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A multicenter evaluation of the PCA3 molecular urine test: Pre-analytical effects, analytical performance, and diagnostic accuracy

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Abstract

Background: Measurement of prostate cancer gene 3 (PCA3) mRNA normalized to prostate-specific antigen (PSA) mRNA in urine has been proposed as a marker for prostate cancer.
Methods: We investigated pre-analytical effects, analytical performance, and diagnostic accuracy of a quantitative assay for PCA3.
Results: Urine specimens collected without prostate manipulation demonstrated low informative rates. However, specimens collected following digital rectal examinations of 3 or 8 strokes per prostate lobe demonstrated informative rates >94%. Across all urine specimen types, median PCA3 results did not show statistically significant differences ($P>0.8$). Measurements of controls of known mRNA content demonstrated percent recoveries of 100±15% for both PCA3 and PSA mRNAs. PCA3 mRNA total, intra-assay, inter-assay, and inter-site CVs were ≤17.1%, ≤14.0%, ≤9.9%, and ≤3.2%, respectively. Corresponding CVs for PSA mRNA assay were ≤11.5%, ≤8.6%, ≤7.9%, and ≤8.3%. Blinded assay of urines from 72 men with known prostate biopsy outcomes yielded areas under the curve from receiver-operating characteristic analysis of 0.7 at both research sites. Deming regression of individual PCA3 results between sites yielded slope=0.94, intercept=0.48, $R=0.9677$ ($P<0.0001$).
Conclusions: The PCA3 assay is insensitive to pre-analytical factors, performs well analytically and correctly classifies a high percent of subjects with known prostate cancer status across research sites.

Keywords: PCA3; PSA; Prostate cancer; Molecular urine test

1. Introduction

In the past two decades, clinical assessment of men at risk for prostate cancer has evolved markedly. The modern standard of care includes annual digital rectal examinations (DRE) and, beginning at about age 50 y [1], annual measurement of serum total prostate-specific antigen (tPSA) in men with a life expectancy of at least 10 y [2]. However, serum PSA levels can be increased in benign conditions; thus, a need for prostate markers that are more cancer-specific has been expressed [3].

Prostate cancer gene 3 (PCA3), also known as $PCA3^{DD3}$ or $DD3^{PCA3}$, is a new prostate-specific gene that is highly over-expressed in prostate cancer tissue. It was first identified using differential display analysis comparing mRNA expression in normal and tumor-bearing prostate tissues [4]. It was found to be undetectable in non-prostate normal and tumorous tissues (including bladder and testis) and cancer cell lines from bladder,

Abbreviations: PCA3, Prostate cancer gene 3; RLU, Relative light units; DRE, Digital rectal examination; LOB, Limit of blank; TMA, Transcription-mediated amplification; FMV, First morning void; CLSI, Clinical and Laboratory Standards Institute; NASBA, Nucleic acid sequence-based amplification.

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breast, kidney, and ovaries [4]. In addition, PCA3 has very low expression in normal and benign prostate tissues [5]. A quantitative real-time reverse transcription PCR research method [6] subsequently developed and applied to prostate tissues demonstrated very high sensitivity and specificity with over-expression in >95% of primary prostate cancer specimens and in prostate metastasis. Further application of this method to sedimented urines collected from men with prostate carcinomas following prostate massage supported the potential clinical value of the marker [6]. Subsequently, a commercially available highly sensitive transcription-mediated amplification (TMA) method (PCA3, Gen-Probe Incorporated) has become available [7]. The method measures both PCA3 messenger RNA (mRNA) and PSA mRNA in first-catch urines collected following a DRE. The function of the DRE is to enhance the release of prostate cells through the prostate duct system into the urinary tract and thus into the urine.

