Policy Statement

The following genetic and protein biomarkers for the diagnosis of prostate cancer are considered investigational:

- Autoantibodies ARF 6, NKX3-1, 5’-UTR-BMI1, CEP 164, 3’-UTR-Ropporin, Desmocollin, AURKAI-P-1, and CSNK2A2 (e.g., Apifiny)
- Candidate gene panels
- Gene hypermethylation testing (e.g., ConfirmMDx®)
- HOXC6 and DLX1 testing (e.g., SelectMDx)
- Kallikrein markers (e.g., 4Kscore® Test)
- Mitochondrial DNA variant testing (e.g., Prostate Core Mitomics Test™)
- PCA3 testing (e.g., Progensa PCA3 Assay)
- PCA3, ERG, and SPDEF RNA expression in exosomes (e.g., ExoDx Prostate IntelliScore)
- Prostate Health Index (phi)
- TMPRSS: ERG fusion genes

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

Policy Guidelines

Genetics Nomenclature Update

The Human Genome Variation Society nomenclature is used to report information on variants found in DNA and serves as an international standard in DNA diagnostics. It is being implemented for genetic testing medical evidence review updates starting in 2017 (see Table PG1). The Society’s nomenclature is recommended by the Human Variome Project, the Human Genome Organization, and by the Human Genome Variation Society itself.

The American College of Medical Genetics and Genomics and the Association for Molecular Pathology standards and guidelines for interpretation of sequence variants represent expert opinion from both organizations, in addition to the College of American Pathologists. These recommendations primarily apply to genetic tests used in clinical laboratories, including genotyping, single genes, panels, exomes, and genomes. Table PG2 shows the recommended standard terminology: “pathogenic,” “likely pathogenic,” “variant of uncertain significance,” “likely benign,” and “benign” - to describe variants identified that cause Mendelian disorders.

Table PG1. Nomenclature to Report on Variants Found in DNA

<table>
<thead>
<tr>
<th>Previous</th>
<th>Updated</th>
<th>Definition</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mutation</td>
<td>Disease-associated variant</td>
<td>Disease-associated change in the DNA sequence</td>
</tr>
<tr>
<td></td>
<td>Disease-associated change in the DNA sequence</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Familial variant</td>
<td>Disease-associated variant identified in a proband for use in subsequent targeted genetic testing in first-degree relatives</td>
</tr>
</tbody>
</table>

Table PG2. ACMG-AMP Standards and Guidelines for Variant Classification

<table>
<thead>
<tr>
<th>Variant Classification</th>
<th>Definition</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pathogenic</td>
<td>Disease-causing change in the DNA sequence</td>
</tr>
<tr>
<td>Likely pathogenic</td>
<td>Likely disease-causing change in the DNA sequence</td>
</tr>
<tr>
<td>Variant of uncertain significance</td>
<td>Change in DNA sequence with uncertain effects on disease</td>
</tr>
<tr>
<td>Likely benign</td>
<td>Likely benign change in the DNA sequence</td>
</tr>
<tr>
<td>Benign</td>
<td>Benign change in the DNA sequence</td>
</tr>
</tbody>
</table>

ACMG: American College of Medical Genetics and Genomics; AMP: Association for Molecular Pathology.
**Genetic Counseling**

Experts recommend formal genetic counseling for patients 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 patients; 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 specific 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

**Effective January 1, 2018**, 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

**Effective October 1, 2019**, there is a new CPTPLA 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

Other than codes listed in the Codetable, 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 the 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. Magnetic resonance imaging-targeted biopsy of suspicious lesions is assessed in Blue Shield of California Medical Policy: Magnetic Resonance Imaging—Targeted Biopsy of the Prostate.
**Related Policies**

- Gene Expression Profiling and Protein Biomarkers for Prostate Cancer Management
- Magnetic Resonance Imaging–Targeted Biopsy of the Prostate

**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. Laboratories that offer laboratory-developed tests must be licensed under the Clinical Laboratory Improvement Amendments for high-complexity testing. The following laboratories are certified under the Clinical Laboratory Improvement Amendments: 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 this test.

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 one 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, with a predicted 174,650 incidence cases and 31,620 deaths expected in the U.S. in 2019.¹
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%. 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. 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, 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. It is an architectural grading system ranging from 1 (well-differentiated) to 5 (poorly-differentiated); 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. A cross-walk of these grading systems is shown in Table 1.

<table>
<thead>
<tr>
<th>Grade Group</th>
<th>Gleason Score (Primary and Secondary Pattern)</th>
<th>Cells</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>6 or less</td>
<td>Well-differentated (low grade)</td>
</tr>
<tr>
<td>2</td>
<td>7 (3+4)</td>
<td>Moderately differentiated (moderate grade)</td>
</tr>
<tr>
<td>3</td>
<td>7 (4+3)</td>
<td>Poorly differentiated (high grade)</td>
</tr>
<tr>
<td>4</td>
<td>8</td>
<td>Undifferentiated (high grade)</td>
</tr>
<tr>
<td>5</td>
<td>9-10</td>
<td>Undifferentiated (high grade)</td>
</tr>
</tbody>
</table>

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
This evidence review has been updated regularly with searches of the MEDLINE database.

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.

DRE has relatively low interrater agreement among urologists, with estimated sensitivity, specificity, and positive predictive value (PPV) for diagnosis of prostate cancer of 59%, 94%, and 28%, respectively.8 DRE might have a higher PPV in the setting of elevated PSA.9

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 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.10,11 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.12-16 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%.17

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%.18,19

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 PICOTS were used to select literature to inform this review.

Patients
The relevant population 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.20 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 ten 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 Candidate for Initial Prostate Biopsy

