2.04.33	Genetic and Protein Biomarkers for the Diagnosis and Cancer Risk Assessment of Prostate Cancer		
Original Policy Date:	July 6, 2012	**Effective Date:**	June 1, 2023
Section:	2.0 Medicine	**Page:**	Page 1 of 48

Policy Statement

Note: Starting on July 1, 2022 (per CA law SB 535) for commercial plans regulated by the California Department of Managed Healthcare and California Department of Insurance (PPO, and HMO), health care service plans and insurers shall not require prior authorization for biomarker testing, including biomarker testing for cancer progression and recurrence, if a member has stage 3 or 4 cancer. Health care service plans and insurers can still do a medical necessity review of a biomarker test and possibly deny coverage after biomarker testing has been completed and a claim is submitted (post service review).

I. The following genetic and protein biomarkers for the diagnosis of prostate cancer are considered **investigational**:
   A. Autoantibodies ARF 6, NKX3-1, 5' -UTR-BMI1, CEP 164, 3' -UTR-Ropporin, Desmocollin, AURKAIP-1, and CSNK2A2 (e.g., Apifiny)
   B. Candidate gene panels
   C. Gene hypermethylation testing (e.g., ConfirmMDx®)
   D. *HOXC6* and *DLX1* testing (e.g., SelectMDx)
   E. Kallikrein markers (e.g., 4Kscore® Test)
   F. Mitochondrial DNA variant testing (e.g., Prostate Core Mitomics Test™)
   G. PanGIA Prostate
   H. *PCA3* testing (e.g., Progensa PCA3 Assay)
   I. PCA3, ERG, and SPDEF RNA expression in exosomes (e.g., ExoDx Prostate IntelliScore)
   J. Prostate Health Index (phi)
   K. *TMPRSS:ERG* fusion genes (e.g., MyProstate Score)

II. Single nucleotide variant testing for cancer risk assessment of prostate cancer is considered **investigational**.

NOTE: Refer to Appendix A to see the policy statement changes (if any) from the previous version.

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.

Coding

There are specific CPT codes for PCA3 and 4Kscore™ testing:

- **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

The following CPT code is specific to the ConfirmMDx® test:

- **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

The following CPT code would be used for the ExoDx Prostate Intelliscore:

- **0005U**: Oncology (prostate) gene expression profile by real-time RT-PCR of 3 genes (ERG, PCA3, and SPDEF), urine, algorithm reported as risk score

The following CPT code would be used for the Apifiny test:

- **0021U**: 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

The following CPT PLA code would be used for MiPS (Mi-Prostate Score):

- **0113U**: 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

Effective October 1, 2022, the following CPT code would be used for SelectMDx for Prostate Cancer by MDxHealth, Inc.

- **0339U:** 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

Effective October 1, 2022, the following CPT code would be used for miR Sentinel™ Prostate Cancer Test, miR Scientific, LLC.

- **0343U:** Oncology (prostate), exosome-based analysis of 442 small noncoding RNAs (sncRNAs) by quantitative reverse transcription polymerase chain reaction (RT-qPCR), urine, reported as molecular evidence of no-, low-, intermediate- or high-risk of prostate cancer

Other than codes listed in the Codes table, for the other types of testing mentioned, in this policy, there are no specific CPT codes. The unlisted molecular pathology code 81479 would be used. If the test includes multiple assays, uses an algorithmic analysis, and is reported as a numeric score or a probability, the unlisted multianalyte assay with algorithmic analysis (MAAA) code 81599 would be reported.

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. Testing to determine cancer aggressiveness after a tissue diagnosis of cancer is addressed in Blue Shield of California Medical Policy: Gene Expression Profiling and Protein Biomarkers for Prostate Cancer Management.

Related Policies

- Gene Expression Profiling and Protein Biomarkers for Prostate Cancer Management

Benefit Application

Benefit determinations should be based in all cases on the applicable contract language. To the extent there are any conflicts between these guidelines and the contract language, the contract language will control. 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.

Some state or federal mandates (e.g., Federal Employee Program [FEP]) prohibits plans from denying Food and Drug Administration (FDA)-approved technologies as investigational. In these instances, plans may have to consider the coverage eligibility of FDA-approved technologies on the basis of medical necessity alone.

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™), 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.

Rationale

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 age 55 and 60% of men age 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	9-10	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.