It is intended that the PCA3 assay will be used in conjunction with serum PSA measurements and DRE in men undergoing evaluation for prostate cancer as an aid in the decision to perform prostate biopsy. To assess the capability of the marker to fulfill this clinical role, an evaluation of the performance of the PCA3 TMA method at 2 independent laboratories has been performed. Specific objectives of this study included evaluations of pre-analytical effects of urine specimen collection (first morning void vs. pre- and post-DRE) and DRE procedural methods (none, attentive, and aggressive); analytical performance parameters; and ability of each research site to accurately classify patients of known prostate cancer status, and to so classify in concordance and correlation with each other.

2. Materials and methods

2.1. Research sites

Two research sites participated in the study: the clinical chemistry division of the pathology department at Johns Hopkins Medical Institutions in Baltimore, MD (site 1) and the department of urology at the University of Washington in Seattle, WA (site 2). A single technologist was trained in running the assay at each site and subsequently performed PCA3 assays to evaluate analytical performance parameters and classification accuracy following a standardized assay protocol and using common reagents.

2.2. Pre-analytical effects

Separate from the analytical performance testing and classification accuracy evaluations occurring at the 2 research sites, pre-analytical effects were investigated in a concurrently performed study at site 2 by collection of urine specimens from apparently healthy men ($n=50$), men scheduled for prostate biopsy ($n=99$), and men scheduled for prostatectomy ($n=30$). The population included 144 Caucasian subjects, 10 African Americans, 7 Asians, 4 subjects of Hispanic descent, and 14 subjects of unspecified ethnicity. The population ranged in age from 40.3–88.3 y (median=62.0 y), and their serum tPSA ranged from 0.2–500 ng/ml (median=4.3 ng/ml). Table 1 presents the demographic and serum tPSA data by the 3 sub-populations of subjects (apparently healthy, prostate biopsy, and prostatectomy). Each subject was asked to provide a first morning void (FMV) specimen and a pre-DRE specimen. Additionally, within each sub-population of subjects, equal numbers of subjects were assigned to provide a urine specimen either following an attentive DRE of exactly 3 strokes per prostate lobe or a more aggressive DRE of exactly 8 strokes per prostate lobe. The DRE of 3 strokes per prostate lobe was previously established as the standard protocol for the assay [7]. Specimens were obtained according to an Institutional Review Board-approved protocol under informed consent. Specimens were stored at −70 °C from 0.3–8.9 months (median=1.5 months) and were shipped frozen. For this evaluation only, specimens collected were analyzed in the PCA3 assay at Gen-Probe Incorporated.

2.3. PCA3 molecular urine assay

The PCA3 assay (Gen-Probe Incorporated) quantitatively measures PCA3 and PSA mRNA using nucleic acid amplification. The PCA3 and PSA mRNA measurements use like protocols and reagents with components specific for the 2 analytes. The PCA3 assay was performed as previously described [7]. In brief, the assay employs target capture via purification of target mRNA by hybridization to magnetic particles coated with target-specific oligonucleotides, transcription-mediated amplification of target RNA sequences, and a hybridization protection assay to specifically detect amplification products using probes labeled covalently with acridinium ester dyes. The method detects both PCA3 mRNA and PSA mRNA. PSA mRNA is used as a housekeeping gene to which PCA3 mRNA copy numbers are normalized and from which the PCA3 Score

Table 1
Study populations

	Pre-analytical effects study population				Diagnostic classification accuracy population
	Overall population	Apparently healthy sub-population	Sub-population scheduled for prostate biopsy	Sub-population scheduled for prostatectomy	
N	179	50	99	30	72
Median age, yrs	62.0	56.3	64.1	61.8	61.6
Age range, yrs	40.3–88.3	40.4–70.9	40.3–88.3	44.1–72.8	43.9–79.1
Median serum tPSA, ng/ml	4.3	0.6	5.5	5.4	7.0
Serum tPSA range, ng/ml	0.2–500	0.2–1.0	0.6–500	0.6–19.0	0.38–31.1
# Caucasians (%)	144 (80.4)	40 (80.0)	81 (81.8)	23 (76.7)	67 (9.3)
# African Americans (%)	10 (5.6)	5 (10)	5 (5.1)	0 (0)	1 (1.4)
# Asians (%)	7 (3.9)	0 (0)	6 (6.1)	1 (3.3)	1 (1.4)
# Hispanics (%)	4 (2.2)	3 (6.0)	1 (1.0)	0 (0)	0 (0)
# Unspecified (%)	14 (7.8)	2 (4.0)	6 (6.1)	6 (20.0)	3 (4.2)

