National Academy of Clinical Biochemistry Guidelines for the Use of Tumor Markers in Prostate Cancer

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Abbreviations: ACS, American Cancer Society; ACP, American College of Physicians; ASTRO, American Society for Therapeutic Radiology and Oncology; AUA, American Urological Association; BPH, benign prostatic hyperplasia; cPSA, complexed PSA; DRE, digital rectal examination; EGTM, European Group on Tumor Markers; ESMO, European Society for Medical Oncology; FISH, fluorescence *in situ* hybridization; hK, human kallikrein; IHC, immunohistochemistry; LOE, levels of evidence; NACB, National Academy of Clinical Biochemistry; NCCN, National Comprehensive Cancer Network; NR, No recommendation published; PCa, Prostate cancer; PCR, polymerase chain reaction; PSA, prostate specific antigen; %fPSA, percent free: total PSA; USPSTF, U.S. Preventive Services Task Force.

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INTRODUCTION

Prostate cancer is the most common tumor in men in the United States. In 2005, 232,090 new cases and 30,350 deaths were predicted. While prostate cancer is unequivocally lethal in some patients, most men die with rather than of their cancer (1). According to autopsy data, histologically apparent cancer may be found in the prostates of more than 42% of men over 50 years of age who die of other causes. At present, the frequency of histological cancer is more than 4-fold higher for American men than the lifetime risk of being diagnosed with prostate cancer (about 16%) while the risk of dying from this disease is far less (about 4%) (2). Current incidence rates of clinical disease are 15-fold higher in the United States than in Japan despite similar frequencies of histological cancer. Hence, the far greater prevalence of histological than incident cancer has been cited to support a conservative, noninterventionist approach to this disease. However, once prostate cancer reaches advanced stages either locally or systemically with bone metastases, or becomes refractory to hormone therapy, there is little if any therapeutic means for cure.

CURRENTLY AVAILABLE MARKERS FOR PROSTATE CANCER

PSA markers helpful in the management of patients with prostate cancer are listed in Table 1, together with the phase of development for each marker as well as the level of evidence (LOE) for their clinical use. The levels of evidence grading system used is based on that described by Hayes et al *[see Section 1]*.

TUMOR MARKERS IN PROSTATE CANCER: NACB RECOMMENDATIONS

Table 2 summarises the National Academy of Clinical Biochemistry (NACB) guidelines for the use of PSA markers in prostate cancer together with recommendations from other representative guidelines published on the use of tumor markers in prostate cancer. While other markers have been investigated (Table 3), based on currently available evidence only the use of PSA and its isoforms can be recommended in prostate cancer. Below we present a more detailed discussion of the use of these measurements.

PSA markers in the screening and early detection of prostate cancer

The widespread measurement of serum PSA is largely responsible for the reported increase in incidence of prostate cancer. As epidemiological data demonstrate both a

marked increase in the number of men diagnosed with prostate cancer, and a profound migration towards earlier stage disease at the time of diagnosis (3), there is growing concern that such "stage migration" causes over-diagnosis and over-treatment of men with indolent cancer, a condition that may pose little threat to the life or health of the patient. Screening with PSA has also been questioned due to lack of specificity when serum concentrations are moderately elevated (4). While elevations of PSA in serum are exclusively associated with disease conditions in the prostate, they are not cancer-specific, occurring in other conditions such as benign prostatic hyperplasia (BPH) and prostatitis. Such non-specificity is less critical when monitoring established prostate cancer where PSA is the most important marker in evaluating response to therapeutic interventions and in detecting tumor relapse. [Measurement of prostatic acid phosphatase (PAP) does not add any clinically useful information to PSA (5-6), and therefore is not recommended by the NACB.]

