Clinical Chemistry 52:6
1089–1095 (2006)

Cancer Diagnostics

APTIMA PCA3 Molecular Urine Test: Development of a Method to Aid in the Diagnosis of Prostate Cancer

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Background: **Prostate cancer gene 3 (*PCA3*) encodes a prostate-specific mRNA that has shown promise as a prostate cancer diagnostic tool. This report describes the characterization of a prototype quantitative *PCA3*-based test for whole urine.**

Methods: **Whole-urine specimens were collected after digital rectal examination from 3 groups: men scheduled for prostate biopsy (n = 70), healthy men (<45 years of age with no known prostate cancer risk factors; n = 52), and men who had undergone radical prostatectomy (n = 21). PCA3 and prostate-specific antigen (PSA) mRNAs were isolated, amplified, and quantified by use of Gen-Probe DTS400® Systems. Prostate biopsy results were correlated with the PCA3/PSA mRNA ratio, and PSA mRNA concentrations were used to normalize PCA3 signals and confirm the yield of prostate-specific RNA. Assay precision, specimen stability, and mRNA yield were also evaluated.**

Results: **The specimen informative rate (fraction of specimens yielding sufficient RNA for analysis) was 98.2%. In this clinical research study, ROC curve analysis of prebiopsy specimens yielded an area under the curve of 0.746; sensitivity was 69% and specificity 79%. Serum PSA assay specificity was 28% for this same group. PCA3 and PSA mRNAs were undetectable in postprostatectomy specimens except for one man with recurrent prostate cancer. Assay interrun CVs were ≤12%. Both mRNAs were stable in processed urine up to 5 days at 4 °C and after 5 freeze–thaw cycles.**

Conclusion: **The APTIMA® PCA3 assay combines simple specimen processing with precise assays and existing instruments and could add specificity to the current algorithm for prostate cancer diagnosis.**

Use of the serum prostate-specific antigen (PSA)[3] test has led to the biopsy-based diagnosis of smaller, previously undetected tumors *(1)*, thus creating a new diagnostic dilemma: Only a fraction of men with increased serum PSA have detectable prostate cancer. Men with at least one negative biopsy often have persistently increased serum PSA, primarily attributable to enlarged prostates and benign prostatic hyperplasia (BPH). However, a significant proportion of men with slightly increased serum PSA (2.5–4.0 μg/L) either have, or will develop, clinically significant prostate cancer *(1)*. Although biopsy remains the gold standard for prostate cancer detection, more accurate tests with better specificity are needed to help guide decisions to biopsy the prostate.

Among the newer molecular biomarkers for prostate cancer diagnosis *(2–5)* is the product of prostate cancer gene 3 (*PCA3*),[4] a prostate-specific noncoding mRNA *(6)*. PCA3 is overexpressed in 95% of prostate cancers tested, with a median 66-fold up-regulation compared with adjacent nonneoplastic prostatic tissue *(4)*. In contrast, *KLK3*, the gene that encodes for PSA, is not up-regulated in

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Received November 8, 2005; accepted March 28, 2006.
Previously published online at DOI: 10.1373/clinchem.2005.063289

[3] Nonstandard abbreviations: PSA, prostate-specific antigen; BPH, benign prostatic hyperplasia; PCA3, prostate cancer gene 3; DRE, digital rectal examination; UTM, Urine Transport Medium; RLU, relative light unit; c/rxn, copies/reaction; and AUC, area under the curve.

[4] Human genes: *PCA3*, prostate cancer gene 3; *KLK3*, kallikrein 3 (prostate-specific antigen).

cancer *(7, 8)*, and PSA mRNA concentrations may therefore be used to normalize for the amount of prostate-specific RNA in molecular test samples. PCA3 expression is mostly undetectable in other tissues, including bladder and testis *(9)*. The feasibility of a quantitative PCA3-based molecular test for urine sediments has been demonstrated *(4)*.