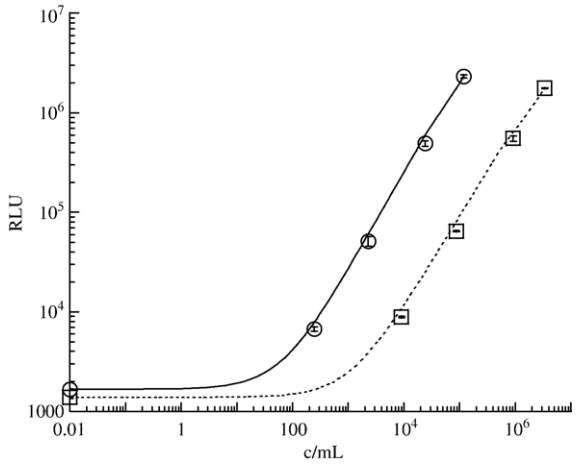

Fig. 1. Representative calibration curves for PCA3 mRNA measurements (○) and PSA mRNA measurements (□). Error bars = 1 standard deviation.

(PCA3 mRNA/PSA mRNA × 1000) is determined. PSA mRNA is suited to this task as its expression is relatively constant in normal prostate cells and only a weak (~1.5 fold) down-regulation of expression occurs in prostate cancer [6]. Primers for PCA3 are directed to exons 3 and 4 of the gene and the PCA3 probe spans the exon 3/4 junction. Primers for PSA target exons 2 and 3 in the PSA mRNA. For both PCA3 and PSA, unspliced mRNA is not detected.

The calibrators and controls used in this study were in-vitro transcripts of PCA3 and PSA in a buffered detergent solution. They were value-assigned using reference transcripts at known copy levels, based on A260 nm measurement. Five calibrators were used for each assay at levels of 0 copies (c)/ml, 245, 2310, 23,957, and 118,835 c/ml for PCA3 and 0, 9,082, 87,342, 921,365, and 3,402,182 c/ml for PSA, respectively. Measurements of calibrators, controls, and specimens were made in triplicate. The raw output from each assay is in relative light units (RLU).

2.4. Analytical performance

Accuracy was assessed for both the PCA3 and PSA mRNA measurements via analysis of 3 controls of known PCA3 and PSA mRNA copy numbers in 5 determinations per assay over 20 non-consecutive days of testing with 1 run per day of testing at each site. Intra-assay, inter-assay, and inter-site precision was evaluated for both PCA3 and PSA mRNA measurements via analysis of 3 controls in 5 replicates per assay over a period of 20 non-consecutive days of testing with 1 run per day of testing at each site. The limits of blank (LOB, the highest measurement likely to be observed for a blank sample), limits of detection (LOD, the lowest amount of analyte in a sample that can be detected with type I and II error rates set to 5%), and limits of quantitation (LOQ, the lowest amount of analyte in a sample that can be reliably detected and at which the total error meets the pre-specified requirement for accuracy) were determined according to CLSI EP-17A for both assays at both research sites. In this study, the limits of blank were determined as the 95th percentiles of replicate determinations of the 0 calibrator ($n=5$ in each of 12 assays over 12 non-consecutive days) in both the PCA3 and PSA mRNA assays. Raw RLUs from the assays were converted to apparent c/ml based on the calibration curve for each assay and the LOB subsequently estimated from the mean RLU value from the 60 determinations. LODs were estimated from 5 determinations of control samples in each of 12 assays over 12 non-consecutive days of testing. The control samples with the lowest measurable concentration of PCA3 or PSA mRNA were used for the LOD estimation. For the LOQ analyses, the prospectively-defined goals for accuracy were <130% recovery and <35% CV. If the measurements at the LOD satisfied these requirements, then the LOQ was set equal to the LOD. If not, then the next highest level of samples tested was evaluated for conformance to these requirements. This process was repeated until the requirements were met.