Population-based median levels are approximately 0.5 µg/L for men ≤50 years, the vast majority of whom have yet to develop any signs or symptoms of prostate cancer or benign enlargement of the gland (7). An upper limit of normal according to the 95th percentile for men ≤50 years has never been implemented in clinical practice, but would correspond to a PSA-level of about 1.5 µg/L. Biopsy confirms prostate cancer in only 25-30% of the men who present with moderately elevated levels of PSA in serum (*i.e.* 4-10 µg/L) (8-9). When serum PSA levels rise above 10 µg/L, the cancer-specificity of the test is 40-50% or higher. Current recommendations in the United States suggest that most men over the age of 50 years should have annual prostate cancer screening with PSA and digital rectal examination (DRE), and that men should be advised to have biopsies when the DRE is abnormal or when the PSA level in serum is ≥4.0 µg/L (10).

These recommendations have their limitations. The PSA cut-off of ≥4.0 µg/L represents a clinical decision limit that was introduced on the basis of a single report evaluating the optimal combination of sensitivity and specificity of the PSA test in a study cohort (11), and the distribution of values observed in this original study may no longer apply (11). There is ongoing debate about recommending a PSA cut-point lower than 4 µg/L, which would increase the cancer detection rate at the expense of increasing the number of men advised to undergo biopsy. Population-based demographics of PSA levels for 50-70 year old men show that 8-9% of these men have PSA levels ≥4.0 µg/L, while 11-12% have PSA levels ≥3.0 µg/L, and as many as 20% of all men have serum PSA levels ≥2.0 µg/L. However, it has also been demonstrated beyond any reasonable

doubt that 20-25% of all men who have PSA levels from 3.0 (or 2.5) up to 4.0 µg/L are found with prostate cancer by biopsy (12). Hence, the positive predictive value of the PSA test in terms of biopsy-proven (histological) prostate cancer is similar from 2-4 and from 4-10 µg/L (8). The across-the-board recommendation of annual PSA testing for men over the age of 50 years (10) is overly simplistic and fails to alter testing frequency based on the individualized risk imparted by previously determined PSA levels. For example, a 55 year old male with a baseline PSA of 0.4 µg/L is much less likely to develop prostate cancer in the future than a similarly aged man with a baseline PSA of 3.3 µg/L. Gann *et al* used information from the Physicians' Health Study to examine the ability of PSA to identify men who subsequently were or were not clinically diagnosed with prostate cancer (13). These data suggest that men with PSA levels between 2.0 and 3.0 µg/L have 5.5-fold higher relative risk for diagnosis of prostate cancer than men with PSA levels less than 1.0 µg/L. In the former group, serum PSA levels reached 2-3 µg/L on average more than 5 years before the cancer was detected by DRE. Some additional issues of particular relevance in the context of a screening program are discussed below.

Age-specific reference intervals for PSA. Since serum PSA levels gradually increase with age in men over 40 years old, age-specific reference ranges have been proposed with the expectation that their implementation should increase cancer detection rates in younger men by lowering the cut-point and increase specificity in older men by raising the cut-point (14). Although there is no consensus, many experts - including a majority of opinion of the National Comprehensive Cancer Network – favor the use of clinical decision limits lower than 4.0 µg/L for serum PSA in younger men. The NACB, however, is not yet convinced of the net benefit in doing this, and at the same time advises caution in increasing the decision limit above 4.0 µg/L, since this could result in failure to diagnose clinically significant tumors in men who might potentially benefit from early treatment (15). Contrary to previously issued recommendations, the NACB does not endorse the use of age-specific reference ranges (16).

Increasing PSA specificity in screening for prostate cancer. The total PSA in circulation roughly corresponds to the sum of circulating free PSA (fPSA) and PSA bound as a stable complex to alpha-1-antichymotrypsin (complexed PSA, cPSA). The free fraction constitutes from 5-30% of the total (35). Free and bound forms may be specifically

