid Synth. Jun 2021; 19(6): 1263-1291. PMID 33741840
34. Catalona WJ, Partin AW, Sanda MG, et al. A multicenter study of [-2]pro-prostate specific antigen combined with prostate specific antigen and free prostate specific antigen for prostate cancer detection in the 2.0 to 10.0 ng/ml prostate specific antigen range. J Urol. May 2011; 185(5): 1650-5. PMID 21419439
35. Tosoian JJ, Druskin SC, Andreas D, et al. Use of the Prostate Health Index for detection of prostate cancer: results from a large academic practice. Prostate Cancer Prostatic Dis. Jun 2017; 20(2): 228-233. PMID 28117387
36. White J, Shenoy BV, Tutrone RF, et al. Clinical utility of the Prostate Health Index (phi) for biopsy decision management in a large group urology practice setting. Prostate Cancer Prostatic Dis. Apr 2018; 21(1): 78-84. PMID 29158509
37. Sanda MG, Feng Z, Howard DH, et al. Association Between Combined TMPRSS2:ERG and PCA3 RNA Urinary Testing and Detection of Aggressive Prostate Cancer. JAMA Oncol. Aug 01 2017; 3(8): 1085-1093. PMID 28520829
38. Tomlins SA, Day JR, Lonigro RJ, et al. Urine TMPRSS2:ERG Plus PCA3 for Individualized Prostate Cancer Risk Assessment. Eur Urol. Jul 2016; 70(1): 45-53. PMID 25985884
39. Ankerst DP, Goros M, Tomlins SA, et al. Incorporation of Urinary Prostate Cancer Antigen 3 and TMPRSS2:ERG into Prostate Cancer Prevention Trial Risk Calculator. Eur Urol Focus. Jan 2019; 5(1): 54-61. PMID 29422418
40. Tosoian JJ, Trock BJ, Morgan TM, et al. Use of the MyProstateScore Test to Rule Out Clinically Significant Cancer: Validation of a Straightforward Clinical Testing Approach. J Urol. Mar 2021; 205(3): 732-739. PMID 33080150
41. Newcomb LF, Zheng Y, Faino AV, et al. Performance of PCA3 and TMPRSS2:ERG urinary biomarkers in prediction of biopsy outcome in the Canary Prostate Active Surveillance Study (PASS). Prostate Cancer Prostatic Dis. Sep 2019; 22(3): 438-445. PMID 30664734

42. Van Neste L, Hendriks RJ, Dijkstra S, et al. Detection of High-grade Prostate Cancer Using a Urinary Molecular Biomarker-Based Risk Score. Eur Urol. Nov 2016; 70(5): 740-748. PMID 27108162
43. McKiernan J, Donovan MJ, O'Neill V, et al. A Novel Urine Exosome Gene Expression Assay to Predict High-grade Prostate Cancer at Initial Biopsy. JAMA Oncol. Jul 01 2016; 2(7): 882-9. PMID 27032035
44. Tutrone R, Donovan MJ, Torkler P, et al. Clinical utility of the exosome based ExoDx Prostate(IntelliScore) EPI test in men presenting for initial Biopsy with a PSA 2-10 ng/mL. Prostate Cancer Prostatic Dis. Dec 2020; 23(4): 607-614. PMID 32382078
45. Tutrone R, Lowentritt B, Neuman B, et al. ExoDx prostate test as a predictor of outcomes of high-grade prostate cancer - an interim analysis. Prostate Cancer Prostatic Dis. Sep 2023; 26(3): 596-601. PMID 37193776
46. Schipper M, Wang G, Giles N, et al. Novel prostate cancer biomarkers derived from autoantibody signatures. Transl Oncol. Apr 2015; 8(2): 106-11. PMID 25926076
47. Shore ND, Pieczonka CM, Henderson RJ, et al. Development and evaluation of the MiCheck test for aggressive prostate cancer. Urol Oncol. Aug 2020; 38(8): 683.e11-683.e18. PMID 32305266
48. Gillatt D, Polikarpov D, Smith I, Lau H, Kim L, Huynh CC, et al. MP17-07VALIDATION OF MICHECK PROSTATE FOR SIGNIFICANT PROSTATE CANCER IN AN AUSTRALIAN POPULATION. Journal of Urology [Internet]. 2023 Apr 1; 209(Supplement 4):e215.
49. Wysock JS, Becher E, Persily J, et al. Concordance and Performance of 4Kscore and SelectMDx for Informing Decision to Perform Prostate Biopsy and Detection of Prostate Cancer. Urology. Jul 2020; 141: 119-124. PMID 32294481
50. Cui Y, Cao W, Li Q, et al. Evaluation of prostate cancer antigen 3 for detecting prostate cancer: a systematic review and meta-analysis. Sci Rep. May 10 2016; 6: 25776. PMID 27161545
51. Rodríguez SVM, García-Perdomo HA. Diagnostic accuracy of prostate cancer antigen 3 (PCA3) prior to first prostate biopsy: A systematic review and meta-analysis. Can Urol Assoc J. May 2020; 14(5): E214-E219. PMID 31793864
52. Nicholson A, Mahon J, Boland A, et al. The clinical effectiveness and cost-effectiveness of the PROGENSA® prostate cancer antigen 3 assay and the Prostate Health Index in the diagnosis of prostate cancer: a systematic review and economic evaluation. Health Technol Assess. Oct 2015; 19(87): i-xxxi, 1-191. PMID 26507078
53. Wei JT, Feng Z, Partin AW, et al. Can urinary PCA3 supplement PSA in the early detection of prostate cancer?. J Clin Oncol. Dec 20 2014; 32(36): 4066-72. PMID 25385735
54. Hennenlotter J, Neumann T, Alperowitz S, et al. Age-Adapted Prostate Cancer Gene 3 Score Interpretation - Suggestions for Clinical Use. Clin Lab. Mar 01 2020; 66(3). PMID 32162868
55. Vickers AJ, Gupta A, Savage CJ, et al. A panel of kallikrein marker predicts prostate cancer in a large, population-based cohort followed for 15 years without screening. Cancer Epidemiol Biomarkers Prev. Feb 2011; 20(2): 255-61. PMID 21148123
56. Ruffion A, Devonec M, Champetier D, et al. PCA3 and PCA3-based nomograms improve diagnostic accuracy in patients undergoing first prostate biopsy. Int J Mol Sci. Aug 29 2013; 14(9): 17767-80. PMID 23994838
57. Ruffion A, Perrin P, Devonec M, et al. Additional value of PCA3 density to predict initial prostate biopsy outcome. World J Urol. Aug 2014; 32(4): 917-23. PMID 24500192