Table 2
Effects of specimen and DRE type on PCA3 Scores

	N	Informative rate (%)	Median PCA3 Score (PCA3/PSA × 1000) [a]	P [b]
Specimen type				
FMV	56	80.4	23.8	0.8349
Pre-DRE	180	74.4	22.7	
Post-DRE	179	95.5	22.1	
Cancer positive	92	96.7	12.4	
Cancer negative	87	94.3	36.6	
DRE type				
None	236*	75.9	23.2	0.8473
3 strokes/lobe	90	96.7	23.2	
8 strokes/lobe	89	94.4	20.1	

No pairwise differences were found to be significant by Wilcoxon Rank sum tests.
*Some subjects contributed more than one specimen in the absence of DRE.
[a] All values are following removal of samples which were not informative.
[b] Kruskal–Wallis test.

2.5. Diagnostic classification accuracy based on known biopsy results

Urine specimens from 72 men with known biopsy outcomes ($n=17$ prostate cancer positive, $n=55$ negative) were obtained from L'Hôtel-Dieu de Québec clinic (Quebec, Canada) according to an IRB-approved protocol and under informed consent. The population included 67 Caucasians, 1 African American, 1 Asian, and 3 subjects of unspecified racial ethnicity. Subject age ranged from 43.9–79.1 y (median = 61.6 y) and serum tPSA values ranged from 0.38–31.1 ng/ml (median = 7.0 ng/ml). The specimens were stored at −70 °C for up to 12 months and were shipped frozen to the research sites for testing. All specimens were tested in a blinded fashion by both research sites in triplicate determinations.

2.6. Statistical analysis

Both the PCA3 and PSA mRNA assays were run with 5-point calibration curves. Raw RLU data was transferred to a verified Microsoft Excel worksheet for analysis. A weighted least-squares fit was applied to generate a 4-parameter logistic dose-response curve. Representative calibration curves of PCA3 or PSA mRNA c/ml vs. RLU are presented in Fig. 1. Urine samples with copy numbers higher than the highest calibration point were diluted 10–30 fold in supplied transport medium, a detergent-containing solution identical in formulation to the zero calibrator. The PCA3/PSA ratio was calculated using the mean of the assayed replicates multiplied times 1000. Pre-analytical effects of specimen type

Table 3a
Accuracy of PCA3 mRNA measurements by research site

	Assigned c/mL	N	Average measured c/mL	% Recovery
Site 1				
Control A	1180	100	1228	104.1
Control B	11,397	100	12,020	105.5
Control C	55,987	100	61,108	109.1
Site 2				
Control A	1180	100	1267	107.4
Control B	11,397	100	12,627	110.8
Control C	55,987	100	62,026	110.8

Table 3b
Accuracy of PSA mRNA measurements by research site

	Assigned c/mL	*N*	Average measured c/mL	% Recovery
Site 1				
Control A	45,750	100	48,091	105.1
Control B	469,135	100	484,457	103.3
Control C	1,769,010	100	2,001,430	113.1
Site 2				
Control A	45,750	100	42,638	93.2
Control B	469,135	100	445,236	94.9
Control C	1,769,010	100	1,925,530	108.8

(FMV, pre-DRE, or post-DRE) and DRE type (none, attentive DRE of exactly 3 strokes per lobe, and more aggressive DRE of exactly 8 strokes per lobe) were assessed via computation of descriptive statistics and via two non-parametric tests, the Kruskal–Wallis test and the Wilcoxon rank sum test for all pairwise comparisons. For evaluation of analytical accuracy, percent recovery (average measured value divided by assigned value × 1000) based on the previously assigned copy numbers was determined for each of the three controls. Assay precision was assessed according to CLSI EP5-A2. A linear random effect model with *run* and *site* as random factors was used to estimate the precision parameters. In this analysis, run was nested within the variable of site.

