

Physician Brochure for the PROGENSA[®] PCA3 Assay

INTENDED USE

The PROGENSA PCA3 Assay is an *in vitro* nucleic acid amplification test. The assay measures the concentration of prostate cancer gene 3 (PCA3) and prostate-specific antigen (PSA) RNA molecules and calculates the ratio of PCA3 RNA molecules to PSA RNA molecules (PCA3 Score) in post-digital rectal exam (DRE) first-catch male urine specimens. The PROGENSA PCA3 Assay is indicated for use in conjunction with other patient information to aid in the decision for repeat biopsy in men 50 years of age or older who have had one or more previous negative prostate biopsies and for whom a repeat biopsy would be recommended by a urologist based on current standard of care, before consideration of PROGENSA PCA3 Assay results.

A PCA3 Score <25 is associated with a decreased likelihood of a positive biopsy. Prostatic biopsy is required for diagnosis of cancer.

WARNING:

The PROGENSA PCA3 Assay should not be used for men with atypical small acinar proliferation (ASAP) on their most recent biopsy. Men with ASAP on their most recent biopsy should be treated in accordance with current medical guidelines.

Warning: the Clinical Study only included men who were recommended by urologists for repeat biopsy. Therefore, the performance of the PROGENSA PCA3 Assay has not been established in men for whom a repeat biopsy was not already recommended.

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LIMITATIONS OF THE CLINICAL STUDY RESULTS

- The PCA3 Score is intended to be used in conjunction with serum prostate-specific antigen (PSA) and other risk indicators to guide appropriate patient management in the “at risk” population of men who have had one or more previous negative prostate biopsies and for whom a repeat biopsy would be recommended based on current standard of care.
- Performance of the PROGENSA PCA3 Assay has not been established in men who undergo repeat biopsy less than three months or more than seven years after their most recent negative biopsy.
- The effect of medications known to affect serum PSA levels such as finasteride (Proscar, Propecia), dutasteride (Avodart) and anti-androgen therapy (Lupron) on PROGENSA PCA3 Assay performance was not evaluated.
- Certain therapeutic and diagnostic procedures, such as prostatectomy, radiation, prostate biopsy and others, may affect the viability of prostatic tissue and subsequently impact the PCA3 Score. The effect of these procedures on assay performance has not yet been evaluated. Samples for PROGENSA PCA3 Assay testing should be collected when the clinician believes prostate tissue has recovered.
- Results from the PROGENSA PCA3 Assay should be interpreted in conjunction with other laboratory and clinical data available to the clinician and relevant guidelines in the decision for repeat biopsy.

INTRODUCTION

The PROGENSA PCA3 Assay is an *in vitro* nucleic acid amplification test that measures the concentration of PCA3 RNA, a specific molecular marker for prostate cancer, and PSA RNA in male urine specimens. The assay result (called the "PCA3 Score") correlates with the likelihood of repeat biopsy outcome, meaning that a lower PCA3 Score is associated with a decreased likelihood of a positive repeat biopsy. The PCA3 Score is intended to be used in conjunction with serum prostate-specific antigen (PSA) and other risk indicators to guide appropriate patient management in the "at risk" population of men who have had one or more previous negative prostate biopsies and for whom a repeat biopsy would be recommended based on current standard of care.

OVERVIEW

Prostate cancer (PCa) is the most common cancer and a leading cause of cancer death among men in the United States (American Cancer Society, 2011). Current practices for detecting PCa utilize serum PSA and DRE as indications for biopsy and approximately 25% of patients with elevated serum PSA are found to have PCa (Catalona, 1994; Schröder, 2009). This means that 75% of first biopsies are negative. The fear that cancer was missed often leads to repeat biopsies—most of which will also be negative because there is not enough clear information provided by serum PSA and DRE to decide whether to proceed with or delay an additional biopsy. Men with one or more previous negative biopsies present a clinical dilemma and there is a medical need for additional tests to help physicians and patients make more informed repeat biopsy decisions.

PCA3 is a prostate-specific gene that is highly over expressed in 95% of prostate cancers. Prostate cancer cells express 60 to 100 times more PCA3 RNA than normal cells (Hessels, 2003). The PROGENSA PCA3 Assay is highly-specific and uses Transcription Mediated Amplification (TMA) to quantify PCA3 RNA in a patient sample.