<table>
<thead>
<tr>
<th>Test</th>
<th>Manufacturer</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>4Kscore</td>
<td>OPKO lab</td>
<td>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.</td>
</tr>
<tr>
<td>Prostate Health Index (phi)</td>
<td>Beckman Coulter</td>
<td>Blood assay that combines several components of PSA (total PSA, free PSA, 21, proPSA) in an algorithm that includes patient age.</td>
</tr>
<tr>
<td>Mi-Prostate (MiPS)</td>
<td>University of Michigan MLabs</td>
<td>Measures TMPRSS2-ERG gene fusion and calculates a probability score that incorporates serum PSA or the PCPT, and urine TMPRSS2-ERG and PCA3 scores.</td>
</tr>
<tr>
<td>SelectMDx</td>
<td>MDxHealth</td>
<td>Three-gene post-DRE urinary panel for HOXC6 and DLX1.</td>
</tr>
<tr>
<td>ExoDx Prostate IntelliScore (EPI)</td>
<td>Exome Diagnostics</td>
<td>Urine panel for PCA3, ERG, and SPDEF RNA expression in exosomes.</td>
</tr>
<tr>
<td>Apifiny</td>
<td>Amune BioScience (acquired by Exact Sciences in 2017)</td>
<td>Algorithm with detection of 8 autoantibodies (ARF 6, NXX3-1, 5'-UTR-BMI1, CEP 164, 3'-UTR-Rpporin, Desmocollin, AURKAIP-1, CSNK2A2) in serum.</td>
</tr>
<tr>
<td>PCA3score (e.g., Progensa)</td>
<td>Hologic Gen-Probe</td>
<td>Measures PCA3 mRNA in urine samples after prostate massage. PCA3 mRNA may be normalized using PSA level to account for prostate cells.</td>
</tr>
</tbody>
</table>

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 four 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 four days, who have received 5α reductase inhibitor therapy in the previous six months, or who have undergone treatment for symptomatic benign prostatic hypertrophy in the previous six months.

The Prostate Health Index (phi; Beckman Coulter) is an assay that combines results of three blood serum immunoassays (total PSA, free PSA, 21, proPSA [p2PSA]) numerically to produce a “phi score.” This score is calculated with the phi algorithm using the following formula: (21, 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 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., Mi-Prostate) 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. 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 or rearrangement.

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 (ARF6, NKX3-1, 5'-UTR-BMI1, CEP164, 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.

**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.

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 and the Rotterdam Prostate Cancer risk calculator (also known as the ERSPC-Risk Calculator 4 [ERSPC-RC]). The American Urological Association and the Society of Abdominal Radiology (2016) 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.

**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.
Timing
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).

Setting
Initial screening using PSA levels and DRE may be performed in the primary care setting with referral to specialists (urologists) for suspicious findings and biopsy. Clinical practice on screening methods and frequency varies widely.

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 reference standard was unclear, did not adequately describe the patient characteristics or did not adequately describe patient selection criteria.

Technically Reliable
Assessment of technical reliability focuses on specific tests and operators and requires a review of unpublished and often proprietary information. Review of specific tests, operators, and unpublished data are outside the scope of this evidence review and alternative sources exist. This evidence review focuses on the clinical validity and clinical utility.

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).

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 (see Table 3). Results of the DRE were not described. The NPV to exclude any type of cancer ranged from 28% to 64% (see Table 4). The NPV of the 4Kscore test to exclude high-grade (Gleason score ≥7) cancer ranged from 95% to 99%.

<table>
<thead>
<tr>
<th>Study</th>
<th>Studies</th>
<th>Dates</th>
<th>Key Inclusion Criteria</th>
<th>Design</th>
<th>Reference Studies</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>Russo et al (2017)</td>
<td>10</td>
<td>2010-2015</td>
<td>Blood samples were collected before biopsy; indication for biopsy was independent of 4K results</td>
<td>Mostly prospective, observational</td>
<td>Biopsy for prostate cancer detection (overall or high grade with Gleason score ≥7)</td>
<td>PSA range, 2-20 ng/mL</td>
</tr>
</tbody>
</table>

PSA: prostate-specific antigen.
Table 4. Results of Systematic Reviews Assessing the Clinical Validity of 4Kscore for Diagnosing Prostate Cancer

<table>
<thead>
<tr>
<th>Study</th>
<th>N (Range)</th>
<th>Outcomes</th>
<th>Sens (95% CI), %</th>
<th>Spec (95% CI), %</th>
<th>PPV Range %</th>
<th>NPV Range %</th>
<th>OR (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Russo et al (2017)28</td>
<td>10 NR</td>
<td>Diagnostic performance for any prostate cancer</td>
<td>74 (73 to 76)</td>
<td>60 (59 to 61)</td>
<td>59-92</td>
<td>28-64</td>
<td>4.6</td>
</tr>
<tr>
<td>Russo et al (2017)28</td>
<td>10 NR</td>
<td>Diagnostic performance for high-grade prostate cancer</td>
<td>87 (85 to 89)</td>
<td>61 (60 to 62)</td>
<td>8-43</td>
<td>95-99</td>
<td>10.2</td>
</tr>
</tbody>
</table>

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

Prospective Studies

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. (see Tables 5 and 6). As reported by Parekh et al (2015)29, 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.

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 17000 Swedish men, estimated that, for men ages 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% CI, 0.64% to 4.66%).

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

<table>
<thead>
<tr>
<th>Study</th>
<th>Study Population</th>
<th>Design</th>
<th>Reference Standard</th>
<th>Timing of Reference and Index Tests</th>
<th>Blinding of Assessors</th>
<th>Comment</th>
</tr>
</thead>
<tbody>
<tr>
<td>Parekh et al (2015)29</td>
<td>Patients scheduled for a prostate biopsy independent of age, PSA level, DRE, or prior prostate biopsy</td>
<td>Prospective, 26 U.S. centers</td>
<td>Prostate biopsy with ≥10 cores</td>
<td>Blood sample taken prior to biopsy</td>
<td>Yes</td>
<td>247 (24%) men had an abnormal DRE, 348 (34%) had PSA level &lt;4 ng/mL, and 104 (10%) had PSA level &gt;10 ng/mL</td>
</tr>
</tbody>
</table>

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

Table 6. Clinical Validity Studies Assessing the 4Kscore Test

<table>
<thead>
<tr>
<th>Study</th>
<th>Initial N</th>
<th>Final N</th>
<th>4Kscore</th>
<th>Performance Characteristics (95% CI)</th>
<th>Comparators</th>
</tr>
</thead>
<tbody>
<tr>
<td>Parekh et al (2015)29</td>
<td>1012</td>
<td>AUC = 0.82 (0.79 to 0.85)</td>
<td>• PCPT Risk model without intact PSA and hK2</td>
<td>• PCPT modified risk calculator</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>• AUC = 0.75 (0.71 to 0.79)</td>
<td>• AUC = 0.74 (NR)</td>
<td></td>
</tr>
</tbody>
</table>

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

The purpose of the limitations tables (see Tables 7 and 8) is to display notable limitations identified in each study. This information is synthesized as a summary of the body of evidence following each table and provides the conclusions on the sufficiency of the evidence supporting the position statement.
The study limitations stated in this table are those notable in the current review; this is not a comprehensive limitations 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).