The ability to accurately classify subjects based on known biopsy outcomes was determined at each site by receiver-operating characteristic (ROC) curve analysis. The area under the ROC curve (AUC) and associated 95% confidence intervals (CIs) were calculated. To compare results between sites, the difference in AUC was determined along with its associated 95% CI and *P*-value for significance of the difference in areas using the non-parametric method of DeLong [8]. Additionally, the accuracy of binary classification of patients above or below a diagnostic cutpoint was computed at each site. A pre-specified PCA3 Score cutpoint of 35 was used to categorize patients. This cutpoint was determined in previous clinical studies of the PCA3 molecular urine test [9]. The difference in diagnostic accuracy at the 2 sites was tested using the unconditional test of equivalence for 2 correlated binomial samples [10] ($p<0.05$ indicates equivalence). To further assess inter-site effects, a Deming regression was performed on the matched values following natural log transformation of the data. Transformation was required due to the log-normal distribution of the fractional PCA3 Scores.

TableCurve 2D software (Systat; Ver. 5.01) was used to generate the 4-parameter dose-response curves. SAS Ver. 9.1 and JMP Ver 5.01 (SAS Institute, Cary NC) were used for pre-analytical effects analyses, precision, Deming regression, and ROC analyses. All comparisons were performed at the type I error rate of 5%.

Table 4
PCA3 and PSA mRNA assay precision by individual site

Site	Analyte	Control	*N*	Average c/mL	CV%		
					Total	Intra-assay	Inter-assay
1	PCA3	A	100	1228	15.2	11.8	9.7
		B	100	12,020	8.9	6.7	6.0
		C	100	61,108	5.7	4.1	4.1
	PSA	A	100	48,092	10.8	7.7	7.7
		B	100	484,457	11.4	8.5	7.7
		C	100	2,001,430	11.6	6.6	9.8
2	PCA3	A	100	1267	18.6	15.7	10.2
		B	100	12,627	8.6	7.0	5.0
		C	100	62,026	6.1	4.3	4.4
	PSA	A	100	42,638	8.5	7.4	4.2
		B	100	445,236	9.5	8.6	3.9
		C	100	1,925,530	7.6	5.6	5.1

3. Results

3.1. Pre-analytical effects

To assess the effects of specimen collection type, FMV, pre-DRE and post-DRE specimens were collected. Table 2 displays the informative rate, defined as the percentage of samples that provided PSA mRNA copy numbers greater than the second assay calibrator (9082 c/ml). Whereas post-DRE specimens provided a high informative rate of 95.5% (96.7% and 94.3% in cancer positive and cancer negative patients, respectively), both FMV and pre-DRE specimens provided markedly lower rates (80.4% and 74.4%, respectively), indicating the need to manipulate the prostate in order to shed enough prostate cells to accurately quantify transcripts. After excluding non-informative specimens, median PCA3 Scores were determined (Table 2). For those samples that were informative, no significant differences were found in median PCA3 Scores across specimen types ($P=0.8349$) or via pairwise comparisons (all $P>0.05$).

Similarly, the effect of DRE procedure was evaluated. *No DRE* was compared to an *attentive DRE* of exactly 3 strokes per prostate lobe and a more *aggressive DRE* of exactly 8 strokes per prostate lobe (Table 2). Again, the informative rate for specimens collected absent a DRE was low (75.9%). However, informative rates for all other procedures were >94%. As with the specimen collection type analysis, median PCA3 Scores of informative samples were not significantly different across DRE types ($P=0.8473$) and via all pairwise comparisons (all $P>0.05$).