The PROGENSA PCA3 Assay is the first FDA-approved urine-based molecular test that detects the over-expression of the PCA3 gene. The specific information provided by the test (the PCA3 score) can be used in conjunction with other patient history to decide whether a repeat biopsy is necessary in men with one or more previous negative biopsies. Data on the PCA3 marker has been cited in over 100 publications (Salagierski, 2012) and more than 200,000 tests have been used in clinical practice throughout the world.

DEVICE DESCRIPTION

The PROGENSA PCA3 Assay utilizes whole urine collected following a digital rectal examination (DRE) consisting of three strokes per lobe. The DRE releases prostate cells through the prostate duct system into the urinary tract, where they can be collected in the first catch urine. The urine is processed by addition of Urine Transport Medium (UTM), which lyses the cells and stabilizes the

RNA. PCA3 and PSA RNAs are quantified and the PCA3 Score is determined based on the ratio of PCA3 RNA to PSA RNA multiplied by 1000. In addition to normalizing PCA3 signal, measurement of PSA RNA also serves to confirm that the yield of prostate-specific RNA is sufficient to generate a valid result.

TECHNOLOGY

The PROGENSA PCA3 Assay is comprised of two quantitative nucleic acid amplification tests. The assay combines the technologies of target capture, TMA and Hybridization Protection Assay (HPA) to streamline urine specimen processing, amplify target RNA and detect amplicon, respectively.

When the PROGENSA PCA3 Assay is performed in the laboratory, the target RNA molecules are isolated from the urine specimens by target capture. Capture oligonucleotides that are complementary to sequence specific regions of the targets are hybridized to the targets in the urine specimen. A separate capture oligonucleotide is used for each target. The hybridized target is then captured onto magnetic microparticles that are separated from the urine specimen in a magnetic field. Wash steps are utilized to remove extraneous components from the reaction tube. Magnetic separation and wash steps are performed with a target capture system.

Target amplification occurs via TMA, which is a transcription-based nucleic acid amplification method that utilizes two enzymes, Moloney murine leukemia virus (MMLV) reverse transcriptase and T7 RNA polymerase. A unique set of primers is used for each target. The reverse transcriptase is used to generate a deoxyribonucleic acid (DNA) copy (containing a promoter sequence for T7 RNA polymerase) of the target sequence. T7 RNA polymerase produces multiple copies of RNA amplicon from the DNA copy template.

Detection is achieved by HPA using single-stranded, chemiluminescent-labeled nucleic acid probes that are complementary to the amplicon. Separate probes are used for each target amplicon. The labeled nucleic acid probes hybridize specifically to the amplicon. The selection reagent differentiates between hybridized and unhybridized probes by inactivating the label on unhybridized probes. During the detection step, the chemiluminescent signal produced by the hybridized probe is measured in a luminometer and is reported as Relative Light Units (RLU).

PCA3 and PSA RNAs are quantified in separate tubes and the PCA3 Score is determined. Calibrators containing known amounts of PCA3 or PSA RNA transcript are included in every assay run and used to generate a standard curve. PCA3 and PSA controls are also included to verify the accuracy of results interpolated from the standard curve.

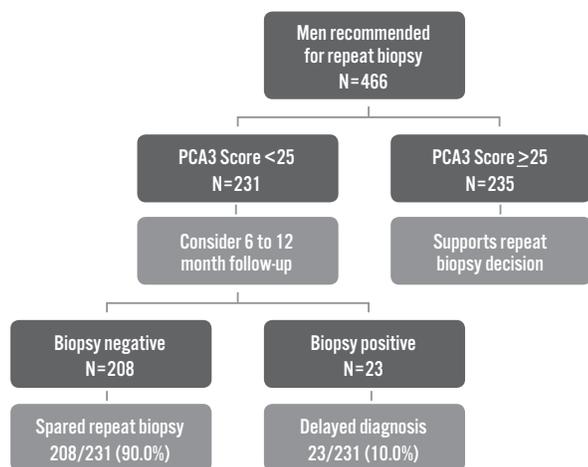
CLINICAL SENSITIVITY AND SPECIFICITY OF THE PROGENSA PCA3 ASSAY

A pivotal, prospective, multicenter clinical study was conducted to evaluate the performance of the PROGENSA PCA3 Assay for assessing the likelihood of repeat biopsy outcome. In order to evaluate the clinical performance of PROGENSA PCA3 Assay, the clinical study data were analyzed to determine how the use of the test might have affected the repeat biopsy recommendation for the clinical study subjects.