The paradigm of direct detection of cancer cells in biological fluids is attractive because of the expected improvement in specificity compared with the current immunodiagnostic tests for surrogate markers such as serum PSA. This approach presents several challenges, however: The procedure for collecting and stabilizing specimens under standardized conditions must be defined. In addition, the method must be able to detect low concentrations of RNA in clinical samples [in previous molecular marker studies, ~20% of the clinical specimens contained insufficient prostate cells for testing *(4, 10)*]. Finally, the test must use a robust and reproducible approach that can be implemented in a clinical laboratory.

We describe here the development of prototype transcription-mediated amplification assays to quantify PCA3 and PSA mRNA in whole urine.

Materials and Methods

specimen source, collection, and processing

All urine specimens were obtained according to an Institutional Review Board–approved protocol from the Urological Sciences Research Foundation (Culver City, CA). Written informed consent was obtained from all study participants. Specimens were collected between July 28, 2004, and November 13, 2004. Biopsies were performed by licensed urologists within 6 months of specimen collection. APTIMA® PCA3 testing was performed at Gen-Probe in March 2005 by 4 trained technicians who were not aware of the biopsy results at the time of testing.

The patient population consisted of 3 groups. The prebiopsy group consisted of 70 men scheduled for prostate biopsy because of increased serum PSA (≥2.5 μg/L) and/or abnormal diagnostic digital rectal examination (DRE) and/or other risk factors: mean (SD) age, 67 (11) years (range, 45–93 years); serum PSA, 7.7 (14.1) μg/L (0.4–101.7 μg/L); prostate gland volume, 40.0 (17.2) cm^3 (8.6–92.8 cm^3). Biopsies were performed according to the procedure described by Marks et al. *(11)*. The second group (normal) included 52 men <45 years of age with no known prostate cancer risk factors; prostate biopsies were not performed on these men. Finally, urine specimens were also collected from 21 men who had undergone radical prostatectomy (postprostatectomy) at least 3 months before specimen collection. As additional negative controls, whole-urine specimens were collected from 6 women.

Male urine samples (20- to 30-mL first catch) were collected after an attentive DRE. The DRE was performed by applying firm pressure (enough to depress the prostate surface ~1 cm) from the base to apex and from the lateral to the median line for each lobe. Exactly 3 strokes per lobe were performed. Urine was kept on ice and processed within 4 h by mixing with an equal volume of detergent-based stabilization buffer (Urine Transport Medium; UTM), which lyses the cells and stabilizes the mRNA. The UTM was the same formulation that is found in the APTIMA Urine Specimen Collection Kit for Male and Female Urine Specimens (Gen-Probe; cat. no. 1040). The processed samples were shipped overnight on cold packs and stored at −70 °C for up to 8 months before testing. PCA3 and PSA mRNA stability in processed urine specimens was assessed by monitoring copy numbers after incubation at defined temperatures or after freeze–thaw cycles.

assay description

The quantitative APTIMA PCA3 assay uses Gen-Probe technologies *(12–15)*, including target capture (purification of target mRNA by hybridization to magnetic particles via target-specific oligonucleotides), transcription-mediated amplification (amplification of target RNA sequences), and a hybridization protection assay (specific detection of amplification products by use of target-specific acridinium ester–labeled probes). The components for the APTIMA PCA3 assay include analyte-specific (PCA3 and PSA) target capture, amplification, and probe reagents, as well as calibrators and controls. All other reagent formulations are identical to the corresponding Gen-Probe APTIMA Combo 2® reagents (package insert IN0037-05, Rev. A.1). The PCA3 and PSA assay protocols are identical and use the reagent addition volumes and incubation times and temperatures specified in IN0037-05 Rev. A.1 *(16)*. Assays were run with Gen-Probe DTS400® Systems; this allowed both PCA3 and PSA mRNA assays to be run in parallel by a single operator.

primers and probes

The PCA3 primers target exons 3 and 4, giving an amplification product of ~75 nucleotides that includes the splice junction *(6)*. The PCA3 probe spans the exon 3/4 junction; as such, the assay does not detect the unspliced forms of the PCA3 transcript. The PSA primers target exons 2 and 3 in the PSA mRNA *(17)*, and as in the PCA3 assay, unspliced mRNA is not detected.