Literature Review

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 (e.g., bleeding or infection requiring hospitalization) have estimated rates ranging from less than 1% to 3%.[16,17]

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 question addressed in this evidence review is: Does the use of testing for genetic protein biomarkers improve the net health outcome in men being considered for an initial prostate 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 (e.g., 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.[18] 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 (e.g. Progensa)	• Hologic Gen-Probe • Many labs offer PCA3 tests (e.g., ARUP Laboratories, Mayo Medical Laboratories, LabCorp)	Measures *PCA3* mRNA in urine samples after prostate massage. *PCA3* mRNA may be normalized using PSA level to account for prostate cells.
PanGIA Prostate	Genetics Institute of America	Analysis of a signature of small molecules, proteins, and cells with a proprietary machine learning algorithm.

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

Prostate-specific kallikreins (e.g., 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 (e.g., 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.[19,] 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.[20,]

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 (e.g., 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.

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.[21,]

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[22,] and the Rotterdam Prostate Cancer risk calculator (also known as the ERSPC-Risk Calculator 4 [ERSPC-RC]).[23,] 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.[24,]

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 4K score to detect high grade prostate cancer using cutoff values of 7.5% to 10%.[25,] Pooled analysis found acceptable diagnostic accuracy (see Table 4). However, significant heterogeneity among the included studies lowered confidence in the results.

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,]	Observational cohort 10	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)[25,]	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)

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)	59-92	28-64	4.6 (3.5 to 6.1)	
Russo et al (2017)[26,](subgroup analysis)	10	NR	Diagnostic performance for high-grade prostate cancer	87 (85 to 89)	61 (60 to 62)	8-43	95-99	10.2 (8.1 to 12.8)	
Mi et al (2021)[25,]	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)

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

Prospective Studies

Two prospective validation studies of the 4Kscore test conducted in different populations have been published (Tables 5 and 6).

The performance of the 4Kscore test was validated in 1012 patients enrolled in a blinded, prospective study of all patients scheduled for a prostate biopsy at 26 urology centers in the U.S. (Tables 5 and 6). As reported by Parekh et al (2015),[27,] biopsies were negative in 54% (n=542) of cases, and showed low-grade (all Gleason grade 6) prostatic cancer in 24% (n=239) and high-grade cancer in 23% (n=231) of cases. Statistical analysis of 4Kscore test clinical data had an area under the receiving operating curve of 0.82 for the detection of high-grade prostate cancer; the area under the receiving operating curve for the PCPT risk calculator model was 0.74, but a precision estimate was not given.

Punnen et al (2018) reported on a second prospective validation study of the 4Kscore test conducted at 8 US Veterans Affairs hospitals from July 2015 to October 2016 (Tables 5 and 6).[28,] One aim of the study was to evaluate test performance in African American men; of 366 men enrolled and evaluated, 205 (56%) were African American. In a comparative analysis, there was no difference in test performance in African American and non-African American men (P =.32).

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. [29,]

Tables 7 and 8 summarize relevance and design and conduct limitations for each study. A major limitation of the validation studies 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%).[30,]

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
Parekh et al (2015)[27,] (U.S.)	Patients scheduled for a prostate biopsy independent of age, PSA level, DRE, or prior prostate biopsy	Prospective, 26 U.S. centers	Prostate biopsy with ≥10 cores	Blood sample taken prior to biopsy	Yes	247 (24%) men had an abnormal DRE, 348 (34%) had PSA level <4 ng/mL, and 104 (10%) had PSA level >10 ng/mL 8.4% of men were African American
Punnen et al (2018)[28,]	Men who were referred for prostate biopsy	Prospective, 8 Veterans Affairs hospitals in the US	Prostate biopsy with ≥10 cores	Blood sample taken prior to biopsy	Yes	61 (17%) men had an abnormal DRE No exclusions for PSA level- % with PSA below 4 or over 10 not reported- median was 7.1 (interquartile range 5.3 to 10.0) 56% of men were African American
Bhattu et al (2021)[29,]	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	Same as above	Same as above	Same as above	