The flow chart below summarizes the results of this analysis. In the clinical study of 466 subjects, 49.6% (231 out of 466) of subjects had PCA3 Scores <25. This indicates decreased likelihood of a positive repeat biopsy result, so the clinician and patient might have considered delaying the repeat biopsy. Of these 231 men, 208 (90%) subsequently had a negative biopsy result, while 23 (10%) had a biopsy positive for prostate cancer. For the 235 men with PCA3 Scores ≥25, the PROGENSA PCA3 Assay result supports the decision to repeat biopsy (34% (79/235) of these men had positive biopsies).

The potential clinical benefit is that 44.6% (208 out of 466) of men in the study may have been spared an unnecessary repeat biopsy. Instead, these men would have been monitored closely for any change in risk factors that would suggest disease. The associated risk is that 23 of the men who had a biopsy positive for prostate cancer may have had their diagnosis delayed. The standard timeframe for conducting follow up with the intended use population is 6 to 12 months. In terms of risk versus benefit, nine men may have avoided an unnecessary repeat biopsy for every one man whose diagnosis may be delayed. In the context of the entire study population (466 total subjects), 49.6% (231/466) of prostate biopsies would have been avoided and 4.9% (23/466) of men who harbored biopsy-detectable prostate cancer would have been monitored instead of receiving an immediate repeat biopsy. The PROGENSA PCA3 Assay should not be used for men with ASAP on their most recent biopsy. Men with ASAP on their most recent biopsy should be treated in accordance with current medical guidelines.

SUMMARY OF CLINICAL STUDY RESULTS



CLINICAL STUDY RESULTS

Four hundred ninety-five (495) male subjects were enrolled from a total of 14 clinical sites, including academic institutions, community-based urology clinics and group health organizations. Men who had at least one previous negative prostatic biopsy, who had never had a positive prostatic biopsy, and who had been recommended for a repeat biopsy by their urologists were eligible for study participation. A questionnaire was used to collect information regarding prostate cancer risk factors from men recommended for a repeat biopsy by their clinician (i.e., the enrolled population) and from men not recommended for a repeat biopsy (i.e., the non-enrolled population). Age, prostate volume and most recent free PSA test result were not significantly different between enrolled and non-enrolled populations. Serum PSA test results and the time since the most recent negative biopsy were significantly different ($P < .0001$) between enrolled and non-enrolled populations, where the non-enrolled men (men not recommended for repeat biopsy by their clinician) had 2.2 ng/mL lower mean serum PSA test results and approximately 60% shorter time since their most recent previous negative biopsy. Clinical study analysis included men who had been recommended for a repeat biopsy by their urologists.

Blood, urine and prostatic biopsy specimens were collected from each subject. The blood specimen was tested with a total serum PSA test at the collection site's associated testing facility. The total serum PSA test used varied by collection site. The urine specimen was collected following a digital rectal exam (DRE) and was a first-catch urine specimen. The urine specimen was processed at the collection site by aliquotting into PROGENSA PCA3 Urine Specimen Transport Tubes and shipped to a testing site for PROGENSA PCA3 Assay testing. The prostatic biopsy was performed per the collection site's standard procedure. The biopsy specimens were evaluated by the collection site's associated pathology facility(ies).

For the 495 eligible subjects, the median age was 67.0 years; ages ranged from 44 years to 92 years. Race was reported as White for 433 subjects (87.5%), Black or African American for 45 subjects (9.1%), Asian for 11 subjects (2.2%), American Indian/Alaska Native for 2 subjects (0.4%) and unknown for 5 subjects (1.0%). Four hundred eighty (480) of the eligible subjects provided a urine sample for PROGENSA PCA3 Assay testing (3.0% (15/495) of subjects did not provide a urine sample); 1.3% (6/480) of sample results were excluded because of sample qualification failure (insufficient RNA for accurate analysis), leaving 474 subjects with a valid and reportable PCA3 Score.