calibrators, controls, and assay setup

Calibrators and controls consisted of PCA3 or PSA transcripts in detergent solution and were included in each assay run. Copy numbers were chosen to encompass the range of PCA3 and PSA mRNA concentrations observed in processed urine specimens (Table 1). Calibrators and controls were value-assigned with the PCA3 or PSA assays by use of reference transcripts at known copy concentrations (based on $A_{260\,nm}$ measurements). For assay precision studies, calibrators, controls, and test samples were run in triplicate. For determination of clinical sensitivity and specificity relative to prostate biopsy,

Table 1. Target copy numbers for PCA3 and PSA calibrators[a] and controls.

PCA3 calibrators			PSA calibrators, c/rxn		
c/rxn	Weight	PCA3 controls, c/rxn	c/rxn	Weight	PSA controls, c/rxn
0	1.00×10^{-4}		0	1.00×10^{-4}	
100	1.00×10^{-6}	500	3000	1.00×10^{-6}	15 000
1000	3.00×10^{-8}	5000	30 000	3.00×10^{-8}	150 000
10 000	5.00×10^{-10}	25 000	300 000	5.00×10^{-10}	600 000
50 000	7.00×10^{-11}		1 200 000	7.00×10^{-11}	

[a] For calibrators, the weight values used to generate the calibration curve are also shown.

processed urine specimens were tested in triplicate; these data were also analyzed to determine the impact of running a single replicate of each specimen. For calibrators and controls, each reaction contained 400 μL; for processed urine specimens, each reaction contained the equivalent of 200 μL of whole urine (200 μL whole urine + 200 μL of UTM).

TARGET CAPTURE

In a preamplification area, PCA3 or PSA Target Capture Reagent (100 μL) was added to strips of ten 12 × 75 mm polypropylene tubes held in racks. Well-mixed processed urine specimens or calibrators/controls (400 μL) were added, and the tubes were covered with sealing cards, vortex-mixed thoroughly on a multitube vortex-mixer, and incubated in a 62 °C water bath for 30 min. The racks were cooled at room temperature for 30 min and then placed on a Gen-Probe Target Capture System for 5–10 min to allow the magnetic particles to migrate to the sides of the tubes. The sealing cards were removed, and the liquid in the tubes was aspirated while the magnetic particles remained on the side of the tubes. Wash Reagent (1 mL) was added to each tube, and the tubes were covered, vortex-mixed for 10 s, and placed back on the Target Capture System. After 5 min, the Wash Reagent was aspirated, and the racks were transferred to the amplification area.

TRANSCRIPTION-MEDIATED AMPLIFICATION

PCA3 or PSA Amplification Reagent (75 μL) was added to each tube with a repeat pipettor, followed by Oil Reagent (200 μL). The tubes were covered, vortex-mixed for 10 s, and incubated in a 62 °C water bath for 10 min. The racks were transferred to a 42 °C water bath for 5 min. Enzyme Reagent (25 μL) was pipetted into each tube within 90 s while the rack remained in the water bath. The tubes were covered, shaken by hand to completely mix the contents, immediately returned to the 42 °C water bath, and incubated for 1 h.

DETECTION

The racks were transferred to a postamplification area, and PCA3 or PSA Probe Reagent (100 μL) was added to each tube. The racks were covered, vortex-mixed until thoroughly mixed (10–20 s), incubated in a 62 °C water bath for 20 min, and then allowed to cool at room temperature for 5 min. Selection Reagent (250 μL) was pipetted into each tube, after which the tubes were covered, vortex-mixed until thoroughly mixed (10–20 s), and incubated in the 62 °C water bath for 10 min. The tubes were cooled at room temperature for 15 min and then transferred to a Gen-Probe LEADER® HC+ Luminometer. The signal in relative light units (RLUs) was determined over a 3-s read time after addition of Auto Detect Reagents I and II.