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*		
Parekh et al (2015)[27] (U.S.)	1012		AUC=0.82 (0.79 to 0.85)	PCPT Risk model without intact PSA and hK2 • AUC=0.75 (0.71 to 0.79)		PCPT modified risk calculator • AUC=0.74 (NR)
Punnen et al (2018)[28]	403	366	All patients: AUC=0.81 (0.77 to 0.86) African American men vs non-African American men: AUC = 0.80 (0.74 to 0.86) vs : 0.84 (.78 to.91); P =.32	Base model AUC = 0.74 (0.69 to 0.79); P <.01 vs 4K score		
Bhattu et al (2021)[29]			**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	• • • •	• 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]
Parekh et al (2015)[27] (U.S.)	4. Study population included patients outside of the indeterminate range of PSA				
Punnen et al (2018)[28]	4. Study population included patients outside of the indeterminate range of PSA				
Bhattu et al (2021)[29]					

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]
Parekh et al (2015)[27,] (U.S.)						1. Study did not provide confidence intervals of validity vs the standard clinical models
Punnen et al (2018)[28,]						
Bhattu et al (2021)[29,]				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 (i.e., 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 4K score compared to the Rotterdam Prostate Cancer Risk Calculator.[31,] 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=0.41). 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.

Subsection Summary: Clinically Valid

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.

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.[27] 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.[32] 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.

Section Summary: Kallikreins Biomarkers and 4Kscore Test
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.

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)[33] 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 included studies in men of any age with any range of PSA levels and reported results according to different cutoffs.[34]

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.[33,] Russo et al (2017) included 23 studies that were mostly prospective and rated as moderate quality.[26,] There was high heterogeneity across studies but pooled estimates showed generally low NPV (5% to 63%) and low specificity (25% to 35%) when sensitivity was 90% to 93% (Table 10).

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)[33,]	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)[34,]	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

PCa: prostate cancer; 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)[33,]	17/6912 (63-1091)	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)	76-98	15-63	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)	88-99	5-31	3.5 (2.5 to 5.0)
Anyango et al (2021)[34,]	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)			

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 phi.[31,] The population for this retrospective analysis included 728 men from the prospective multicenter clinical trial of phi (Catalona et al, 2011).[35,] 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).

Subsection Summary: Clinically Valid

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 35% and NPV, which would indicate an absence of disease and allow patients to forego biopsy, ranged from 5% to 63%. For high-grade disease, the sensitivity of the phi test was 93%, with a NPV ranging from 5% to 31%.

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 of 345 men who underwent phi testing compared with 1318 men who did not.[36,] 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.[37,] 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.

Section Summary: proPSA and Prostate Health Index

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.[38,] 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).[39,] 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.[40,] 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.[41,] 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).[42,] 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)[38,]	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)[39,]	1244 men who had initial (80%)	7-center prospective	Any cancer or HG cancer		Samples collected after DRE	Yes	A MiPS score threshold was not provided, so

Study; Trial	Study Population	Design	Reference Standard	Threshold for Positive Index Test	Timing of Reference and Index Tests	Blinding of Assessors	Comment
	or repeat biopsy due to elevated PSA		(Gleason score ≥7)		and prior to biopsy		sensitivity and NPV were not calculated

DRE: digital rectal exam; HG; high-grade; MiPS: Mi-Prostate Score; NPV: negative predictive value; 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)[38,]	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)				
			Excluded Samples	PSA Alone	PCPT Risk Calculator	PSA Plus *PCA3*	MiPS	
Tomlins et al (2016)[39,]	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: *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)[38,]	4. Some patients were 70 y, 16% had an abnormal DRE; median PSA level was 4.8 ng/mL				
Tomlins et al (2016)[39,]	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		

The study limitations stated in this table are those notable in the current review; this is not a comprehensive gaps assessment.
DRE: digital rectal exam; PCPT: Prostate Cancer Prevention Trial; 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 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)[38,]						
Tomlins et al (2016)[39,]						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.[38,] 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.[39,] 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.

Section 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 *TMPRSS*2*-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

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 15).[43] 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 16). Limitations of this study is the inclusion of men with an abnormal DRE (Tables 17 and 18), 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).

Development and validation studies on a revised risk model that included HOXC6 and DLX1 expression along with patient age, DRE, and PSA density in men undergoing initial biopsy was reported by Haese et al (2019).[44] The new analysis included data from the Dutch patients in the report by Van Neste et al (2016) along with additional cohorts from France and Germany. In the validation cohort of men with all PSA levels, the AUC was 0.82 with 89% sensitivity and 53% specificity. The PCPTRC AUC was 0.76. Since some clinicians will proceed to biopsy when there is a positive DRE, results were also calculated for patients who had a PSA <10 ng/ml and a negative DRE. For this cohort (n=591), the AUC was 0.80 with sensitivity of 84% and specificity of 57%. Comparison with the PCPTRC in this subgroup was not reported.