detected by commercially available assays. Several composite measures have been proposed to improve the specificity of a single serum total PSA concentration for the early detection of prostate cancer. PSA density (17-19), PSA velocity (20), PSA doubling time (21-22), and percent free PSA (%fPSA) (23-27) have all been evaluated in this context, but only %fPSA has been widely validated and implemented in clinical practice. Men with benign disease generally present with higher %fPSA than men with prostate cancer (and no benign enlargement). Unfortunately, concurrent benign prostatic enlargement and prostate cancer complicates interpretation of %fPSA data (28). Nevertheless, the use of %fPSA has been suggested as a means of decreasing the number of unnecessary biopsies, particularly for men with PSA levels from 4 to 10 µg/L. The NACB and European Group on Tumor Markers (EGTM) recommend the use of %fPSA as an aid in distinguishing men with prostate cancer from men with benign disease in selected high-risk groups, e.g. when total PSA is <10 µg/L and digital rectal examination (DRE) is negative (16). In particular, %fPSA may be useful in identifying men who have prostate cancer despite initial negative biopsy findings. In men suspected of being at high risk of harboring malignant disease due to low %fPSA, a cancer diagnosis may become evident after a repeat biopsy. This recommendation is tempered by the need to validate the medical decision limit for each free and total PSA commercial assay combination (29).

The cPSA fraction is bound 95% to alpha-1-antichymotrypsin and 5% to other complex ligands (33). Levels of cPSA in blood can be determined either directly by the cPSA specific assay (30-32) or indirectly by subtracting fPSA from tPSA levels (34) using assays standardized against one another, The cPSA fraction provides comparable cancer detection to total PSA, but appears to give somewhat better specificity in a narrow concentration range (32).

Pre-analytical specimen processing and storage. It is desirable to collect blood prior to any manipulation of the prostate by digital rectal examination (DRE), cystoscopy, or prostate biopsy (36). If prior collection is not possible, then it is prudent to delay several days after DRE before drawing blood for PSA, although in most men DRE does not cause a clinically relevant change in circulating PSA concentration (36). Following prostate biopsy or surgery, the recommended delay is several weeks to permit sufficient time for the kidneys to clear from the blood any PSA that was liberated from the prostate by the procedure. (37-38).

Taking into account the intra-individual biological variation of PSA in blood, a change of 20-30% between serially collected specimens is required before the difference can be considered to be clinically significant (39).

In order to eliminate *in vitro* artifacts, blood should be centrifuged within 3 hours of collection to isolate the serum or plasma (40). Serum and plasma may be kept at refrigerated temperatures for up to 24 hours without loss of PSA. If analysis is delayed longer, then it is vital to store specimens frozen, preferably at least at -30^{o}C to avoid the eutectic point. Long-term storage is best carried out at temperatures of at least -70^{o}C. Data show that fPSA is more susceptible to decay than cPSA (36, 41), and that the fPSA decay is slower in plasma than in serum (40).

Analytical and reporting concerns

PSA is most frequently used in conjunction with physical examination to screen for prostate cancer. A single positive PSA screen should always be verified, by repeating the PSA measurement in a separately collected specimen, before ordering confirmatory investigations. Cancer is confirmed by histopathological tissue examination.

Analytical assay performance should be monitored with quality control material containing PSA at concentrations near clinically relevant decision points. Information on assay characteristics and utility, including the functional detection limit of the assay and its coefficient of variation (CV) at concentrations corresponding to these decision points, should be available to clinicians through laboratory test information sources. It is prudent to include with the PSA result a reminder that a single screening blood test result should not be used as the sole evidence of the presence or absence of malignant disease. The laboratory report should include the manufacturer of the PSA assay used, document the recommended clinical decision limits, and warn that the results cannot be used interchangeably with those generated by other assays unless the interchange of assay values has previously been validated.

PSA markers in the post-treatment monitoring of prostate cancer

When monitoring patients post-treatment, a single PSA measurement at or near the lower detection limit of the assay should not be used to diagnose biochemical recurrence of prostate cancer. Rising levels demonstrated by repeat or serial measurements provide much more reliable evidence (42). Following radical prostatectomy, circulating PSA declines to undetectable levels if the prostate cancer was organ-confined and all

residual prostate tissue surgically excised. Sustained detection of PSA suggests either incomplete resection or metastatic deposits. If “ultra”-sensitive assays are used in this setting, the functional detection limit of the assay should be established and should correspond to the lower reporting limit.