3.2. Analytical performance

Percentage recoveries for the PCA3 mRNA assay ranged from 104.1% to 109.1% at site 1 and 107.4% to 110.8% at site 2 (Table 3a). For the PSA mRNA assay, percent recoveries ranged from 103.3% to 113.1% at site 1 and 93.2% to 108.8% at site 2 (Table 3b). For total, intra-assay, inter-assay, and inter-site CVs, a linear random effect model was used to estimate the precision parameters. When evaluating data by individual site (Table 4), the PCA3 mRNA total, intra-assay, and inter-assay CVs ranged from 5.7% to 18.6%, 4.1% to 15.7%, and 4.1% to 10.2%, respectively. Corresponding CVs for the PSA mRNA assay were 7.6% to 11.6%, 5.6% to 8.6%, and 3.9% to 9.8%. For the combined site data (Table 5), PCA3 mRNA total, intra-assay,

Table 5
PCA3 and PSA mRNA assay precision — combined site data

	N	Mean c/mL	CV %			
			Total	Intra-assay	Inter-assay	Inter-site
PCA3 control						
A	200	1248	17.1	14.0	9.9	0.0
B	200	12,323	9.1	6.9	5.5	3.2
C	200	61,567	5.9	4.2	4.3	0.2
PSA control						
A	200	45,365	11.5	7.6	6.4	8.3
B	200	464,846	11.3	8.6	6.3	5.7
C	200	1,963,480	10.1	6.1	7.9	2.0

Table 6
PCA3 and PSA LOB, LOD, and LOQ

	Site 1 (c/mL)		Site 2 (c/mL)		Combined Sites (c/mL)	
	PCA3	PSA	PCA3	PSA	PCA3	PSA
LOB	126	646	181	915	176	831
LOD	194	2050	287	2516	259	2338
LOQ	194	2050	287	2516	259	2338

inter-assay, and inter-site CVs ranged from 5.9% to 17.1%, 4.2% to 14.0%, 4.3% to 9.9%, and 0.0% to 3.2%, respectively. Corresponding CVs for the PSA mRNA assay were 10.1% to 11.5%, 6.1% to 8.6%, 6.3% to 7.9%, and 2.0% to 8.3%. In both assays, the factor of *site* was a generally minor contributor to total variability. The LOB, LOD, and LOQ values are presented in Table 6. For the PCA3 mRNA assay, results for LOB were ≤176 c/ml when the data from both sites were combined. For LOD and LOQ, the combined site data generated results of ≤259 c/ml. For the PSA mRNA assay, combined site data resulted in LOB ≤831 c/ml and LOD and LOQ ≤2338 c/ml.

3.3. Diagnostic classification accuracy based on known biopsy results

To evaluate the capability of each site to apply the PCA3 assay to discrimination of samples from prostate cancer patients vs. apparently healthy individuals, blinded samples from 72 subjects were analyzed ($n=17$ prostate cancer positive based on biopsy, $n=55$ negative). ROC analysis was performed on site-specific results taking the PCA3 Score as a continuous variable. Fig. 2 presents the ROC curves by site. For site 1, an AUC of 0.706 (95% CI: 0.578 to 0.834) was generated whereas site 2 achieved an AUC of 0.703 (95% CI: 0.558 to 0.847). The difference in areas of 0.003 (95% CI: −0.067 to 0.074) was not significant ($P=0.9289$). Applying a binary PCA3 Score cutpoint of 35 to the data, both sites correctly classified the same number of subjects (49/72 [68.1%]), which demonstrated a statistically significant equivalence of classification accuracy between sites ($P=0.0085$).