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. The cohort included 2872 men with PSA > 3.0 from the European Randomized Study of Screening for Prostate Cancer Rotterdam. The 4K panel was measured in frozen serum samples. The 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 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 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, or 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 three probability score were used in decision-curve analyses to estimate the number of biopsies vs 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. 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. All 611 patients of participating urologists to whom men were referred for 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%-19.9%, high-risk: ≥20%) correlated highly (p<0.001) 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 positive DRE in the validation studies are likely, not reflective of the intended use population. The chain of evidence is incomplete.

**proPSA and Prostate Health Index**
**Clinically Valid**
A test must detect the presence or absence of a condition, the risk of developing a condition in the future, or treatment response (beneficial or adverse).
Systematic Reviews
Several systematic reviews and meta-analyses have evaluated the clinical validity of p2PSA (proPSA) and phi tests. The characteristics of the most comprehensive reviews from 2016 and 2017 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) restricted eligibility to studies including PSA levels between 2 ng/mL and 10 ng/mL, while Russo et al (2017) restricted eligibility to studies including PSA levels between 2 ng/mL and 20 ng/mL.

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. Russo et al (2017) included 23 studies that were mostly prospective and rated as moderate quality. 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% (see Table 10).

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

<table>
<thead>
<tr>
<th>Study</th>
<th>Studies Included</th>
<th>Dates</th>
<th>Key Inclusion Criteria</th>
<th>Design</th>
<th>Reference Studies Included</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pecoraro et al (2016)</td>
<td>17</td>
<td>2003-2014</td>
<td>PSA level 2-10 ng/mL</td>
<td>Prospective, retrospective, and mixed prospective/retrospective, observational</td>
<td></td>
</tr>
<tr>
<td>Russo et al (2017)</td>
<td>23</td>
<td>2010-2015</td>
<td>Blood samples were collected before biopsy; PSA level 2-20 ng/mL; indication for biopsy was independent of phi results</td>
<td>Mostly prospective, observational</td>
<td>Biopsy for prostate cancer detection (overall or high grade with Gleason score ≥7)</td>
</tr>
</tbody>
</table>

PSA: prostate-specific antigen.

* 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

<table>
<thead>
<tr>
<th>Study</th>
<th>Studies/N (Range)</th>
<th>Outcomes</th>
<th>Sens (95% CI), %</th>
<th>Spec (95% CI), %</th>
<th>PPV Range, %</th>
<th>NPV Range, %</th>
<th>OR (95% CI), %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pecoraro et al (2016)</td>
<td>17/6912 (63-1091)</td>
<td>Diagnostic performance for any prostate cancer</td>
<td>Set at 90</td>
<td>Phi: 31 (29 to 33)</td>
<td>76-98</td>
<td>15-63</td>
<td>4.4 (3.3 to 5.8)</td>
</tr>
<tr>
<td>Russo et al (2017)</td>
<td>23</td>
<td>Diagnostic performance for any prostate cancer</td>
<td>89 (88 to 90)</td>
<td>34 (32 to 35)</td>
<td>76-98</td>
<td>15-63</td>
<td>4.4 (3.3 to 5.8)</td>
</tr>
<tr>
<td>Russo et al (2017)</td>
<td>7</td>
<td>Diagnostic performance for high-grade prostate cancer</td>
<td>93 (90 to 95)</td>
<td>26 (25 to 28)</td>
<td>88-99</td>
<td>5-31</td>
<td>3.5 (2.5 to 5.0)</td>
</tr>
<tr>
<td>Summary</td>
<td></td>
<td></td>
<td>88 to 95</td>
<td>25 to 35</td>
<td>76-99</td>
<td>5-63</td>
<td></td>
</tr>
</tbody>
</table>

CI: confidence interval; NPV: negative predictive value; OR: odds ratio; 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. The population for this retrospective analysis included 728 men from the prospective multicenter clinical trial of phi (Catalona et al [2011]). The probability of aggressive prostate cancer was evaluated at each value of phi from 1 to 100. The addition of phi to the PCPT2.0 risk calculator increased the AUC for the discrimination of aggressive prostate cancer.
cancer from 0.575 to 0.696 (p<0.001), while the addition of phi to the ERSPC 4 plus DRE risk
calculator increased the AUC from 0.650 to 0.711 (p=0.014).

**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 forgo biopsy, ranged from 5% to 63% For high-grade disease, the sensitivity of the phi test was 93% with an 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, or 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. 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. 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 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 Mi-Prostate 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).

Validation studies on the combined 2-gene test (TMPRSS2-ERG and PCA3) are shown in Table 11. Sanda et al (2017), from the National Cancer Institute Early Detection Research Network,
reported separate developmental and validation cohorts for high-grade prostate cancer in men undergoing initial prostate biopsy.\(^{37}\) For the validation cohort, any of the following was considered a positive result: PSA level greater than 10 ng/mL, urine \text{TMPRSS2-ERG} score greater than 8, or urine \text{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 0.04 (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 (see Table 11).\(^{38}\) Thresholds were not defined and the AUCs for predicting any cancer using PSA alone, PCPT risk calculator alone, or the Mi-Prostate Score (MiPS) alone are shown in Table 12. The AUC for MiPS was significantly improved compared with the PCPT risk calculator \((p<0.001)\). However, a study by Ankerst et al (2019) found that adding \text{TMPRSS2-ERG} to a PCPT risk calculator plus \text{PCA3} did not improve the AUC.\(^{39}\) The online PCPT risk calculator now includes both the \text{PCA3} and \text{TMPRSS2-ERG} scores, which will be used for further validation.

### Table 11. Characteristics of Studies Assessing the Clinical Validity of the Combined \text{TMPRSS2-ERG} and \text{PCA3} Score

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

DRE: digital rectal exam; HG: high-grade; MiPS: Mi-Prostate Score; NPV: negative predictive value; PSA: prostate-specific antigen; \text{T2:ERG}: \text{TMPRSS2-ERG}.