At present, evidence is equivocal regarding the clinical benefit of reporting biochemical recurrence of prostate cancer at PSA levels below 0.4 μg/L (43). Recently, however, salvage radiation therapy following prostatectomy has been shown to yield best results when PSA levels are still very low (≤0.5 μg/L) (44). The recurrence limit is less clear following radiation therapy because of the typically slower decline in circulating PSA concentration. The American Society for Therapeutic Radiation and Oncology (ASTRO) has defined biochemical recurrence as three consecutive rises in PSA above the nadir (45).

Guidelines for the early detection of prostate cancer

The American Cancer Society has issued guidelines related to the early detection of prostate cancer. The guidelines recommend an annual screening with DRE and serum PSA measurement beginning at the age of 50 in men at average risk with at least 10 years of life expectancy (10). Although PSA is considered the best biochemical test currently available to detect prostate cancer, a DRE should be included whenever possible. Screening at an earlier age (45 years, even 40 years) is warranted in men at increased risk including those of African-American descent and those with one or more first-degree relatives with prostate cancer. Both of these groups often develop prostate cancer several years earlier than the general population and tend to present with a more aggressive type of cancer (46).

The recommended follow-up testing of high risk individuals initially screened at 40 years of age depends on the PSA result. Those with PSA levels <1 μg/L would resume testing at 45 years of age, those with levels >1 but <2.5 μg/L would be tested annually, while those with levels ≥2.5 μg/L would be evaluated further and considered for biopsy (10).

These guidelines do not endorse a general recommendation on mass screening, but support the notion that men should be informed of the benefits and limitations of prostate cancer screening prior to making a decision. Much greater emphasis than previously is being placed on informed decision-making by the individual. There are many issues to consider including the disparity between incidence and mortality associated with prostate

cancer since many more men are diagnosed with prostate cancer than eventually die from it. However, early detection affords the opportunity to detect organ-confined disease when curative treatment is possible. Metastatic disease now constitutes only about 5% of initial diagnoses in the United States, a dramatic fall from the 50% incidence rate of the pre-PSA era (3). Nevertheless there are still many uncertainties concerning treatment of early stage disease including the preferred treatment for clinically localized prostate cancer.

Merits of early detection of prostate cancer

Consequently, there is still considerable debate regarding the merits of early detection of prostate cancer, and not all physician organizations advocate routine screening (47). While the American Urological Association endorses the American Cancer Society policy statement on the early detection of prostate cancer, other organizations differ over the benefit of prostate cancer screening (48-49). Arguments against screening are based on the fact that there is no conclusive evidence that early detection and treatment influence overall mortality, while the standard treatments for organ confined prostate cancer are associated with significant and frequently irreversible side effects. Currently, the US Preventive Task Force, the American Academy of Family Physicians, the American College of Physicians, the National Cancer Institute (NCI) and the EGTM do not recommend population-based prostate cancer screening (48-49). The overriding concern is that screening will result in over-diagnosis and over-treatment of early stage disease that may not be clinically significant.

The NACB and the EGTM espouse the view that wide-spread implementation of screening for prostate cancer in the general population should await the final outcome of on-going prospective randomized studies, in particular the European Randomized Screening for Prostate Cancer (ERSPC) trial (50), which are sufficiently powered to establish whether early detection and treatment decreases prostate cancer mortality. The ERSPC has been underway for ten years and is scheduled to run for several more (51). Long term multi-center trials to determine the impact of prostate cancer screening on survival are also ongoing in the United States under the aegis of the National Cancer Institute and the U.S. Public Health Service (52).

With no clear-cut evidence as yet that prostate cancer screening is of net benefit, proponents of screening have pointed to the association among prostate cancer testing, earlier cancer stage at detection and reduced mortality arising from prostate cancer.

Registry data from heavily and sparsely screened male populations in Austria provide a case in point. The expected death rate from prostate cancer (53) declined much more in the Tyrol, a heavily screened section of the country, than in less intensely screened areas The decrease in observed mortality was associated with a shift towards a more favorable stage at diagnosis, in particular an increase in the proportion of organ-confined disease The inference is that early detection and the availability of effective treatment resulted in a corresponding improvement in disease specific survival. A similar trend has been observed in data from the National Cancer Institute's Surveillance, Epidemiology and End Results (SEER) program and from a study conducted in Olmsted County, Minnesota (54).