Hendriks et al (2021) evaluated the SelectMDx test to detect high-grade prostate cancer in biopsy-naive men.[45] In total, 599 men in the Netherlands with PSA level of 3 ng/mL or greater scheduled for their initial biopsy were included in the study. All subjects underwent a multi-parametric magnetic resonance imaging (MRI) test and biopsy after urine sample and DRE were complete. The primary outcome was the detection rates of low- and high-grade prostate cancer and the number of biopsies avoided in 4 distinct diagnostic strategies: (1) SelectMDx test only, (2) MRI only, (3) SelectMDx test followed by MRI when SelectMDx test was positive (conditional strategy), and (4) SelectMDx and MRI in all (joint strategy). Decision curve analysis was performed to assess clinical utility. Overall, prevalence of high-grade prostate cancer was 31% (183/599). Thirty-eight percent of patients had negative SelectMDx tests in whom biopsy could be avoided. Decision curve analysis showed the highest net benefit for the MRI only strategy, followed by the conditional strategy at risk thresholds over 10%. Investigators also found that SelectMDx test led to 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.

Table 15. 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)[43,]	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
Haese et al (2019)[44,]	916 men scheduled for initial biopsy, of whom 715 had PSA < 10 ng/ml. The new analysis included participants in the Van Neste et al (2016) study.	Prospective	Prostate cancer on biopsy		Urine sample taken after DRE and prior to biopsy	
Hendriks et al (2021)[45,]	599 men ages 50 to 75 years, with PSA level $\geq$3 ng/mL scheduled for initial biopsy in hospitals in the Netherlands	Prospective	Prostate cancer on biopsy	Positive SelectMDx test outcome was a risk score of $\geq$-2.8 (corresponding with percent liklihood of 13% that subsequent biopsy would identify high-grade prostate cancer)	Urine sample taken after DRE and prior to biopsy	Yes

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

Table 16. 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)[43,]	386	264		0.90 (0.85 to 0.96)	0.78 (0.68 to 0.88)	0.66 (0.57 to 0.75)	.001

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)				
Haese et al (2019)[44,]	916	715	591	0.80 (0.76 to 0.85) for the 591 patients		0.76 for all 916 patients		
Hendriks et al (2021)[45,]	599	NR, median PSA, 6.4 ng/mL (IQR, 5.0 to 8.7)	NR, normal DRE in 72% of patients	**Biopsy avoidance, % (n)**	**NPV, % (95% CI)**	**PPV, % (95% CI)**	**Sens, % (95% CI)**	**Spec, % (95% CI)**
SelectMDx only				38 (227)	92 (0.88 to 0.95)	44 (0.39 to 0.50)	90 (0.85 to 0.94)	50 (0.45 to 0.55)
MRI only				49 (295)	97 (0.94 to 0.99)	57 (0.51 to 0.63)	95 (0.91 to 0.98)	69 (0.64 to 0.73)
Conditional strategy				60 (357)	93 (0.90 to 0.96)	66 (0.59 to 0.72)	87 (0.81 to 0.91)	80 (0.76 to 0.84)
Joint strategy				28 (165)	98 (0.95 to 0.99)	41 (0.37 to 0.46)	98 (0.95 to 0.99)	39 (0.34 to 0.44)

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 17. Study Relevance Limitations

Study	Population[a]	Intervention[b]	Comparator[c]	Outcomes[d]	Duration of Follow-Up[e]
Van Neste et al (2016)[43,]	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			
Haese et al (2019)[44,]	4. The study was conducted in Europe and not representative of the U.S. population		3. Comparison with %fPSA and PCPTR was not reported for the subgroup of interest of men with a PSA < 10 ng/ml and negative DRE		

Study	Population[a]	Intervention[b]	Comparator[c]	Outcomes[d]	Duration of Follow-Up[e]
Hendriks et al (2021)[45,]	3. Number of patients with appropriate target PSA levels was not defined; 4. The study was conducted in the Netherlands and not representative of the U.S. population				

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 18. 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)[43,]		1. Blinding not reported			1. Inadequate description of indeterminate samples	
Haese et al (2019)[44,]		1. Blinding not reported			1. Inadequate description of indeterminate samples	
Hendriks et al (2021)[45,]						

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%.[43,]

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.