### Table 12. Results of Studies Assessing the Clinical Validity of the Combined \text{TMPRSS2-ERG} and \text{PCA3} Score

<table>
<thead>
<tr>
<th>Study; Trial</th>
<th>Initial N</th>
<th>Final N</th>
<th>Threshold</th>
<th>Sens (95% CI)</th>
<th>Spec (95% CI)</th>
<th>PPV (95% CI)</th>
<th>NPV (95% CI)</th>
<th>p(^a)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sanda et al (2017)(^{37}) PSA level, ng/mL</td>
<td>561</td>
<td>561</td>
<td>3</td>
<td>91.2 (\text{(86.6 to 95.8)})</td>
<td>16.7 (\text{(13.1 to 20.3)})</td>
<td>28.2 (\text{(28.9 to 29.5)})</td>
<td>84.1 (\text{(75.1 to 90.3)})</td>
<td></td>
</tr>
<tr>
<td>PCA3</td>
<td>7</td>
<td>96.6 (\text{93.7 to 99.5)})</td>
<td>18.4 (\text{91.2 to 22.1)})</td>
<td>29.8 (\text{28.6 to 30.9)})</td>
<td>93.8 (\text{86.2 to 97.3)})</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PCA3, \text{T2:ERG}</td>
<td>20, 8</td>
<td>90.5 (\text{85.8 to 95.2)})</td>
<td>35.4 (\text{30.8 to 40.0)})</td>
<td>33.4 (\text{31.5 to 35.4)})</td>
<td>91.2 (\text{86.1 to 94.6)})</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PSA level &gt;10 ng/mL; \text{T2:ERG} score &gt;8; or \text{PCA3} score &gt;20</td>
<td>Excluded Samples</td>
<td>PSA Alone</td>
<td>PCPT Risk Calculator Plus PCA3</td>
<td>MiPS</td>
<td>AUC (95% CI not reported)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tomlins et al (2016)(^{38}) 1244</td>
<td>1225</td>
<td>19 with insufficient samples for analysis</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Any cancer</td>
</tr>
</tbody>
</table>

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### Table 13. Relevance Limitations

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

The study limitations stated in this table are those notable in the current review; this is not a comprehensive limitations assessment.

\(^a\) Population key: 1. Intended use population unclear; 2. Clinical context is unclear; 3. Study population is unclear; 4. Study population not representative of intended use.

\(^b\) Intervention key: 1. Classification thresholds not defined; 2. Version used unclear; 3. Not intervention of interest.

\(^c\) Comparator key: 1. Classification thresholds not defined; 2. Not compared to credible reference standard; 3. Not compared to other tests in use for same purpose.

\(^d\) Outcomes key: 1. Study does not directly assess a key health outcome; 2. Evidence chain or decision model not explicated; 3. Key clinical validity outcomes not reported (sensitivity, specificity, and predictive values); 4. Reclassification of diagnostic or risk categories not reported; 5. Adverse events of the test not described (excluding minor discomforts and inconvenience of venipuncture or noninvasive tests).

\(^e\) Follow-Up key: 1. Follow-up duration not sufficient with respect to natural history of disease (true-positives, true-negatives, false-positives, false-negatives cannot be determined).

### Table 14. Study Design and Conduct Limitations

<table>
<thead>
<tr>
<th>Study</th>
<th>Selection(^a)</th>
<th>Blinding(^b)</th>
<th>Delivery of Test</th>
<th>Selective Reporting(^d)</th>
<th>Data Completeness(^e)</th>
<th>Statistical(^f)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sanda et al (2017)(^{37})</td>
<td>1. Confidence intervals not reported</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tomlins et al (2016)(^{38})</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

The study limitations stated in this table are those notable in the current review; this is not a comprehensive limitations 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.


\(^e\) 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.

### Subsection Summary: Clinically Valid

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%).

**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, or 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.

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

Tomlins et al (2016) also used decision-curve analysis to estimate the number of biopsies that would have been performed and cancers that would have been missed using a MiPS risk cutoff for biopsy in their cohort.38, Compared with a biopsy-all strategy, using a MiPS cutoff for aggressive cancer of 15% would have avoided 36% of biopsies while missing 7.0% 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 positive DRE who would not likely forgo biopsy.

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

**SelectMDx for Prostate Cancer**
Clinically Valid
A test must detect the presence or absence of a condition, the risk of developing a condition in the future, or treatment response (beneficial or adverse).
Van Neste et al (2016) evaluated a risk calculator that added HOXC6 and DLX1 expression to a clinical risk model (see Table 15). 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 (see Table 16). A limitation of this study is the inclusion of men with an abnormal DRE (see Tables 17 and 18), which was the strongest predictor of prostate cancer in the training set (OR=5.53; 95% CI, 2.89 to 10.56). The OR for HOXC6 and DLX1 expression in this model was 1.68 (95% CI, 1.38 to 2.05; p<0.003).

### Table 15. Characteristics of Clinical Validity Studies Assessing SelectMDx for Prostate Cancer

<table>
<thead>
<tr>
<th>Study</th>
<th>Study Population</th>
<th>Design</th>
<th>Reference Standard</th>
<th>Threshold for Positive Index Test</th>
<th>Timing of Reference and Index Tests</th>
<th>Blinding of Assessors</th>
<th>Comment</th>
</tr>
</thead>
<tbody>
<tr>
<td>Van Neste et al (2016)</td>
<td>386 men with PSA level &gt;3 ng/mL scheduled for initial (89%) or repeat biopsy</td>
<td>Prospective</td>
<td>Prostate cancer on biopsy</td>
<td>Urine sample taken after DRE and prior to biopsy</td>
<td>NR</td>
<td>NR</td>
<td></td>
</tr>
</tbody>
</table>

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

### Table 16. Results of Clinical Validity Studies Assessing SelectMDx for Prostate Cancer

<table>
<thead>
<tr>
<th>Study</th>
<th>Total N</th>
<th>N With PSA Level &lt;10 ng/mL</th>
<th>N with No or Low-Grade Cancer</th>
<th>AUC for the Risk Score in Patients With PSA Level &lt;10 ng/mL (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Van Neste et al (2016)</td>
<td>386</td>
<td>264</td>
<td>226/264</td>
<td>0.90 (0.85 to 0.96) 0.78 (0.68 to 0.88) 0.66 (0.57 to 0.75)</td>
</tr>
</tbody>
</table>

AUC: area under the curve; CI: confidence interval; HG: high-grade; PCPTRC: Prostate Cancer Prevention Trial Risk Calculator; PSA: prostate-specific antigen.

### Table 17. Relevance Limitations

<table>
<thead>
<tr>
<th>Study</th>
<th>Population</th>
<th>Intervention</th>
<th>Comparator</th>
<th>Outcomes</th>
<th>Duration of Follow-Up</th>
</tr>
</thead>
<tbody>
<tr>
<td>Van Neste et al (2016)</td>
<td>4.31% of men had abnormal DRE</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

The study limitations stated in this table are those notable in the current review; this is not a comprehensive limitations assessment.

DRE: digital rectal exam.