Even though recent data suggest that the apparent stage shift to early disease and subsequent treatment of localized prostate cancer detected with PSA has positively influenced mortality rates, it is still an open question whether early intervention alters the natural history of the disease. Observed benefits may be the result of lead-time bias (55). The stage at diagnosis may be more dependent on the biological behavior of the tumor (aggressiveness) than on delay in presentation, and early detection may not have a significant impact on mortality. An increase in the proportion of localized prostate cancers that are being treated may account for some of the change in the mortality statistics (51).

PSA in patient management

The optimal treatment of early stage disease has yet to be established. Treatment options include expectant management (watchful waiting), radical prostatectomy, cryosurgery and radiation therapy (external beam radiation or brachytherapy). Patients with advanced metastatic disease are typically offered hormonal therapy to deprive the prostate of androgen stimulation. PSA synthesis by differentiated prostate cells is typically impaired by such treatment and circulating PSA levels reflect tumor burden differently than before androgen deprivation. When the disease becomes refractory to androgen deprivation, patients may be entered into experimental chemotherapy protocols. PSA plays a cardinal role in all aspects of the management of prostate cancer from surveillance to selection of optimal treatment to estimation of prognosis to post-therapeutic monitoring. Free PSA measurement offers no advantages over total PSA in the follow-up of prostate cancer (56).

The treatment selected after detecting prostate cancer depends critically on whether the disease is confined to the prostate Radical prostatectomy is an option only for patients with organ-confined disease. However, the extent of disease is difficult to predict accurately. PSA alone is not informative (57), but in combination with the clinical stage and Gleason score predicts reasonably well the pathological stage of localized prostate cancer. Predictive tables that incorporate these parameters have been published (58-60) and are used by physicians to estimate the probability of organ-confined disease and to determine whether radical prostatectomy is indicated.

Following successful surgery, PSA should decrease to undetectable levels (61-62). Persistently elevated PSA provides evidence of residual disease. However, the converse does not always hold, namely that undetectable PSA post-op indicates a surgical cure. Considerable time may elapse before residual disease becomes evident through detectable PSA. Most commonly, residual disease will declare within three years of surgery. About 35% of the men who undergo radical prostatectomy present with residual disease in the first ten years after surgery.

A rising PSA level after radical prostatectomy is a biochemical sign of recurrent disease that typically predates other signs of progression by many years. However, not all patients with biochemical recurrence will progress to symptoms of clinical disease and metastatic spread in their lifetimes and require treatment (43, 63). Factors reported to predict the time course to the development of metastatic disease include time to biochemical recurrence, tumor grade (Gleason score), and PSA-doubling time (22, 27). These parameters can be used to estimate the likelihood of patients remaining free of overt metastatic disease and allow physicians to stratify patients into low-risk and high-risk categories and to make better treatment decisions.

Monitoring response after initial treatment and evaluating outcome during subsequent therapy are significant clinical applications of PSA determinations. Measurement of PSA provides essential information about the efficacy of surgery or radiation therapy, helps establish the possibility of residual disease (local or distant), signals recurrent metastatic disease before it can be detected by other conventional diagnostic procedures, and provides a useful adjunct in the evaluation of therapeutic response.

PSA may provide the earliest measure of treatment efficacy or disease recurrence, and as such influence the patient's perception of well-being. For some patients, it may be most appropriate to stop measuring PSA, particularly if effective alternative treatments to counter adverse findings are not available (16).

Use of nomograms incorporating PSA to manage prostate cancer

Nomograms incorporating one or more factors provide the most accurate means of individualizing therapy and predicting outcome, and reflect the most recent advances in patient management (64). Rather than relying on physician experience or general risk assessments of patient populations with similar characteristics, the nomograms assess treatment options or prognosis based on computerized models of Cox proportional hazards regression analysis. Predictive outcomes provided by computer models are not perfect, but nomograms can be extremely useful in assisting with treatment decisions. On occasion, it may be difficult to select the best nomogram when several competing versions apply to the same clinical decision. Kattan and co-workers (64, 65) have developed pre- and post-operative nomograms, incorporating PSA together with Gleason score and other variables, in order to predict disease recurrence following radical prostatectomy.