Section Summary: SelectMDx for Prostate Cancer

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 19).[46] 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 20). Diagnostic performance is shown in Table 20, with sensitivity of 97% and NPV of 96%.

Table 19. 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)[46]	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 20. 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)[46]	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 21 and 22 summarize relevance and design and conduct limitations in each study.

Table 21. Study Relevance Limitations

Study	Population[a]	Intervention[b]	Comparator[c]	Outcomes[d]	Duration of Follow-Up[e]
McKiernan et al (2016)[46]	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 22. 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)[46]			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 23 through 26)[47]. Thise 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 of 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.

Table 23. Summary of Key RCT Characteristics

Study; Trial	Countries	Sites	Dates	Participants	Interventions	
					Active	*Comparator*
Tutrone et al (2020)[47]	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.

Table 24. 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)[47,]				
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%)

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

Table 25. Study Relevance Limitations

Study	Population[a]	Intervention[b]	Comparator[c]	Outcomes[d]	Follow-Up[e]
Tutrone et al (2020)[47,]	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 26. Study Design and Conduct Limitations

Study	Allocation[a]	Blinding[b]	Selective Reporting[c]	Data Completeness[d]	Power[e]	Statistical[f]
Tutrone et al (2020)[47,]	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.

Section 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.[48] When the algorithm was applied to an independent validation set, the AUC was 0.69 (95% CI, 0.62 to 0.75).

Section 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

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 (e.g., 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 27. 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. Cui et al (2016) reported on results of a systematic review of case-control or cohort studies.[50,] The studies assessed both initial and repeat biopsy and had a quality rating of moderate to high. 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 28. 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. 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 27. 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
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 28. 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
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%)	• Pooled: 73%	0.75 (0.74 to 0.77)

Study	Studies	N (Range)	Outcomes	Sens (95% CI), %	Spec (95% CI), %	AUC (95% CI) or Range
				Range: 47% to 95%	(72% to 74%) • Range: 22%-100%	
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 29 and 30).[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.[40] 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)[42] 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 29. 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 30. 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 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.

Section Summary: PCA3 Score (e.g., 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 in the moderate range (e.g., 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 question addressed in this evidence review is: Does the use of testing for genetic protein biomarkers improve the net health outcome in men being considered for a repeat prostate 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 31.

Table 31. 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 (e.g., 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, such as Life Technologies, LabCorp (23andme), and ARUP Laboratories (deCODE)	Panel tests for prostate cancer risk are offered as laboratory-developed tests

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, review of the history of PSA values, along with 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.[21,]

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[22,] and the ERSPC-RC.[23,] 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.[24]

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 32 and 33).[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 32. 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 prostate biopsy core tissue samples from 498 men from the U.K. and Belgium	Retrospective ConfirmMDx performed on first biopsy	Repeat biopsy	NR	<30 mo	Yes	73% of men had benign 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 33. 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	68 (57 to 77)	64 (59 to 69)		90 (87 to 93)

Study; Trial	Initial N	Final N	Excluded Samples	Prevalence of Condition	Clinical Validity (95% CI), %			
				negative second biopsy (controls)				
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 34 and 35 summarize the relevance and design and conduct limitations in each study.

Table 34. 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 35. 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)[33]	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 unneccessary 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.

Section 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 unneccessary 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.

Section 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 36 and 37).[71,] Validation of this panel is needed.

Table 36. 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 37. 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 unneccessary 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.

Section 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.

Summary of Evidence

For individuals who are being considered for an initial prostate biopsy who receive testing for genetic and protein biomarkers of prostate cancer (e.g., kallikreins biomarkers and 4Kscore Test, proPSA and Prostate Health Index, TMPRSS fusion genes and MyProstateScore, SelectMDx for Prostate Cancer, ExoDx Prostate, Apifiny, PCA3 score, and PanGIA Prostate), the evidence includes systematic reviews, meta-analyses, and primarily observational studies. Relevant outcomes are overall survival, disease-specific survival, test validity, resource utilization, and quality of life. The evidence supporting clinical utility varies by the test but has not been directly shown for any biomarker test. Absent direct evidence of clinical utility, a chain of evidence might be constructed. However, the performance of biomarker testing for directing biopsy referrals is uncertain. While some studies have shown a reduction or delay in biopsy based on testing, a chain of evidence for clinical utility cannot be constructed due to limitations in clinical validity. Test validation populations have included men with a positive DRE, a PSA level outside of the gray zone (between 3 or 4 ng/mL and 10 ng/mL), or older men for whom the information from test results are less likely to be informative. Many biomarker tests do not have standardized cutoffs to recommend a biopsy. In addition, comparative studies of the many biomarkers are lacking. The evidence is insufficient to determine that the technology results in an improvement in the net health outcome.