Four hundred sixty-six (466) subjects with valid and reportable PCA3 Scores and disease status (determined by biopsy result) and who were 50 years of age or older were included in the analyses. Prevalence of positive repeat biopsy was 21.9% (102/466). For the subjects with a study total serum PSA test result ($n=464$), the

median total serum PSA test result was 5.80 ng/mL (results ranged from 0.3 ng/mL to 49.2 ng/mL). Prostatic biopsies consisted of 6 to 24 cores with 93% of subjects having 12 to 21 cores taken.

Figure 1. Shows the percentage of subjects with positive prostatic biopsy results by PCA3 Score interval (with 95% confidence).

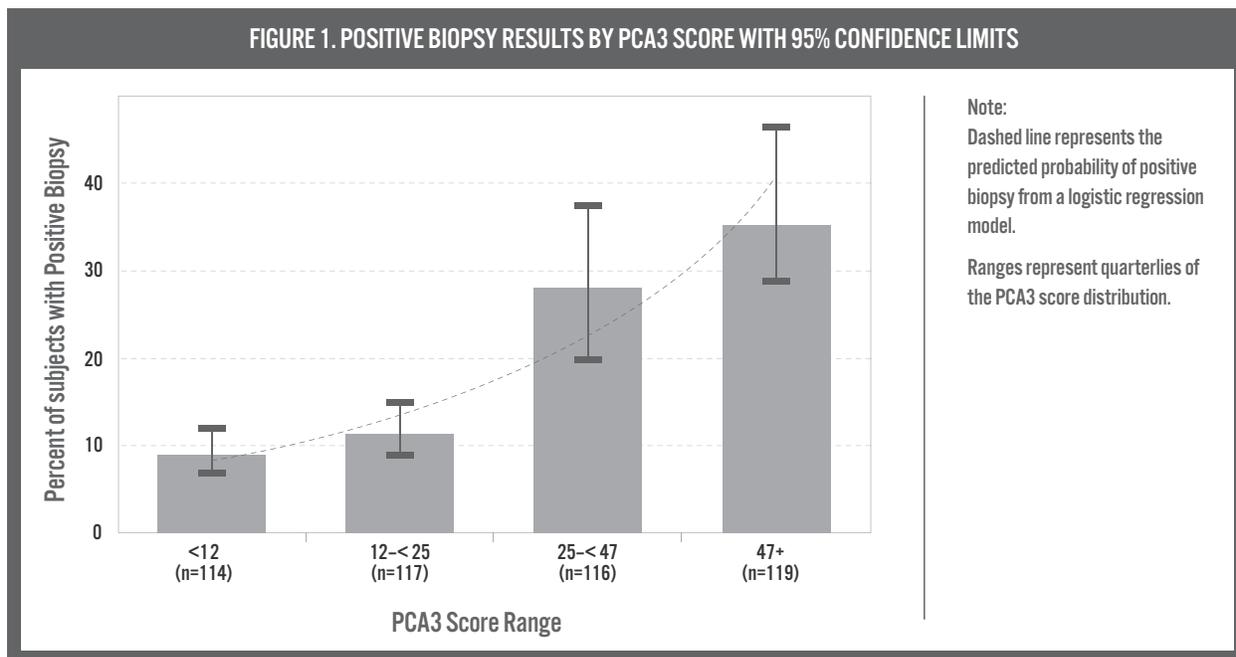


Table 1. Shows the performance characteristics of the PROGENSA PCA3 Assay relative to prostatic biopsy outcome at a PCA3 Score cut-off value of 25.

TABLE 1. PERFORMANCE CHARACTERISTICS OF THE PROGENSA PCA3 ASSAY						
	BIOPSY POSITIVE	BIOPSY NEGATIVE	TOTAL	PERFORMANCE CHARACTERISTICS	ESTIMATE	95% CI
PCA3 SCORE ≥ 25	79	156	235	SENSITIVITY %	77.5 (79/102)	68.4-84.5
PCA3 SCORE < 25	23	208	231	SPECIFICITY %	57.1 (208/364)	52.0-62.1
TOTAL	102	364	466	PPV%	33.6 (79/235)	30.0-37.2
				NPV %	90.0 (208/231)	86.5-93.1
POSITIVE BIOPSY PREVALENCE %	21.9 (102/466)			PLR	1.81	1.53-2.11
				NLR	0.40	0.26-0.56
				ODDS RATIO	4.58	2.75-7.62

CI = Confidence Interval, PPV = Positive Predictive Value, NPV = Negative Predictive Value, PLR = Positive Likelihood Ratio, NLR = Negative Likelihood Ratio

PROGENSA PCA3 ASSAY PERFORMANCE IN SUBGROUPS

Table 2. Shows the performance characteristics of the PROGENSA PCA3 Assay relative to prostatic biopsy outcome for subgroups of the study population.