CONCLUSIONS

Measurements of serum PSA markers have an important role in both diagnosis and management of patients with prostate cancer. Some key NACB recommendations relating to the optimal use of these markers are summarized in Table 4.

Table 1. Clinical use of PSA serum markers in the management of prostate

Marker	Application	NACB Recommendations 2005	LOE	References
PSA	Screening/Early Detection (with DRE)	Yes	I, III, IV	8, 53, 66, 67
	Early Detection: Age-Specific Reference Ranges	No	III	14, 68
	Early Detection: PSA Velocity	Yes	III	20, 69
	Staging	Yes[1]	I	59, 64, 65, 70
	Follow-up negative biopsy (with DRE)	Yes	III, IV	71-73
	Management and Monitoring	Yes	III	74, 75
	Prognosis	Yes	II, III, IV	63, 76-78
cPSA	Screening/Early Detection (with DRE)	Yes	I	35, 79
	Follow-up negative biopsy (with DRE)	Yes	III	80, 81
	Staging	Yes[1]	IV	82
	Management and Monitoring	Yes	IV	83
% fPSA	Differentiation of PCa and benign prostatic disease	Yes	I, III	26, 84
	Follow-up negative biopsy (with DRE) or patients with increased biopsy risk	Yes	III, IV	85-87

[1]As part of nomograms with DRE and biopsy Gleason grade (e.g. Partin tables or Kattan nomogram);

LOE, levels of evidence

Table 2. Recommendations by different Expert Groups for use of PSA, complexed PSA (cPSA) and percent free: total PSA (%fPSA) as tumor markers for prostate cancer

Marker	Application	ACS (10)	ACP (88)	ASTRO (74)	AUA (75)	EAU (89)	EGTM (90)	ESMO (91)	NACB/ EGTM 2002 (92)	NCCN (93, 94)	USPSTF (95)	NACB 2005
PSA	Screening/Early detection (with DRE)	**Yes**	**No[1]**	None published	**Yes**	**Yes**	**No[1]**	**No[2]**	**Yes (NACB)**	**Yes**	Ins	**Yes**
	Early detection: Age-specific reference ranges	None published	None published	None published	None published	None published	**No**	None published	**Yes (NACB)**	None published	None published	**No**
	Early detection: PSA velocity	None published	None published	None published	None published	None published	None published	None published	None published	**Yes**	None published	**Yes**
	Staging	None published	None published	None published	**Yes**	**Yes[3]**	None published	**Yes**	None published	**Yes[3]**	None published	**Yes[3]**
	Follow-up negative biopsy (with DRE)	None published	None published	None published	None published	None published	None published	None published	None published	**Yes**	None published	**Yes**
	Management and monitoring	None published	None published	**Yes[4]**	**Yes**	**Yes**	**Yes**	**Yes**	None published	**Yes**	None published	**Yes**
	Prognosis	None published	None published	None published	None published	None published	**Yes**	None published	None published	**Yes**	None published	**Yes**
cPSA	Screening/Early detection (with DRE)	None published	None published	None published	None published	None published	None published	None published	None published	**Yes**	None published	**Yes**
	Follow-up negative biopsy (with DRE)	None published	None published	None published	None published	None published	None published	None published	None published	**Yes**	None published	**Yes**
	Staging	None published	None published	None published	None published	None published	None published	None published	None published	None published	None published	**Yes**
	Management and monitoring	None published	None published	None published	None published	None published	None published	None published	None published	None published	None published	**Yes**
% fPSA [5]	Differentiation of PCa and benign prostatic disease	None published	None published	None published	None published	None published	**Yes**	None published	**Yes**	**Yes**	None published	**Yes**
	Follow-up negative biopsy (with DRE) or patients with increased biopsy risk	None published	None published	None published	None published	None published	None published	None published	None published	**Yes**	None published	**Yes**

ACS, American Cancer Society; ACP, American College of Physicians; ASTRO, American Society for Therapeutic Radiology and Oncology; AUA, American Urological Association; EGTM, European Group on Tumor Markers; ESMO, European Society for Medical Oncology; Ins, Insufficient evidence for recommendation; NACB, National Academy of Clinical Biochemistry; NCCN, National Comprehensive Cancer Network; PCa, Prostate cancer; USPSTF, U.S. Preventive Services Task Force.