DATA ANALYSIS

Both the PCA3 and PSA assays were run with corresponding 5-point calibration curves. The RLU data were transferred to an Excel spreadsheet for analysis. A weighted least-squares fit was used to generate a 4-parameter logistic dose–response curve for the 5 calibrator points with TableCurve 2D software (Systat; Ver. 5.01). The weight values used for each calibrator point are shown in Table 1. The curve [RLU vs (calibrator copies/reaction)] is defined by the equation $y = a + b/[1 + (x/c)^d]$, where x is the calibrator copies/reaction, y is RLU output, and a, b, c, and d are the 4 parameters defining the logistic dose–response curve as determined by the software. The values for a, b, c, and d were entered into the equation, which was then solved for copies/reaction (c/rxn) based on RLU values obtained for the controls and specimens. The normalized PCA3/PSA ratio result was calculated by use of the mean c/rxn of the 3 replicates of each target. Samples with copy numbers greater than the highest calibrator were diluted in APTIMA Transport Medium (detergent solution of the same formulation as the negative calibrator; also contained in the APTIMA Adaptor Kit; Gen-Probe cat. no. 1087) to bring the sample within the dynamic range of the assay and retested. The measured copy numbers for controls were compared with their known values to verify the results interpolated from the calibration curve.

Assay precision was assessed by use the method described in NCCLS EP5-A2 *(18)*. Confidence intervals for the area under the ROC curve were determined by a nonparametric approach using SAS/STAT software.

Results

ASSAY PRECISION

To assess the precision of the prototype assays, 18 assay runs were performed by 3 different operators using 3

different reagent lots and Gen-Probe DTS400 Systems. Three replicates of each calibrator, control, and test sample (previously characterized pooled processed urine specimens) were included in each run. The actual and mean measured RNA concentrations of each PCA3 and PSA control are shown in Table 2, as are the measured values as a percentage of the expected values (c/rxn divided by assigned c/rxn) and intrarun, interrun, and total CVs. The measured values of all controls were within 11% of the target values, and interrun CVs ranged from 4% to 12%. The results obtained for the pooled processed urine specimens are shown in Table 3. Interrun CVs for PCA3 and PSA mRNA were similar to those obtained for the assay controls, and interrun CVs for the PCA3/PSA ratio were 15%–24%.

specimen stability

The stability of PCA3 and PSA mRNA in processed urine was evaluated by use of 3 clinical specimens. Frozen processed urine specimens were thawed, then stored at 4 °C or 30 °C for 2 weeks and assayed at different time points (Fig. 1). Data are shown as the mean of the c/rxn values obtained for the 3 specimens. At 4 °C, mRNA copy numbers remained constant for up to 5 days. Degradation of PCA3 and PSA mRNA was observed at 30 °C; the SD also increased, reflecting the variability in room temperature stability from specimen to specimen (Fig. 1, A and B). The PCA3/PSA ratio remained within 20% of initial for all 3 samples up to 2 weeks at 4 °C (Fig. 1C). PCA3 and PSA mRNAs were also stable in processed urine specimens (n = 4) after 5 freeze–thaw cycles (data not shown).

pca3 and psa mRNA yield from cultured cells and urine

On average, 200 μL of whole urine yielded ~500 000 copies of PSA mRNA (median, 186 198). PCA3 mRNA copy numbers were 20- to 30-fold lower (mean and median of ~22 000 and 5000 c/rxn, respectively). The informative rate from prebiopsy and healthy controls, defined as the fraction of specimens that yielded sufficient PSA mRNA for analysis, was 98.2% (110 of 112). As a point of reference, PCA3 and PSA mRNA concentrations in the LNCaP prostate carcinoma cell line were also measured. On average, LNCaP cells contained ~1000 to 2000 copies of PSA mRNA/cell; these results are in good agreement with previously published data *(19)*. PCA3 concentrations in LNCaP cells were much lower (~1 to 10 copies/cell), which is consistent with the fact that PCA3 promoter activity has been shown to be relatively weak in this cell line *(20)*.