For individuals who are being considered for repeat biopsy who receive testing for genetic and protein biomarkers of prostate cancer (e.g., PCA3 score, Gene Hypermethylation and ConfirmMDx test, Prostate Core Mitomics Test), the evidence includes systematic reviews and meta-analyses and primarily observational studies. Relevant outcomes are overall survival, disease-specific survival, test validity, resource utilization, and quality of life. The performance of biomarker testing for guiding rebiopsy decisions is lacking. The tests are associated with a diagnosis of prostate cancer and aggressive prostate cancer, but studies on clinical validity are limited and do not compare performance characteristics with standard risk prediction models. Direct evidence supporting clinical utility has not been shown. No data are currently available on physician decisions on rebiopsy or on the longer-term clinical outcomes of men who did not have a biopsy based on test results. The evidence is insufficient to determine that the technology results in an improvement in the net health outcome.

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

The American Urological Association (AUA, 2013; confirmed 2018) published guidelines on the early detection of prostate cancer.[72,] The AUA concluded that:
"the literature supporting the efficacy of digital rectal exam (DRE), PSA [prostate-specific antigen] derivatives and isoforms (e.g. free PSA, -2proPSA, prostate health index, hK2 [human kallikrein 2], PSA velocity or PSA doubling time) and novel urinary markers and biomarkers (e.g. PCA3) for screening with the goal of reducing prostate cancer mortality provide limited evidence to draw conclusions. While some data suggest use of these secondary screening tools may reduce unnecessary biopsies (i.e. reduce harms) while maintaining the ability to detect aggressive prostate cancer (i.e. maintain the benefits of PSA screening), more research is needed to confirm this."

National Comprehensive Cancer Network

The National Comprehensive Cancer Network (NCCN) guidelines (v.1.2022) recommend that any man with a PSA level greater than 3 ng/mL undergo workup for benign disease, repeat PSA, and DRE.[73,]

The guidelines recommend as part of the workup for benign disease, consider 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 InteliScore (EPI), MyProstate Score (MPS), and IsoPSA in patients who have not yet had a biopsy. NCCN noted that these tests may be especially useful in men with PSA levels between 3 ng/mL and 10 ng/mL. NCCN also noted that it 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, EPI , MPS, IsoPSA, and ConfirmMDx.

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. [74,]

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. [75,]

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).

Medicare National Coverage

There is no national coverage determination. In the absence of a national coverage determination, coverage decisions are left to the discretion of local Medicare carriers.

Ongoing and Unpublished Clinical Trials

Some currently ongoing and unpublished trials that might influence this review are listed in Table 38.

Table 38. Summary of Key Trials

NCT No.	Trial Name	Planned Enrollment	Completion Date
Ongoing			
NCT00773773	A Study to Assess if a Combination of Serum Measurements of Molecular Biomarkers and Serum Protein Profiling Can be Used to Predict Which Patients Undergoing Prostatic Biopsy Will be Diagnosed With Cancer	500	Oct 2022
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 2023
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
Unpublished			

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

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Documentation for Clinical Review

- No records required

Coding

This Policy relates only to the services or supplies described herein. Benefits may vary according to product design; therefore, contract language should be reviewed before applying the terms of the Policy.

The following codes are included below for informational purposes. Inclusion or exclusion of a code(s) does not constitute or imply member coverage or provider reimbursement policy. Policy Statements are intended to provide member coverage information and may include the use of some codes for clarity. The Policy Guidelines section may also provide additional information for how to interpret the Policy Statements and to provide coding guidance in some cases.