[1] Not routinely, individual decision; [2] Except in men with urinary symptoms; [3] As part of nomograms with DRE and biopsy Gleason grade (Partin Tables); [4]Following radiation therapy; [5] In men with a total PSA of 4-10 μg/L and a negative DRE.

Table 3. Biomarkers currently being explored for prostate cancer[1]

	Proposed use or uses and comments	Phase of development	LOE	References
Circulating biomarkers				
PSA sub-fractions: complexed PSA, free PSA, proPSA, intact PSA, benign PSA	Absolute concentrations in serum and percentage relative to total PSA may help discriminate between malignancy and benign conditions.	Undergoing evaluation. [Clinical assays in development]	IV, V	96-98
Human kallikrein 2 (hK2)	Shares 80% amino acid sequence with PSA and is produced in prostatic epithelium at concentrations 50-100 times less than PSA. Generally elevated in prostate cancer vs BPH, and is more sensitive than PSA at detecting extracapsular extension.	Undergoing evaluation.	IV, V	98, 99
Insulin-like growth factor (IGF-1), insulin-like growth factor binding protein (IGFBP-3)	High serum IGF-1 concentrations associated with increased risk for prostate cancer. IGFBP-3 can be detected in tissue with ProstaScint; serum concentrations elevated in prostate cancer; discriminates between cancer and BPH or no disease; also being investigated as a therapeutic target.	Undergoing evaluation.	IV, V	100, 101
Molecular urine markers				
PCA3	Prostate-specific gene highly expressed in prostate cancer compared to other genitourinary tissues and non-neoplastic prostatic tissues. Urine assays measure PCA3 mRNA following an attentive DRE; the mRNA is non-coding, no protein products are made.	Undergoing evaluation. [Next generation ASR PCA3 test]	IV, V	102, 103
Alpha-methylacyl-CoA racemase (AMACR)	Mitochondrial and peroxisomal enzyme involved in oxidation; over-expressed in prostate cancer; detected in tissue by IHC, and in conjunction with loss of basal cell markers (e.g. basal cytokeratins, p63) can help establish diagnosis of cancer on prostate needle biopsy. Assays to detect a humoral response may supplement PSA screening in identifying significant tumors.	Undergoing evaluation (urine and tissue).	IV, V	104-108
Glutathione S-transferase-pi (GSTPi)	Protects cells from oxidative damage; reduced expression in prostate cancer due to hypermethylation of its promoter region; distinguishes between BPH and cancer; methylation status of GSTPi gene promoter quantified in prostatic tissue, cells derived from serum, urine and seminal plasma by PCR.	Undergoing evaluation in a clinical trial	IV, V	109, 110
Methylation panel	Hypermethylation of a panel of markers in combination with histology may aid in prostate cancer diagnosis; aberrant methylation profiles in prostate tissue samples correlated with clinicopathological features of poor prognosis.	Undergoing evaluation. [ASR in development].	IV, V	111, 112
Telomerase activity	Telomerase activity is detectable in the vast majority of prostate cancers but not in benign prostate tissues. Improved methods of telomerase detection may make this marker useful for early detection of prostate cancer in tissue samples or in urine.	Undergoing evaluation.	IV, V	113, 114

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Table 3, continued.