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

<table>
<thead>
<tr>
<th>Study</th>
<th>Selection</th>
<th>Blinding</th>
<th>Delivery of Test</th>
<th>Selective Reporting</th>
<th>Data Completeness</th>
<th>Statistical</th>
</tr>
</thead>
<tbody>
<tr>
<td>Van Neste et al (2016)</td>
<td></td>
<td>1. Blinding not reported</td>
<td></td>
<td></td>
<td>1. Inadequate description of indeterminate samples</td>
<td></td>
</tr>
</tbody>
</table>

The study limitations stated in this table are those notable in the current review; this is not a comprehensive limitations 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.
Subsection Summary: Clinically Valid
One validation study evaluating the SelectMDx for Prostate Cancer was identified. This study reported that a risk model that added an expression of HOX6 and DLX1 to a clinical risk model increased the AUC for the detection of high-grade cancer for men who were in the “gray zone,” with a PSA level between 3 ng/mL and 10 ng/mL. A limitation of this study is its inclusion of men with an abnormal DRE, which was the strongest predictor of prostate cancer.

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, or 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%.

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
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).
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 (see Table 19). 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<0.001) and 0.62 for the PCPTRC (see Table 20). Diagnostic performance is shown in Table 20, with sensitivity of 97% and NPV of 96%.

<table>
<thead>
<tr>
<th>Table 19. Characteristics of Clinical Validity Studies Assessing ExoDx Prostate (IntelliScore)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Study</strong></td>
</tr>
<tr>
<td>McKiernan et al (2016)41</td>
</tr>
</tbody>
</table>

PSA: prostate-specific antigen.

<table>
<thead>
<tr>
<th>Table 20. Results of Clinical Validity Studies Assessing ExoDx Prostate (IntelliScore)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Study</strong></td>
</tr>
<tr>
<td>McKiernan et al (2016)41</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th><strong>Area Under the Curve (95% CI)</strong></th>
</tr>
</thead>
<tbody>
<tr>
<td>ExoDx + SOC</td>
</tr>
<tr>
<td>(0.68 to 0.77)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th><strong>Diagnostic Performance (95% CI), %</strong></th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Sensitivity</strong></td>
</tr>
<tr>
<td>97.44</td>
</tr>
</tbody>
</table>

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

<table>
<thead>
<tr>
<th>Table 21. Relevance Limitations</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Study</strong></td>
</tr>
<tr>
<td>McKiernan et al (2016)41</td>
</tr>
</tbody>
</table>

The study limitations stated in this table are those notable in the current review; this is not a comprehensive limitations assessment.

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

---

<sup>a</sup> 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.

<sup>b</sup> Intervention key: 1. Classification thresholds not defined; 2. Version used unclear; 3. Not intervention of interest.

<sup>c</sup> 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.

<sup>d</sup> Outcomes key: 1. Study does not directly assess a key health outcome; 2. Evidence chain or decision model not explicited; 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).

<sup>e</sup> 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

<table>
<thead>
<tr>
<th>Study</th>
<th>Selection</th>
<th>Blinding</th>
<th>Delivery of Test</th>
<th>Selective Reporting</th>
<th>Data Completeness</th>
<th>Statistical</th>
</tr>
</thead>
<tbody>
<tr>
<td>McKeirnan et al (2016)</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
</tbody>
</table>

The study limitations stated in this table are those notable in the current review; this is not a comprehensive limitations assessment.

Subsection Summary: Clinically Valid

The ExoDx Prostate (IntelliScore) assay showed a sensitivity of 97% and NPV of 96% for high-grade prostate cancer in men over 50 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.

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, or 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 were identified that compared health outcomes for patients managed with and without the test.

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.

McKeirnan et al (2016) calculated that using a predefined cut point, 138 (27%) of 519 biopsies would have been avoided, missing 8% of patients with high-risk disease. Present studies on clinical validity are insufficient to establish a chain of evidence.

Section Summary: ExoDx Prostate (IntelliScore)

No trials were identified that compared health outcomes for patients managed with and without the ExoDx Prostate. A chain of evidence depends on clinical validity. Current evidence on adding exosome PCA3 and ERG to a clinical risk model is insufficient to support its use. ExoDx Prostate has data suggesting an improved AUC (0.73) compared with the standard of care (0.63) in 1 validation study. That study included men with PSA levels in the indeterminate range. However, the study also included men with suspicious DRE and the comparator did not include DRE in its score. The sensitivity and NPV at the defined threshold were 97% and 96%, respectively.
No prospective data are available on using ExoDx Prostate for decision making. Present studies on clinical validity are insufficient to establish a chain of evidence.

**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. 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.

**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 Food and Drug Administration-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 one 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).

**Systematic Reviews**

Several systematic reviews and meta-analyses have described the clinical validity of the Progensa PCA3 Assay. The characteristics of the reviews are described in Table 23. All primary studies were observational, with one 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. The studies assessed both initial and repeat biopsy and had a quality rating of moderate to high. 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.

Results from the systematic reviews are shown in Table 24. 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). The estimates of AUC were lower for studies that included men having repeated (0.68) vs initial (0.80) biopsies. Nicholson et al (2015) included 13 reports describing 11 cohorts, including one from the placebo arm of an RCT. 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 23. Characteristics of Systematic Reviews Assessing the Clinical Validity of Progena PCA3 Assay for Diagnosing Prostate Cancer

<table>
<thead>
<tr>
<th>Study</th>
<th>Studies Dates</th>
<th>Key Inclusion Criteria</th>
<th>Design</th>
<th>Reference Studies Included</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cui et al (2016)</td>
<td>46</td>
<td>Up to 2014 Initial prostate biopsy negative or equivocal, 6+ cores in initial biopsy, with or without DRE</td>
<td>Prospective, retrospective (case-control or cohort)</td>
<td>Biopsy as reference standard</td>
</tr>
<tr>
<td>Nicholson et al (2015)</td>
<td>11</td>
<td>2000-2014 Initial prostate biopsy negative or equivocal, 6+ cores in initial biopsy, with or without DRE</td>
<td>Prospective and mixed (prospective/retrospective)</td>
<td>Biopsy as reference standard (OBS 1 included a cohort from an RCT)</td>
</tr>
</tbody>
</table>

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

Table 24. Results of Systematic Reviews Assessing the Clinical Validity of Progena PCA3 Assay for Diagnosing Prostate Cancer

<table>
<thead>
<tr>
<th>Study</th>
<th>Studies N (Range)</th>
<th>Outcomes</th>
<th>Sens (95% CI), %</th>
<th>Spec (95% CI), %</th>
<th>AUC (95% CI) or Range</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cui et al (2016)</td>
<td>12,295 (NR)</td>
<td>Any prostate cancer on initial or repeat biopsy</td>
<td>Pooled: 65% (63% to 66%) Range: 47%-95%</td>
<td>• Pooled: 73% (72% to 74%) Range: 22%-100%</td>
<td>0.75 (0.74 to 0.77)</td>
</tr>
<tr>
<td>Nicholson et al (2015)</td>
<td>3336 (41-1072)</td>
<td>Any prostate cancer on repeat biopsy</td>
<td>CA alone range, 44%-48% CA plus PCA3 range, 39%-46%</td>
<td>Fixed at 80%</td>
<td>• CA alone: 0.55-0.75</td>
</tr>
</tbody>
</table>

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 (see Table 25). 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 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≤0.003). 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%; see Table 26). 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. 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.