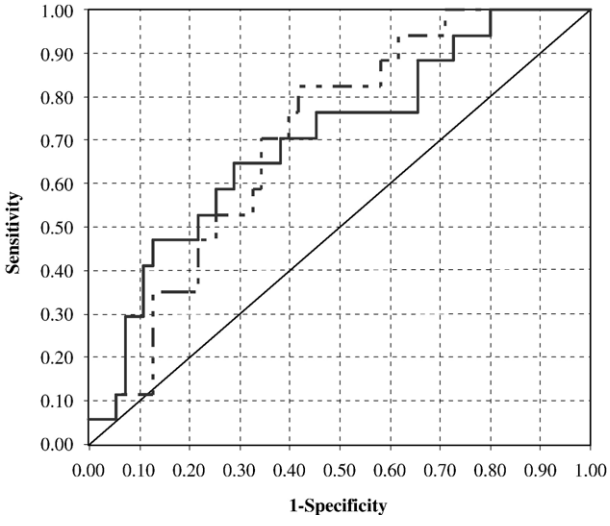

Fig. 2. Receiver operating characteristic (ROC) curves for PCA3 Scores from biopsy positive patients ($n=17$) and biopsy negative subjects ($n=55$) from sites 1(- - - - -) and 2 (———). Each curve displays the relationship between sensitivity and false positive rate (1 — specificity) for each observed value. Areas under the curve and associated 95% confidence intervals were site 1: 0.706 (0.578 to 0.834) and site 2: 0.703 (0.558 to 0.847). The difference in AUCs was 0.003 (−0.067 to 0.074), which was not significant ($P=0.9289$).

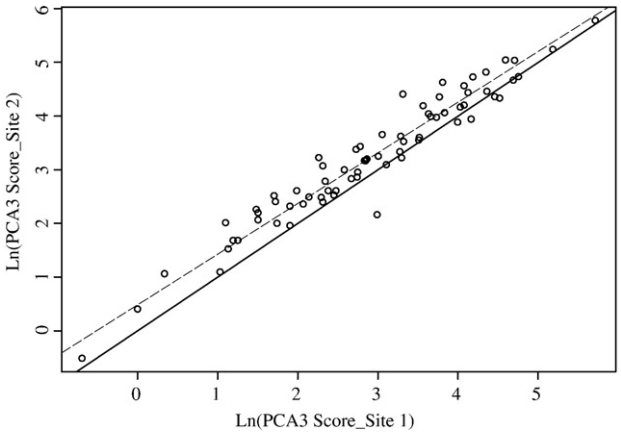

Fig. 3. Deming regression following natural log transformation of PCA3 Scores from biopsy positive patients ($n=17$) and biopsy negative subjects ($n=55$) from sites 1 and 2. Slope=0.94; Intercept=0.48; $R=0.9677$, $P<0.0001$.

To further assess inter-site agreement, the PCA3 Scores from both sites were natural log transformed (to adjust for the log-normal distribution of the data) and regressed via the method of Deming (Fig. 3). The slope of the regression line was 0.94, the intercept was 0.48, and the correlation was 0.9677 ($P<0.0001$) indicating agreement between sites.

4. Discussion

In the present study, we contribute to the ongoing investigation of the capabilities of the PCA3 assay to fulfill a role in the clinical management of men at risk for prostate cancer. We have assessed the pre-analytical effects of specimen collection type and DRE procedure and have found that informative rates in the absence of prostate manipulation are low. Based on this study, at least 20% of patients would have to return to their physician to provide another urine specimen, which represents a clear inconvenience to the patient and to the healthcare system. However, what might be considered a "good urologic DRE" of 3 strokes per prostate lobe provided an informative rate of >94%, which was equivalent to a more aggressive procedure incorporating 8 strokes per prostate lobe. Thus, it may be inferred that any manipulation of the prostate will shed enough cells to provide informative specimens. Moreover, the resulting PCA3 Scores of informative samples are equivalent irrespective of specimen type or DRE procedure, indicating the substantial insensitivity of the method to pre-analytical effects.