Table 3. Shows PROGENSA PCA3 Assay performance results in the subgroup of men with ASAP on their most recent negative biopsy. The clinical study was not designed to evaluate subgroups, so the results for individual subgroups may not be conclusive. However, PROGENSA PCA3 Assay performance in men with prior ASAP indicated that the PROGENSA PCA3 Assay is not informative of biopsy outcome in this subgroup (Table 3).

TABLE 2. PERFORMANCE CHARACTERISTICS OF THE PROGENSA PCA3 ASSAY BY SUBGROUPS												
SUBGROUP	N	TP	FP	TN	FN	SE% (95% CI) ¹	SP% (95% CI)	PPV% (95% CI)	NPV% (95% CI)	PLR (95% CI)	NLR (95% CI)	OR (95% CI)
AGE (YEARS)												
50-59	96	10	19	59	8	55.6 (33.7-75.4)	75.6 (65.1-83.8)	34.5 (20.8-48.0)	88.1 (82.2-93.5)	2.28 (1.14-4.00)	0.59 (0.30-0.94)	3.88 (1.34-11.25)
60-69	193	24	62	94	13	64.9 (48.8-78.2)	60.3 (52.4-67.6)	27.9 (21.4-34.2)	87.9 (82.7-92.5)	1.63 (1.15-2.19)	0.58 (0.34-0.88)	2.8 (1.33-5.91)
70+	177	45	75	55	2	95.7 (85.8-98.8)	42.3 (34.2-50.9)	37.5 (33.6-41.6)	96.5 (89.5-99.5)	1.66 (1.40-1.97)	0.10 (0.02-0.33)	16.50 (3.84-70.94)
PRIOR NEGATIVE BIOPSY RESULT												
HGPIN (NOT ASAP)	101	21	42	35	3	87.5 (69.0-95.7)	45.5 (34.8-56.5)	33.3 (27.1-39.5)	92.1 (81.7-98.2)	1.60 (1.20-2.09)	0.28 (0.06-0.72)	5.83 (1.61-21.20)
NONE/OTHER	316	48	90	163	15	76.2 (64.4-85.0)	64.4 (58.4-70.1)	34.8 (29.8-39.8)	91.6 (87.8-94.7)	2.14 (1.71-2.65)	0.37 (0.22-0.56)	5.80 (3.07-10.93)
NUMBER OF PREVIOUS NEGATIVE BIOPSIES												
1	316	56	101	138	21	72.7 (61.9-81.4)	57.7 (51.4-63.8)	35.7 (31.0-40.4)	86.8 (82.2-90.9)	1.72 (1.39-2.10)	0.47 (0.31-0.67)	3.64 (2.07-6.40)
2+	150	23	55	70	2	92.0 (75.0-97.8)	56.0 (47.2-64.4)	29.5 (24.3-34.6)	97.2 (91.9-99.6)	2.09 (1.61-2.65)	0.14 (0.02-0.44)	14.64 (3.31-64.78)
TIMING OF PREVIOUS BIOPSY RELATIVE TO STUDY ENROLLMENT												
<3 MONTHS ¹	13	2	6	2	3	40.0 (11.8-76.9)	25.0 (7.1-59.1)	25.0 (3.2-49.9)	40.0 (6.9-76.2)	0.53 (0.05-1.59)	2.40 (0.50-21.76)	0.22 (0.02-2.45)
3 MONTHS - <7 YEARS	438	75	145	199	19	79.8 (70.6-86.7)	57.8 (52.6-63.0)	34.1 (30.4-37.8)	91.3 (87.7-94.3)	1.89 (1.60-2.22)	0.35 (0.22-0.51)	5.42 (3.13-9.36)
7+ YEARS	15	2	5	7	1	66.7 (20.8-93.9)	58.3 (32.0-80.7)	28.6 (6.5-54.8)	87.5 (66.6-99.5)	1.60 (0.28-4.85)	0.57 (0.02-2.01)	2.80 (0.20-40.06)
RACE												
BLACK	39	6	16	16	1	85.7 (48.7-97.4)	50.0 (33.6-66.4)	27.3 (14.3-37.9)	94.1 (79.4-99.8)	1.71 (0.76-2.79)	0.29 (0.01-1.19)	6.00 (0.65-55.66)
NON-BLACK	427	73	140	192	22	76.8 (67.4-84.2)	57.8 (52.5-63.0)	34.3 (30.5-38.1)	89.7 (86.0-92.9)	1.82 (1.53-2.15)	0.40 (0.27-0.57)	4.55 (2.69-7.69)
SERUM PSA (NG/ML) AND DIGITAL RECTAL EXAM												
PSA<4 AND DRE NORM	81	13	37	28	3	81.3 (57.0-93.4)	43.1 (31.8-55.2)	26.0 (18.8-32.3)	90.3 (78.5-97.6)	1.43 (0.94-1.94)	0.44 (0.10-1.11)	3.28 (0.85-12.62)
PSA≥4 OR DRE ABN	383	65	119	179	20	76.5 (66.4-84.2)	60.1 (54.4-65.5)	35.3 (31.1-39.6)	89.9 (86.1-93.3)	1.92 (1.58-2.30)	0.39 (0.25-0.56)	4.89 (2.81-8.49)
SERUM PSA (NG/ML) AND NUMBER OF PREVIOUS NEGATIVE BIOPSIES												
PSA>10 AND 1 BX	34	10	9	14	1	90.9 (62.3-98.4)	60.9 (40.8-77.8)	52.6 (38.4-68.4)	93.3 (73.4-99.8)	2.32 (1.31-4.53)	0.15 (0.01-0.78)	15.56 (1.69-143.16)
PSA≤10 OR 2+ BX	430	68	147	193	22	75.6 (65.8-83.3)	56.8 (51.5-61.9)	31.6 (27.9-35.3)	89.8 (86.1-93.0)	1.75 (1.46-2.06)	0.43 (0.29-0.61)	4.06 (2.39-6.87)