Table 25. Characteristics of Clinical Validity Studies Assessing the Progena PCA3 Assay

<table>
<thead>
<tr>
<th>Study</th>
<th>Study Population</th>
<th>Design</th>
<th>Reference Standard</th>
<th>Threshold for Positive Index Test</th>
<th>Timing of Reference and Index Tests</th>
<th>Blinding of Assessors Comment</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wei et al (2014)</td>
<td>910 men scheduled for a diagnostic prostate biopsy</td>
<td>Prospective</td>
<td>Any prostate cancer on biopsy or HG prostate cancer</td>
<td>Determined a priori at thresholds of &lt;20 and &gt;60</td>
<td>Urine samples collected following DRE and prior to biopsy</td>
<td>Yes</td>
</tr>
</tbody>
</table>
Genetic and Protein Biomarkers for the Diagnosis and Cancer Risk Assessment of Prostate Cancer

Table 26. Results of Clinical Validity Studies Assessing the Progensa PCA3 Assay

<table>
<thead>
<tr>
<th>Study</th>
<th>Initial N</th>
<th>Final N</th>
<th>Excluded Samples</th>
<th>Clinical Validity (95% Confidence Interval), %</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Sensitivity</td>
</tr>
<tr>
<td>Wei et al (2014)</td>
<td>46,910</td>
<td>859</td>
<td>27</td>
<td>42 (36 to 48)</td>
</tr>
<tr>
<td>Initial biopsy PCA3 &gt;60</td>
<td>562</td>
<td>42</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Repeat biopsy PCA3 &lt;20</td>
<td>297</td>
<td>76</td>
<td></td>
<td>76 (64 to 86)</td>
</tr>
</tbody>
</table>

NPV: negative predictive value; PPV: positive predictive value.

No notable limitations were identified for relevance or design and conduct.

Subsection Summary: Clinically Valid

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. Another systematic review found an AUC of 0.68 for the PCA3 assay in men having repeat biopsies.

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, or 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 or PCA3 level corrected for prostate volume (PCA3 density). 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. 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. 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)**

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 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 PICOTS were used to select literature that provides evidence relevant to this review.

**Patients**

The relevant population 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 27.

**Table 27. Commercially Available Tests to Determine Candidates for Repeat Prostate Biopsy**

<table>
<thead>
<tr>
<th>Test</th>
<th>Manufacturer</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>PCA3 Score (e.g., Progensa PCA3 Assay)</td>
<td>Hologic Gen-Probe</td>
<td>Measures PCA3 mRNA in urine samples after prostate massage. PCA3 mRNA may be normalized using PSA to account for prostate cells.</td>
</tr>
<tr>
<td></td>
<td>Many labs offer PCA3 tests (e.g., ARUP Laboratories, Mayo Medical Laboratories, LabCorp)</td>
<td></td>
</tr>
</tbody>
</table>
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 Food and Drug Administration 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.

SNVs occur when a single nucleotide is replaced with another, and they 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 for disease, 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.24,25

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 model25, and the ERSPC-RC.26 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.27
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.50,51.

Timing
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 OS.

Setting
Screening using PSA levels and DRE may be performed in the primary care setting with referral to specialists (urologists) for suspicious findings and biopsy. Clinical practice on screening methods and frequency vary widely.

Study Selection Criteria
For the evaluation of clinical validity, studies that met the eligibility criteria outlined for the initial biopsy indication were considered.

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).

Three blinded multicenter validation studies of the ConfirmMDx test have been performed, one of which was conducted in African American men (see Table 28).52,53,54, 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% (see Table 29). Multivariate analysis of potential predictors of cancer on repeat biopsy, corrected for age, PSA, DRE, first biopsy histopathology characteristics, showed that the ConfirmMDx test was the most significant independent predictor of patient outcome in both DOCUMENT (OR=2.69; 95% CI, 1.60 to 4.51) and 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.55,56, 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 28. Characteristics of Clinical Validity Studies Assessing ConfirmMDx

<table>
<thead>
<tr>
<th>Study</th>
<th>Study Population</th>
<th>Design</th>
<th>Reference Standard</th>
<th>Threshold for Positive Index Test</th>
<th>Timing of Reference and Index Tests</th>
<th>Blinding of Assessors</th>
<th>Comment</th>
</tr>
</thead>
<tbody>
<tr>
<td>Waterhouse et al (2018)54</td>
<td>Archived, cancer-negative prostate biopsy core tissue samples from 211 African American men from 7 U.S. urology centers</td>
<td>Retrospective, ConfirmMDx performed on first biopsy</td>
<td>Repeat biopsy</td>
<td>NR</td>
<td>&lt;30 mo</td>
<td>Yes</td>
<td>55% of men had a normal DRE; median PSA level</td>
</tr>
</tbody>
</table>
**Table 29. Results of Clinical Validity Studies Assessing ConfirmMDx**

<table>
<thead>
<tr>
<th>Study; Trial</th>
<th>Prevalence of Condition</th>
<th>Sens (95% Confidence Interval), %</th>
<th>Spec (95% Confidence Interval), %</th>
<th>PPV (95% Confidence Interval), %</th>
<th>NPV (95% Confidence Interval), %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Waterhouse et al (2018)</td>
<td>81 had positive second biopsy (cases), 130 had negative second biopsy (controls)</td>
<td>74 (63 to 83)</td>
<td>60 (51 to 69)</td>
<td>54 (47 to 60)</td>
<td>79 (72 to 85)</td>
</tr>
<tr>
<td>Partin et al (2014); DOCUMENT</td>
<td>92 had positive second biopsy (cases), 228 had negative second biopsy (controls)</td>
<td>62 (51 to 72)</td>
<td>64 (57 to 70)</td>
<td>88 (85 to 91)</td>
<td></td>
</tr>
<tr>
<td>Stewart et al (2013); MATLOC</td>
<td>87 had positive second biopsy, 396 had negative second biopsy (controls)</td>
<td>68 (57 to 77)</td>
<td>64 (59 to 69)</td>
<td>90 (87 to 93)</td>
<td></td>
</tr>
<tr>
<td>Summary</td>
<td>51 to 83</td>
<td>51 to 70</td>
<td>54</td>
<td>72 to 93</td>
<td></td>
</tr>
</tbody>
</table>

NPV: negative predictive value; NR: not reported; PPV: positive predictive value; Sens: sensitivity; Spec: specificity.