mRNA yield from postprostatectomy samples was also determined. Twenty of the 21 specimens yielded PCA3 and PSA assay signals at or near background values, and mRNA copy numbers were below the amount required for analysis. The remaining postprostatectomy specimen yielded PCA3 and PSA mRNA c/rxn values of 2368 and 42 744, respectively, and a PCA3/PSA mRNA ratio of 55×10^{-3}. Follow-up indicated that during the 4 months between the radical prostatectomy and collection of urine for PCA3 testing, this patient had a recurrence and became biopsy-positive. The analytical specificity of the assay was also confirmed by testing 6 female urine samples; all gave RLU values within background (not shown).

pca3/psa ratio and correlation with biopsy results

The prebiopsy population tested included 70 males. Of the 68 that yielded informative specimens, 16 biopsies were positive for prostate cancer, and the remaining 52 were biopsy-negative; 40 of the 52 biopsy-negative males had BPH and/or inflammation. The PCA3/PSA mRNA ratio values obtained for different subject groups are shown in Fig. 2. The median ratios (PCA3/PSA $\times 10^{-3}$) for the healthy, biopsy-negative, and biopsy-positive groups were 4.5, 27.0, and 81.8, respectively. The mean PCA3/PSA mRNA ratios of these 3 groups were significantly different ($P < 0.01$).

ROC curve analysis was performed on the prebiopsy specimens with PCA3/PSA mRNA ratio used as the diagnostic indicator and biopsy as the comparison method (Fig. 3). The area under the curve (AUC) was 0.746 (95% confidence interval, 0.574–0.918). The use of PCA3 copy numbers alone gave an AUC of only 0.575 (not shown), demonstrating the value of normalizing for

Table 2. PCA3 and PSA assay precision (n = 18 assay runs, 3 replicates each sample/run).[a]

	Copy number (c/rxn)	Mean measured c/rxn	Percentage of expected value	CV, %		
				Intrarun	Interrun	Total
PCA3 control						
A	472	508	108	13	12	19
B	4559	4639	102	8	7	11
C	22 395	24 407	109	3	4	6
PSA control						
A	18 300	17 921	98	6	7	8
B	187 654	175 615	94	10	5	8
C	707 604	786 468	111	5	7	8

[a] For each PCA3 and PSA control, the assigned copy number, mean measured c/rxn, measured c/rxn divided by assigned c/rxn, and CVs are shown.

Table 3. Assay precision calculated with pooled processed urine specimens (n = 18 assay runs, 3 replicates each sample/run).

	PCA3 mRNA		PSA mRNA		PCA3/PSA ratio	
Sample	Mean c/rxn	Interrun CV, %	Mean c/rxn	Interrun CV, %	Mean ratio ($\times 10^{-3}$)	Interrun CV, %
Pool 1	101	20	21 079	10	4.9	24
Pool 2	2821	12	86 116	11	33.2	17
Pool 3	12 656	9	426 855	11	30.1	15

[a] For each pool, the mean PCA3 and PSA mRNA copy numbers and PCA3/PSA ratio are shown, along with the interrun CV.