	Proposed use or uses and comments	Phase of development	LOE	References
Cell / Gene tests				
Circulating prostate cells RT-PCR gene targets PSA, hK2, and PMSA mRNAs	Measurements of the frequency in the shedding of circulating prostate/tumor cells in blood – using RT-PCR assays for PSA-, hK2- and/or PSMA-mRNAs – as a means to define invasive and/or systemic disease stage.	Undergoing evaluation in a clinical trial	IV, V	96, 115
PTEN	A lipid phosphatase that functions as a tumor suppressor by inhibiting the phosphatidylinositol 3-kinase/protein kinase B (P13K/Akt) signaling pathway. Gene somatically deleted or mutated in some prostate cancers. Protein can be detected by IHC and decreased levels are associated with higher grade and stage	Undergoing evaluation	IV, V	116, 117
CDKN1B (P27)	Cyclin-dependent kinase inhibitor. Protein decreased in prostate tumor cells and levels correlated with worse outcome.	Undergoing evaluation	IV, V	118,119
Ki-67	Marker of cellular proliferation. Fractions of cells staining positive by IHC associated with worse outcome.	Undergoing evaluation	IV, V	120
Chromosome 8p22 loss and 8q24 (C-MYC) gain	Bq24 over-representation, especially in combination with loss of 8q22 using a FISH assay, is associated with prostate cancer progression in men with stage pT2N0M0, pT3N0M0 and pT23N1-3M0 prostate cancers.	Undergoing evaluation	IV, V	121
Prostate stem cell antigen (PSCA)	Cell surface protein found primarily in the prostate; increased expression in many higher-grade prostate cancers and most metastatic lesions; correlated with late-stage disease; detection in prostatic tissue via FISH, PCR, IHC.	Undergoing evaluation	IV, V	122

BPH, benign prostatic hyperplasia; FISH, fluorescence *in situ* hybridization; IHC, immunohistochemistry; LOE, levels of evidence; PCR, polymerase chain reaction.

[1]Table based on Table 3 of the Prostate Cancer Foundation *Report to the Nation on Prostate Cancer, 2004* [http://www.prostatecancerfoundation.org – accessed 17th July 2005]

Table 4. Summary of NACB Recommendations for use of PSA in prostate cancer

Clinical decision limits • Given the controversy regarding the use of PSA to detect very small tumors, reported benefits arising from lowering the clinical decision limit for biopsy below 4 µg/L are too uncertain to mandate any general recommendation. Cut-points lower than the commonly-used 4 µg/L limit will increase sensitivity with a concomitant decrease in specificity unless other adjunctive tests or measures are employed to increase specificity. Conversely, use of clinical decision limits for PSA higher than 4.0 µg/L decreases the sensitivity, which results in the missed diagnoses of clinically significant tumors in men who might potentially benefit from early treatment.
Use of percent free PSA • The use of percent free PSA is recommended as an aid in distinguishing prostate cancer from BPH when the total PSA level in serum is in the range of 4-10 µg/L and DRE is negative, most frequently in men undergoing repeat biopsy. This recommendation is tempered by the need for proper validation of the medical decision limits for each combination of free and total PSA assays within each institution.
PSA in monitoring patients with prostate cancer • PSA is recommended for management of patients with prostate cancer to monitor disease status following treatment.
Pre-analytical requirements – prostate manipulation • Blood should be drawn before any manipulation of the prostate and several weeks after resolution of prostatitis.
Pre-analytical requirements – sample handling • Samples should be centrifuged and refrigerated within three hours of phlebotomy. • Samples may be stored at refrigerated temperatures for up to 24 hours. • Samples that will not be analyzed within 24 hours of collection should be stored frozen (at least at -20°C, and preferably at -30ºC or lower). • For long-term storage, samples should be frozen at -70°C or lower
Analytical requirements • The lowest reportable concentration should be determined by the laboratory and reported to physicians. Quality control at such levels should be established. The contribution of within-individual biological variation (which may be quite high at these low concentrations should also be taken into account.
Post-analytical requirements – information to be included on each report of clinical results • A statement that PSA for the early detection of prostate cancer should be used in conjunction with digital rectal examination. • The name of the assay. • The functional sensitivity of the assay (concentration at which CV exceeds 20%) • A valid clinical decision limit (ie PSA cut-off or PSA “reference” interval) specifically generated for the assay used and determined in collaboration with requesting clinicians. Ethnic and/or regional differences between reference interval populations should be taken into account.

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