Tables 30 and 31 summarize the relevance and design and conduct limitations in each study.

**Table 30. Relevance Limitations**

<table>
<thead>
<tr>
<th>Study; Trial</th>
<th>Population</th>
<th>Intervention</th>
<th>Comparator</th>
<th>Outcomes</th>
<th>Duration of Follow-Up</th>
</tr>
</thead>
<tbody>
<tr>
<td>Waterhouse et al (2018)</td>
<td>1. Classification thresholds not described (proprietary)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Partin et al (2014); DOCUMENT</td>
<td>1. Classification thresholds not described (proprietary)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Stewart et al (2013); MATLOC</td>
<td>1. Classification thresholds not defined. Training set with a stepwise approach to maximize NPV</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

The study limitations stated in this table are those notable in the current review; this is not a comprehensive limitations assessment.

NPV: negative predictive value.

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 31. Study Design and Conduct Limitations

<table>
<thead>
<tr>
<th>Study; Trial</th>
<th>Selection</th>
<th>Blinding</th>
<th>Delivery of Test</th>
<th>Selective Reporting</th>
<th>Data Completeness</th>
<th>Statistical</th>
</tr>
</thead>
<tbody>
<tr>
<td>Waterhouse et al (2018)</td>
<td></td>
<td></td>
<td></td>
<td>1. Inadequate</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>not described</td>
<td></td>
<td></td>
<td>description of</td>
<td>indeterminate and</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>missing samples</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Partin et al (2014)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>DOCUMENT</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Stewart et al (2013)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>MATLOC</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

The study limitations stated in this table are those notable in the current review; this is not a comprehensive limitations 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.


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.

Subsection Summary: Clinically Valid

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.

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, or more effective therapy, or avoid unnecessary therapy, or avoid unnecessary testing.

Direct Evidence

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

Aubry et al (2013) estimated the reduction in biopsies associated with ConfirmMDx use.58 Using the performance characteristics from MATLOC, the authors estimated that 1106 biopsies per 1
Genetic and Protein Biomarkers for the Diagnosis and Cancer Risk Assessment of Prostate Cancer

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
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).

Robinson et al (2010) assessed the clinical value of a 3.4-kilobase mitochondrial deletion in predicting rebiopsy outcomes. 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%.

Subsection Summary: Clinically Valid
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%.

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, or more effective therapy, or avoid unnecessary therapy, or avoid unnecessary testing.

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

Legisi et al (2016) queried a pathology services database to identify (1) men who had a negative initial prostate biopsy and a negative PCMT (n=644), and (2) men who had a negative initial prostate biopsy and a repeat biopsy (n=823). Of the 644 patients with a negative PCMT, 35 had a repeat biopsy and 5 (14.2%) were false-negatives who were found to have cancer on rebiopsy. The number of false-negatives of the patients who did not have a repeat biopsy cannot be determined from this study. 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**

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).

A 3-gene panel (HOXC6, TDRD1, DLX1) developed by Leyten et al (2015) is now commercially available as SelectMDx (see above). Xia et al (2016) reported the development of an 8-gene panel (PMP22, HPN, LMTK2, FN1, EZH2, GOLM1, PCA3, GSTP1) distinguished high-grade prostate cancer from indolent prostate cancer with a sensitivity of 93% and NPV of 61% (see Tables 32 and 33). Validation of this panel is needed.

**Table 32. Characteristics of Clinical Validity Studies Assessing Candidate Gene Panels**

<table>
<thead>
<tr>
<th>Study</th>
<th>Study Population</th>
<th>Design</th>
<th>Reference Standard</th>
<th>Threshold for Positive Index Test</th>
<th>Timing of Reference and Index Tests</th>
<th>Blinding of Assessors</th>
</tr>
</thead>
<tbody>
<tr>
<td>Xiao et al (2016)</td>
<td>Specimens from 158 men</td>
<td>Retrospective</td>
<td>High-grade prostate cancer on biopsy</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**Table 33. Results of Clinical Validity Studies Assessing Candidate Gene Panels**

<table>
<thead>
<tr>
<th>Study</th>
<th>N</th>
<th>Sensitivity</th>
<th>Specificity</th>
<th>PPV</th>
<th>NPV</th>
</tr>
</thead>
<tbody>
<tr>
<td>Xiao et al (2016)</td>
<td>158</td>
<td>93</td>
<td>70</td>
<td>98</td>
<td>61</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(88 to 97)</td>
<td>(36 to 104)</td>
<td>(95 to 100)</td>
<td>(25 to 97)</td>
</tr>
</tbody>
</table>

NPV: negative predictive value; PPV: positive predictive value.

**Clinically Useful**

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

**Direct Evidence**

Direct evidence of clinical utility is provided by studies that have compared health outcomes for patients managed with and without the test. Because these are intervention studies, the preferred evidence would be from RCTs.
Chain of Evidence
Indirect evidence on clinical utility rests on clinical validity. If the evidence is insufficient to demonstrate test performance, no inferences can be made about clinical utility. Because the clinical validity of these multigene tests has not been established, a chain of evidence supporting the clinical utility of these tests cannot be constructed.

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., kalikreins biomarkers and 4Kscore Test, proPSA and Prostate Health Index, TMPRSS fusion genes and Mi-Prostate Score, SelectMDx for Prostate Cancer, ExoDx Prostate, Apifiny, PCA3 score), the evidence includes systematic reviews, meta-analyses, and primarily observational studies. The relevant outcomes are OS, 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 the effects of the technology on health outcomes.

For individuals who are being considered for repeat biopsy who receive testing for genetic and protein biomarkers of prostate cancer (e.g., Gene Hypermethylation and ConfirmMDx test, Prostate Core Mitomics Test), the evidence includes systematic reviews and meta-analyses and primarily observational studies. The relevant outcomes are OS, 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 did 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 biopsy based on test results. The evidence is insufficient to determine the effects of the technology on health outcomes.