Type	Code	Description
CPT®	0005U	Oncology (prostate) gene expression profile by real-time RT-PCR of 3 genes (ERG, PCA3, and SPDEF), urine, algorithm reported as risk score
	0053U	Oncology (prostate cancer), FISH analysis of 4 genes (ASAP1, HDAC9, CHD1 and PTEN), needle biopsy specimen, algorithm reported as probability of higher tumor grade
	0021U	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	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	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	Oncology (prostate), multianalyte molecular profile by photometric detection of macromolecules adsorbed on nanosponge array slides with machine learning, utilizing first morning voided urine, algorithm reported as likelihood of prostate cancer
	0339U	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 ***(Code effective 10/1/2022)***
	0343U	Oncology (prostate), exosome-based analysis of 442 small noncoding RNAs (sncRNAs) by quantitative reverse transcription polymerase chain reaction (RT-qPCR), urine, reported as molecular evidence of no-, low-, intermediate- or high-risk of prostate cancer ***(Code effective 10/1/2022)***
	0376U	Oncology (prostate cancer), image analysis of at least 128 histologic features and clinical factors, prognostic algorithm determining the risk of distant metastases, and prostate cancer-specific mortality, includes predictive algorithm to androgen deprivation-therapy response ***(Code effective 4/1/2023)***

Type	Code	Description
	81313	PCA3/KLK3 (prostate cancer antigen 3 [non-protein coding]/kallikrein-related peptidase 3 [prostate specific antigen]) ratio (e.g., prostate cancer)
	81479	Unlisted molecular pathology procedure
	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
	81599	Unlisted multianalyte assay with algorithmic analysis
	84153	Prostate specific antigen (PSA); total
	84154	Prostate specific antigen (PSA); free
	86316	Immunoassay for tumor antigen, other antigen, quantitative (e.g., CA 50, 72-4, 549), each
HCPCS	None	

Policy History

This section provides a chronological history of the activities, updates and changes that have occurred with this Medical Policy.

Effective Date	Action
07/06/2012	BCBSA Medical Policy adoption
02/22/2013	Coding update
01/01/2015	Coding update
06/30/2015	Coding update
09/30/2015	Policy title change from Gene Based Tests for Screening, Detection and Management of Prostate Cancer Policy revision without position change
01/01/2016	Coding update
04/01/2016	Coding update
12/01/2016	Policy revision without position change
12/01/2017	Policy revision without position change
01/01/2018	Policy revision without position change
08/01/2018	Coding update
01/01/2019	Policy revision without position change
02/01/2020	Annual review. No change to policy statement. Literature review updated. Coding update.
01/01/2021	Annual review. No change to policy statement. Literature review updated. Coding update.
05/01/2021	Policy statement and literature updated.
02/01/2022	Annual review. Policy statement and literature updated.
11/01/2022	Coding update

Effective Date	Action
02/01/2023	Annual review. No change to policy statement. Policy guidelines and literature review updated.
06/01/2023	Coding update

Definitions of Decision Determinations

Medically Necessary: Services that are Medically Necessary include only those which have been established as safe and effective, are furnished under generally accepted professional standards to treat illness, injury or medical condition, and which, as determined by Blue Shield, are: (a) consistent with Blue Shield medical policy; (b) consistent with the symptoms or diagnosis; (c) not furnished primarily for the convenience of the patient, the attending Physician or other provider; (d) furnished at the most appropriate level which can be provided safely and effectively to the patient; and (e) not more costly than an alternative service or sequence of services at least as likely to produce equivalent therapeutic or diagnostic results as to the diagnosis or treatment of the Member's illness, injury, or disease.

Investigational/Experimental: A treatment, procedure, or drug is investigational when it has not been recognized as safe and effective for use in treating the particular condition in accordance with generally accepted professional medical standards. This includes services where approval by the federal or state governmental is required prior to use, but has not yet been granted.

Split Evaluation: Blue Shield of California/Blue Shield of California Life & Health Insurance Company (Blue Shield) policy review can result in a split evaluation, where a treatment, procedure, or drug will be considered to be investigational for certain indications or conditions, but will be deemed safe and effective for other indications or conditions, and therefore potentially medically necessary in those instances.

Prior Authorization Requirements and Feedback (as applicable to your plan)

Within five days before the actual date of service, the provider must confirm with Blue Shield that the member's health plan coverage is still in effect. Blue Shield reserves the right to revoke an authorization prior to services being rendered based on cancellation of the member's eligibility. Final determination of benefits will be made after review of the claim for limitations or exclusions.

Questions regarding the applicability of this policy should be directed to the Prior Authorization Department at (800) 541-6652, or the Transplant Case Management Department at (800) 637-2066 ext. 3507708 or visit the provider portal at www.blueshieldca.com/provider.