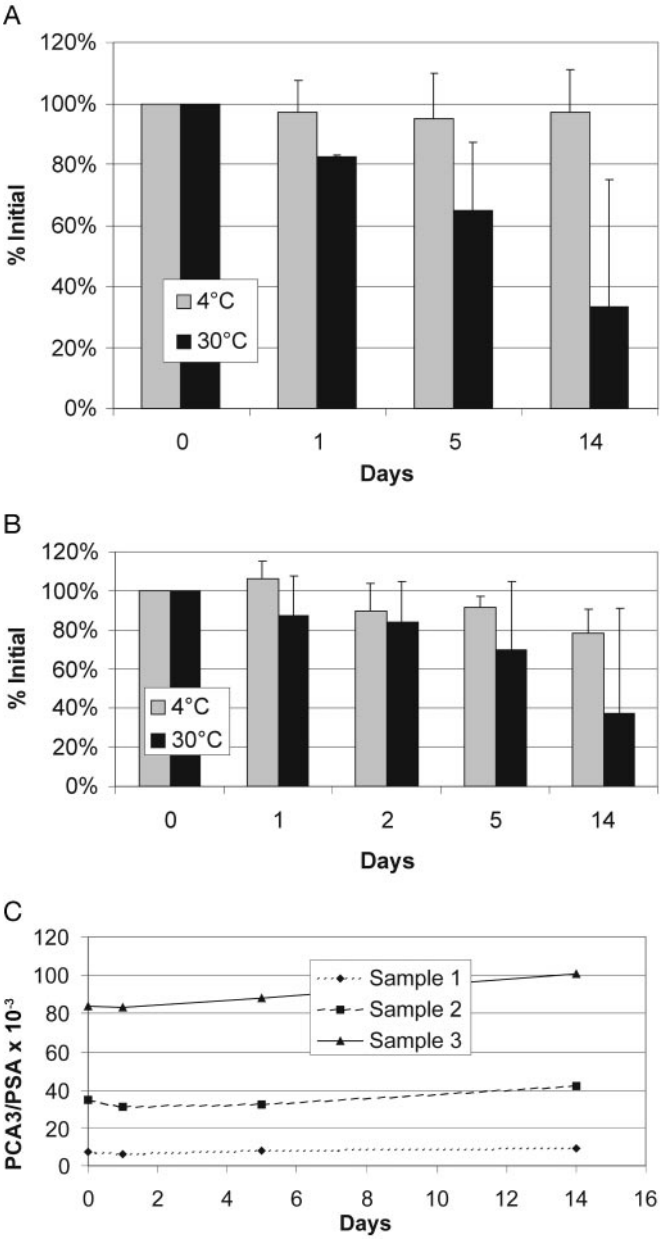

Fig. 1. PCA3 and PSA mRNA stability.

Three processed urine specimens were incubated at 4 °C or 30 °C for 2 weeks and assayed at the time points indicated. The mean copy number for all 3 specimens divided by the mean copy number at day 0 (*% Initial*) is shown for PCA3 mRNA (*A*) and PSA mRNA (*B*) in processed urine. (*C*), PCA3/PSA ratio for the individual specimens stored at 4 °C. *Error bars*, SD.

prostate-specific mRNA concentration by use of the concentration of PSA mRNA. The ROC curve was used to determine sensitivity and specificity relative to prostate biopsy at different PCA3/PSA mRNA ratio cutoffs. At a cutoff of 50×10^{-3}, sensitivity was 69% and specificity 79% (Table 4). Increasing the cutoff to 80×10^{-3} decreased sensitivity to 56% but increased specificity to 94%. For comparison, the serum PSA assay (cutoff, 2.5 μg/L) yielded a specificity of 28% and sensitivity of 81% for this same study group; at 69% sensitivity, serum PSA assay specificity was 60% vs 79% for the APTIMA PCA3. Importantly, the clinical performance of the APTIMA PCA3 assay for prebiopsy samples from men with serum PSA in the "gray zone" of 2.5 to 10 μg/L (n = 44) was similar to that obtained for the entire prebiopsy group (sensitivity, 69%; specificity, 83%). To evaluate the impact of including disease-free persons on clinical performance, we also performed ROC analysis by adding the healthy