Supplemental Information
Practice Guidelines and Position Statements

American Urological Association et al
The American Urological Association (2013; confirmed 2018) published guidelines on the early detection of prostate cancer.63 The association 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, 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.”
The American Urological Association and the Society of Abdominal Radiology (2016) published joint guidelines on prostate magnetic resonance imaging and magnetic resonance imaging-targeted biopsy. The associations recommended:

“In patients with negative or low suspicion magnetic resonance imaging (PI-RADS [Prostate Imaging Reporting and Data System] assessment category of 1 or 2, respectively), other ancillary markers (ie PSA [prostate-specific antigen], PSAD [PSA density], PSAV [PSA velocity], PCA3, PHI, 4K) may be of value in identifying patients warranting repeat systematic biopsy, although further data are needed on this topic.”

Guidelines published by the American Cancer Society and the American Urological Association have endorsed consideration of PSA screening based on age, other risk factors, and estimated life expectancy.

**National Comprehensive Cancer Network**
The NCCN guidelines (v.2.2019) recommend that any man with a PSA level greater than 3 ng/mL undergo workup for benign disease, repeat PSA, and digital rectal examination. The guidelines also recommend consideration of biomarkers that improve the specificity of screening including percent free PSA, phi, ExoDx Prostate (IntelliScore) (EPI), and 4Kscore in patients with a PSA level greater than 3 ng/mL who have not yet had a biopsy, and consideration of percent free PSA, phi, 4Kscore, PCA3, ExoDx Prostate (IntelliScore) (EPI), and ConfirmMDx in men who had a negative biopsy but are thought to be at higher risk (category 2A evidence). The NCCN noted that these tests may be especially useful in men with PSA levels between 3 ng/mL and 10 ng/mL. The NCCN considers the Mi-Prostate Score (MiPS), and SelectMDx to be investigational at the time of the update. NCCN indicated that:

"....no biomarker test can be recommended over any other at this time.... The optimal order of biomarker tests and imaging is unknown; and it remains too unclear how to interpret results of multiple tests in individual patients - especially when results are contradictory.”

**National Institute for Health and Care Excellence**
The National Institute for Health and Care Excellence (2019) 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.

**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.

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. Several MolDx carriers have positive coverage for the ConfirmMDx Epigenetic Molecular Assay and the PCA3 assay. At least one LCD will cover percent free PSA, phi, or 4K score once prior to initial biopsy in men who meet criteria.

**Ongoing and Unpublished Clinical Trials**
Some currently ongoing and unpublished trials that might influence this review are listed in Table 34.
Table 34. Summary of Key Trials

<table>
<thead>
<tr>
<th>NCTNo.</th>
<th>Trial Name</th>
<th>Planned Enrollment</th>
<th>Completion Date</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ongoing</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>NCT00773773</td>
<td>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</td>
<td>500</td>
<td>Oct 2019</td>
</tr>
<tr>
<td>NCT03082274</td>
<td>Prospective Validation of Prostate Biomarkers for Repeat Biopsy: The PRIORITY Study</td>
<td>1000</td>
<td>Dec 2019</td>
</tr>
<tr>
<td>NCT01739062</td>
<td>Prostate Cancer Risk Assessment Using Genetic Markers in General Practice (ProCaRis)</td>
<td>5000</td>
<td>Jan 2024</td>
</tr>
<tr>
<td>NCT01632930</td>
<td>Medical Economics of Urinary PCA3 Test for Prostate Cancer Diagnosis</td>
<td>962</td>
<td>Nov 2020</td>
</tr>
<tr>
<td>NCT04079699</td>
<td>Predicting Prostate Cancer Using a Panel of Plasma and Urine Biomarkers Combined in an Algorithm in Elderly Men Above 70 Years</td>
<td>700</td>
<td>Oct 2039</td>
</tr>
<tr>
<td>Unpublished</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

NCT: national clinical trial.

* Denotes industry-sponsored or cosponsored trial.

References

   Referenced with permission from the NCCN Clinical Practice Guidelines in Oncology (NCCN Guidelines®) for NCCN clinical practice guidelines in oncology: prostate cancer early detection V.2.2019. © National Comprehensive Cancer Network rights reserved. Accessed October 14, 2019. To view the most recent and complete version of the guideline, go online to NCCN.org. NCCN makes no warranties of any kind whatsoever regarding their content, use or application and disclaims any responsibility for their application or use in any way.


### 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. Inclusion or exclusion of codes does not constitute or imply member coverage or provider reimbursement.

#### IE

The following services may be considered investigational.

<table>
<thead>
<tr>
<th>Type</th>
<th>Code</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>CPT®</td>
<td>0005U</td>
<td>Oncology (prostate) gene expression profile by real-time RT-PCR of 3 genes (ERG, PCA3, and SPDEF), urine, algorithm reported as risk score</td>
</tr>
<tr>
<td></td>
<td>0053U</td>
<td>Oncology (prostate cancer), FISH analysis of 4 genes (ASAP1, HDAC9, CHD1 and PTEN), needle biopsy specimen, algorithm reported as probability of higher tumor grade</td>
</tr>
<tr>
<td></td>
<td>0021U</td>
<td>Oncology (prostate), detection of 8 autoantibodies (ARF 6, NXX3-1, 5'-UTR-BMI1, CEP 164, 3'-UTR-Ropporin, Desmocollin, AURKAIP-1, CSNK2A2), multiplexed immunoassay and flow cytometry serum, algorithm reported as risk score</td>
</tr>
<tr>
<td></td>
<td>0047U</td>
<td>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 risk score</td>
</tr>
<tr>
<td></td>
<td>0113U</td>
<td>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 (Code effective 10/1/2019)</td>
</tr>
<tr>
<td></td>
<td>81313</td>
<td>PCA3/KLK3 (prostate cancer antigen 3 [non-protein coding]/kallikrein-related peptidase 3 [prostate specific antigen]) ratio (e.g., prostate cancer)</td>
</tr>
<tr>
<td></td>
<td>81479</td>
<td>Unlisted molecular pathology procedure</td>
</tr>
<tr>
<td></td>
<td>81539</td>
<td>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</td>
</tr>
<tr>
<td></td>
<td>81542</td>
<td>Oncology (prostate) mRNA microarray gene expression profiling of 22 content genes, utilizing formalin-fixed paraffin-embedded tissue, algorithm reported as metastasis risk score (Code effective 1/1/2020)</td>
</tr>
</tbody>
</table>
|      | 81551 | Oncology (prostate), promoter methylation profiling by real-time PCR of 3 genes (GSTP1, APC, RASSF1), utilizing formalin-fixed paraffin-
Policy History

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

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

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 (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.

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.