58. Merdan S, Tomlins SA, Barnett CL, et al. Assessment of long-term outcomes associated with urinary prostate cancer antigen 3 and TMPRSS2:ERG gene fusion at repeat biopsy. Cancer. Nov 15 2015; 121(22): 4071-9. PMID 26280815
59. Djavan B, Waldert M, Zlotta A, et al. Safety and morbidity of first and repeat transrectal ultrasound guided prostate needle biopsies: results of a prospective European prostate cancer detection study. J Urol. Sep 2001; 166(3): 856-60. PMID 11490233
60. Lujan M, Paez A, Santonja C, et al. Prostate cancer detection and tumor characteristics in men with multiple biopsy sessions. Prostate Cancer Prostatic Dis. 2004; 7(3): 238-42. PMID 15289810
61. Stewart GD, Van Neste L, Delvenne P, et al. Clinical utility of an epigenetic assay to detect occult prostate cancer in histopathologically negative biopsies: results of the MATLOC study. J Urol. Mar 2013; 189(3): 1110-6. PMID 22999998
62. Partin AW, Van Neste L, Klein EA, et al. Clinical validation of an epigenetic assay to predict negative histopathological results in repeat prostate biopsies. J Urol. Oct 2014; 192(4): 1081-7. PMID 24747657
63. Waterhouse RL, Van Neste L, Moses KA, et al. Evaluation of an Epigenetic Assay for Predicting Repeat Prostate Biopsy Outcome in African American Men. Urology. Jun 2019; 128: 62-65. PMID 29660369
64. Van Neste L, Partin AW, Stewart GD, et al. Risk score predicts high-grade prostate cancer in DNA-methylation positive, histopathologically negative biopsies. Prostate. Sep 2016; 76(12): 1078-87. PMID 27121847
65. Partin AW, VAN Criekinge W, Trock BJ, et al. CLINICAL EVALUATION OF AN EPIGENETIC ASSAY TO PREDICT MISSED CANCER IN PROSTATE BIOPSY SPECIMENS. Trans Am Clin Climatol Assoc. 2016; 127: 313-327. PMID 28066067
66. Food and Drug Administration. Summary of Safety and Effectiveness Data. PMA P090026. Quantitative test for determination of [-2]proPSA levels. Silver Spring, MD: Food and Drug Administration; 2012.
67. Aubry W, Lieberthal R, Willis A, et al. Budget impact model: epigenetic assay can help avoid unnecessary repeated prostate biopsies and reduce healthcare spending. Am Health Drug Benefits. Jan 2013; 6(1): 15-24. PMID 24991343
68. Robinson K, Creed J, Reguly B, et al. Accurate prediction of repeat prostate biopsy outcomes by a mitochondrial DNA deletion assay. Prostate Cancer Prostatic Dis. Jun 2010; 13(2): 126-31. PMID 20084081
69. Legisi L, DeSa E, Qureshi MN. Use of the Prostate Core Mitomic Test in Repeated Biopsy Decision-Making: Real-World Assessment of Clinical Utility in a Multicenter Patient Population. Am Health Drug Benefits. Dec 2016; 9(9): 497-502. PMID 28465777
70. Leyten GH, Hessels D, Smit FP, et al. Identification of a Candidate Gene Panel for the Early Diagnosis of Prostate Cancer. Clin Cancer Res. Jul 01 2015; 21(13): 3061-70. PMID 25788493
71. Xiao K, Guo J, Zhang X, et al. Use of two gene panels for prostate cancer diagnosis and patient risk stratification. Tumour Biol. Aug 2016; 37(8): 10115-22. PMID 26820133
72. Tosoian JJ, Sessine MS, Trock BJ, et al. MyProstateScore in men considering repeat biopsy: validation of a simple testing approach. Prostate Cancer Prostatic Dis. Sep 2023; 26(3): 563-567. PMID 36585434
73. Wei JT, Barocas D, Carlsson S, et al. Early Detection of Prostate Cancer: AUA/SUO Guideline Part I: Prostate Cancer Screening. J Urol. Jul 2023; 210(1): 46-53. PMID 37096582

74. National Comprehensive Cancer Network (NCCN). NCCN Clinical Practice Guidelines in Oncology: prostate cancer early detection V.2.2025. https://www.nccn.org/professionals/physician_gls/pdf/prostate_detection.pdf. Accessed September 25, 2025.
75. National Institute for Health and Care Excellence (NICE). Prostate cancer: diagnosis and management [NG131]. 2019. Updated December 15, 2021; https://www.nice.org.uk/guidance/ng131/chapter/Recommendations#assessment-and-diagnosis. Accessed September 25, 2025.
76. U. S. Preventive Services Task Force. Prostate Cancer: Screening. 2018; https://www.uspreventiveservicestaskforce.org/uspstf/draft-update-summary/prostate-cancer-screening-adults. Accessed September 25, 2025.