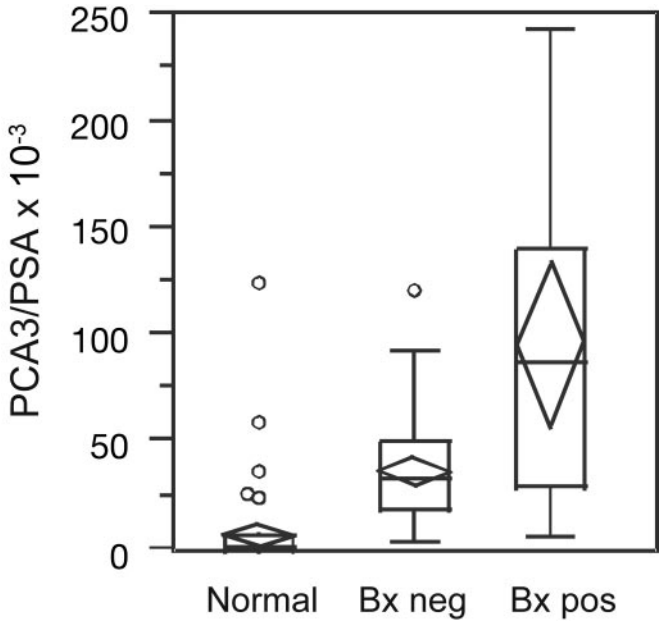

Fig. 2. Box plots of the PCA3/PSA ratio distribution in specimens from healthy (*Normal*; n = 52), biopsy-negative (*Bx neg*; n = 52), and biopsy-positive (*Bx pos*; n = 16) persons.

The *limits* of the *boxes* are the 25th and 75th quantiles (lower and upper quartiles). The *vertical lines* correspond to (1.5 × interquartile range); outliers that exceed this range are shown as *open circles*. The *horizontal line inside* each *box* represents the median PCA3/PSA ratio for the group. The *diamonds* represent the mean (*sides*) and 95% confidence intervals (*top* and *bottom*).

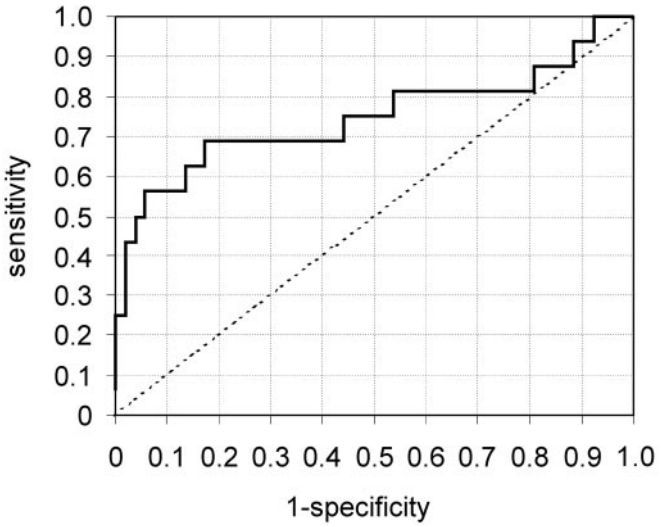

Fig. 3. ROC curve for the assay.

The ROC curve is a plot of sensitivity as a function of false-positive rate (1 − specificity). PCA3/PSA mRNA ratios for the prebiopsy population were analyzed with prostate biopsy used as the comparison method. The area under the curve was 0.746 (95% confidence interval, 0.574–0.918).

group to the biopsy-negative group. The AUC increased from 0.746 to 0.825, sensitivity was 69%, and specificity improved to 89%.

These clinical specimen results were obtained by use of the means of 3 replicates to determine the PCA3/PSA ratio. To determine whether accurate results could be obtained by use of a single replicate of each specimen, we randomly combined the 3 PCA3 and PSA replicates to generate all 9 possible single-replicate ratios. For 59 of the 68 informative specimens (87%), all 9 possible single-replicate PCA3/PSA ratios yielded the same overall interpretation (above or below the cutoff of 50×10^{-3}) as the mean. The remaining 9 specimens (13%) had PCA3/PSA ratios near the cutoff or some PCA3 or PSA replicates that were outliers.

Discussion

The need for prostate cancer diagnostic tests with improved specificity has been well recognized. As a prostate-specific mRNA that is highly overexpressed in prostate cancer cells, PCA3 is an ideal target. Because PCA3 is also expressed in noncancer cells, its content in clinical specimens must be normalized to the amount of prostate-derived RNA. This is achieved by use of the ratio of PCA3/PSA mRNA concentrations as the diagnostic indicator. PSA mRNA yield is also used to verify that the amount of RNA present is sufficient to yield an accurate result.