We are interested in receiving feedback relative to developing, adopting, and reviewing criteria for medical policy. Any licensed practitioner who is contracted with Blue Shield of California or Blue Shield of California Promise Health Plan is welcome to provide comments, suggestions, or concerns. Our internal policy committees will receive and take your comments into consideration.

For utilization and medical policy feedback, please send comments to: MedPolicy@blueshieldca.com

Disclaimer: This medical policy is a guide in evaluating the medical necessity of a particular service or treatment. Blue Shield of California may consider published peer-reviewed scientific literature, national guidelines, and local standards of practice in developing its medical policy. Federal and state law, as well as contract language, including definitions and specific contract provisions/exclusions, take precedence over medical policy and must be considered first in determining covered services. Member contracts may differ in their benefits. Blue Shield reserves the right to review and update policies as appropriate.

Appendix A

<table>
<tr><th colspan="2">POLICY STATEMENT
(No changes)</th></tr>
<tr><th>BEFORE</th><th>AFTER</th></tr>
<tr>
<td>Genetic and Protein Biomarkers for the Diagnosis and Cancer Risk Assessment of Prostate Cancer 2.04.33

Policy Statement:
Note: Starting on July 1, 2022 (per CA law SB 535) for commercial plans regulated by the California Department of Managed Healthcare and California Department of Insurance (PPO, and HMO), health care service plans and insurers shall not require prior authorization for biomarker testing, including biomarker testing for cancer progression and recurrence, if a member has stage 3 or 4 cancer. Health care service plans and insurers can still do a medical necessity review of a biomarker test and possibly deny coverage after biomarker testing has been completed and a claim is submitted (post service review).

I. The following genetic and protein biomarkers for the diagnosis of prostate cancer are considered investigational:
A. Autoantibodies ARF 6, NKX3-1, 5' -UTR-BMI1, CEP 164, 3' -UTR-Ropporin, Desmocollin, AURKAIP-1, and CSNK2A2 (e.g., Apifiny)
B. Candidate gene panels
C. Gene hypermethylation testing (e.g., ConfirmMDx®)
D. HOXC6 and DLX1 testing (e.g., SelectMDx)
E. Kallikrein markers (e.g., 4Kscore® Test)
F. Mitochondrial DNA variant testing (e.g., Prostate Core Mitomics Test™)
G. PanGIA Prostate
H. PCA3 testing (e.g., Progensa PCA3 Assay)
I. PCA3, ERG, and SPDEF RNA expression in exosomes (e.g., ExoDx Prostate IntelliScore)
J. Prostate Health Index (phi)
K. TMPRSS:ERG fusion genes (e.g., MyProstate Score)

II. Single nucleotide variant testing for cancer risk assessment of prostate cancer is considered investigational.</td>
<td>Genetic and Protein Biomarkers for the Diagnosis and Cancer Risk Assessment of Prostate Cancer 2.04.33

Policy Statement:
Note: Starting on July 1, 2022 (per CA law SB 535) for commercial plans regulated by the California Department of Managed Healthcare and California Department of Insurance (PPO, and HMO), health care service plans and insurers shall not require prior authorization for biomarker testing, including biomarker testing for cancer progression and recurrence, if a member has stage 3 or 4 cancer. Health care service plans and insurers can still do a medical necessity review of a biomarker test and possibly deny coverage after biomarker testing has been completed and a claim is submitted (post service review).

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A. Autoantibodies ARF 6, NKX3-1, 5' -UTR-BMI1, CEP 164, 3' -UTR-Ropporin, Desmocollin, AURKAIP-1, and CSNK2A2 (e.g., Apifiny)
B. Candidate gene panels
C. Gene hypermethylation testing (e.g., ConfirmMDx®)
D. HOXC6 and DLX1 testing (e.g., SelectMDx)
E. Kallikrein markers (e.g., 4Kscore® Test)
F. Mitochondrial DNA variant testing (e.g., Prostate Core Mitomics Test™)
G. PanGIA Prostate
H. PCA3 testing (e.g., Progensa PCA3 Assay)
I. PCA3, ERG, and SPDEF RNA expression in exosomes (e.g., ExoDx Prostate IntelliScore)
J. Prostate Health Index (phi)
K. TMPRSS:ERG fusion genes (e.g., MyProstate Score)

II. Single nucleotide variant testing for cancer risk assessment of prostate cancer is considered investigational.</td>
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