Analyses of analytical accuracy for both PCA3 and PSA mRNA measurements demonstrate percentage recoveries of ±15% of assigned values. Precision evaluations from combined site data indicate total, intra-assay, and inter-assay variations of <18%, 15%, and 10%, respectively, results that are in excellent

agreement with those of Groskopf, et al. [7] Furthermore, variation between research sites was minimal in this study and proved to be a generally minor component of total variability.

Both research sites were able to correctly classify the same number of subjects (49 of 72) tested in blinded fashion. The categorical concordance was further supported by the direct linear relationship with a slope close to 1.0 demonstrated on regression analysis. The resulting AUCs from ROC analysis of the data from both sites of ~0.7 are in good agreement with the literature. For example, the first assessment of PCA3 urine measurements was performed by Hessels et al. using an RT-PCR method [6]. In a cohort of 108 patients undergoing prostate biopsy, an AUC of 0.72 was achieved. Similarly, Groskopf et al. [7] demonstrated an AUC of 0.746 in a study group of 70 men scheduled for biopsy using the TMA method. In a recent study of 233 men with serum tPSA levels persistently >2.5 ng/ml but with previous negative biopsies, Marks, et al. [9] demonstrated an AUC of 0.68, a finding superior to the AUC of 0.52 for serum tPSA in the same study. Such observations have been noted by other researchers including Fradet et al. who developed a nucleic acid sequence-based amplification (NASBA) technique [11] for assay of PCA3 in whole urines collected after attentive DRE. In their study, PCA3 demonstrated increased positive and negative predictive values and overall improved accuracy in direct comparison to serum tPSA. These findings have supported the hypothesis that the cancer specificity of PCA3 imparts the capability to better detect cancers than traditional serum PSA measurements and to avoid a greater number of unnecessary biopsies.

Other investigators have been intrigued by the use of PCA3 measurements in or near the diagnostic "grey zone" of serum tPSA (generally considered to be ~4–10 ng/ml). For instance, a recent multicenter study by van Gils et al. [12] in a cohort of 583 men with serum tPSA between 3 and 15 ng/ml demonstrated an AUC of 0.66 for urine PCA3 measured via quantitative RT-PCR in comparison to 0.57 for serum tPSA. The authors determined the best cutpoint for their test and compared specificities of PCA3 and serum tPSA at a fixed sensitivity of 65%. Specificity for PCA3 was greater than that for serum tPSA (66% vs. 47%). Still other studies have evaluated the use of PCA3 measurements as part of a panel of prostate-related genes. For example, Petrovics et al. [13] demonstrated over-expression of PCA3, ETS-related gene-1, and/or AMACR in 54 of 55 prostate cancers tested. In addition, Schmidt et al. [14] evaluated PCA3 in direct comparison to and in addition to AibZIP, D-GPCR, EZH2, PDEF, prostein, PSA, PSCA, and TRPM8 in prostate tissues. All genes were measured by quantitative PCR and were normalized to four housekeeping genes. In the analysis of prostate tissues from 106 excised prostates, PCA3 was demonstrated to have the highest AUC of all genes tested (0.85) and when explored in a multivariate model with EZH2, prostein, and TRPM8 the AUC was increased only marginally to 0.90, indicating the strong and independent power of PCA3 measurements.

In summary, we have found the PCA3 assay to be relatively insensitive to pre-analytical factors, to display acceptable analytical performance, and to correctly classify a high rate of subjects in agreement with previous studies. Further, the results between sites were equivalent, demonstrating the robust performance across sites. Based on the prostate cancer specificity of the gene and the performance of the PCA3 assay, the method continues to demonstrate applicability to the proposed clinical role as an adjunct in the management of men at risk for prostate cancer. Further studies are required to more clearly define the use of the product in relation to existing procedures for initial detection, reflex testing following negative biopsy, recurrence, and ongoing therapeutic management of prostate cancer.

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