Preliminary evaluation of PCA3 and PSA mRNA stability in processed urine indicated that specimens may be held for 5 days at 4 °C. It should be noted that at 30 °C, mRNA stability varied significantly from specimen to specimen; therefore, all shipments from the collection site were overnight on cold packs and specimens were frozen at −70 °C on receipt to ensure integrity. The freeze–thaw stability data indicate that frozen specimens may be thawed and retested several times without a negative impact on assay results.

When we used prostate biopsy as the comparison method, the APTIMA PCA3 assay yielded an AUC_{ROC} of 0.746; sensitivity was 69% and specificity 79% at a PCA3/PSA mRNA ratio cutoff of 50×10^{-3}. This specificity was higher than that for the serum PSA assay for the same study group (28% at cutoff of 2.5 μg/L). Mean PCA3/PSA ratios were significantly different for the healthy, biopsy-negative (including BPH), and biopsy-positive patients. The inclusion of healthy (presumed to be disease-free) persons in the ROC analysis increased the AUC to 0.825 and also improved specificity to 89%; this is consistent with the fact that the mean PCA3/PSA ratio was significantly lower for the healthy group. Although these results are encouraging, data from other ongoing studies are needed to fully establish the clinical utility of the assay.

APTIMA PCA3 clinical performance was similar to that observed in previous studies that used reverse transcription-PCR and urine sediments. Using a quantitative PCA3/PSA ratio, Hessels et al. *(4)* reported an AUC_{ROC} of 0.717 with a sensitivity of 67% and specificity 83% in a cohort of 108 prebiopsy patients. Fradet et al. *(10)* reported a sensitivity and specificity of 66% and 89%, respectively, although direct comparison with that study is less straightforward because their method was semiquantitative and used a more complex data reduction algorithm. The APTIMA PCA3 assay was able to detect recurrence of prostate cancer in 1 man after radical prostatectomy. PCA3 and PSA mRNA concentrations were nearly undetectable in the remaining postprostatectomy specimens (RNA concentrations less than the minimum for informative specimens); these data provide preliminary confirmation of the prostate tissue specificity of the urine assay, using a known negative study population without prostate glands. The results obtained with female urine specimens also verify the analytical specificity of the assay.

The APTIMA PCA3 assay provides several advantages over other PCA3 urine tests *(4, 10)*. The ability to use whole urine (as opposed to urine sediments) simplifies the specimen-processing procedure. Processing is complete with the addition of UTM; specimens can then be frozen and stored at the collection site. Reverse transcription-PCR methods require stabilization of the cells in whole urine and immediate shipment to the testing site. The target-capture technology used in the APTIMA PCA3

Table 4. Two-by-two table for PCA3/PSA ratio cutoff of 50×10^{-3}.

	Biopsy positive	Biopsy negative
APTIMA PCA3 positive	11	11
APTIMA PCA3 negative	5	41

assay is more quantitative and user-friendly than are the RNA extraction methods required for reverse transcription-PCR. Perhaps most importantly, the informative rate of 98.2% is significantly higher than those obtained with the other methods. This is a key point because patients must be called in for another sample if the first specimen is noninformative. The APTIMA PCA3 assay can be completed in <6 h with instrumentation currently used for several US Food and Drug Administration–cleared products. The robustness of PCA3 and PSA mRNA assays has been demonstrated across operators, reagent lots, and equipment/instrument sets and yielded interrun CVs of 20% or less for assay controls and processed urine specimens. The CV for PCA3/PSA ratio in specimen pools was ≤24%.

In summary, a relatively simple specimen-processing procedure and a robust assay that can be run on an existing platform have been developed. Clinical specimen testing to date has yielded specificity greater than that for the serum PSA assay. Studies are ongoing to evaluate larger and more defined populations and to compare the performance of the PCA3 molecular urine test with established prostate cancer markers, including total serum PSA and free PSA. The prototype APTIMA PCA3 assay yielded encouraging results in this study and warrants further evaluation as an aid in the diagnosis of prostate cancer.

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