TP = true positive, FP = false positive, TN = true negative, FN = false negative, CI = confidence interval, SE = sensitivity, SP = specificity, PPV (NPV) = positive (negative) predictive value, PLR (NLR) = positive (negative) likelihood ratio, OR = odds ratio, Bx = biopsy. For calculations in this table, PCA3 Score values ≥ 25 are considered positive and PCA3 score values < 25 are considered negative.

¹ In this subgroup, 84.6% (11/13) had ASAP on their most recent negative biopsy. In the clinical study, the PROGENSA PCA3 Assay was not predictive of repeat biopsy outcome in men with prior ASAP (Table 3).

TABLE 3. PERFORMANCE CHARACTERISTICS OF PROGENSA PCA3 ASSAY IN MEN WITH ASAP ON THEIR MOST RECENT NEGATIVE BIOPSY

	BIOPSY POSITIVE	BIOPSY NEGATIVE	TOTAL	PERFORMANCE CHARACTERISTICS	ESTIMATE	95% CI
PCA3 SCORE \geq 25	10	24	34	SENSITIVITY %	66.7 (10/15)	41.7-84.8
PCA3 SCORE < 25	5	10	15	SPECIFICITY %	29.4 (10/34)	16.8-46.2
TOTAL	15	34	49	PPV%	29.4 (10/34)	19.1-38.2
				NPV %	66.7 (10/15)	44.7-87.0
POSITIVE BIOPSY PREVALENCE %	30.6 (15/49)			PLR	0.94	0.54-1.40
				NLR	1.13	0.34-2.80
				ODDS RATIO	0.83	0.23-3.07

CI = confidence interval, PPV = positive predictive value, NPV = negative predictive value, PLR = positive likelihood ratio, NLR = negative likelihood ratio

*The PROGENSA PCA3 Assay should not be used for men with ASAP on their most recent biopsy. Men with ASAP on their most recent biopsy should be treated in accordance with current medical guidelines.

THE ADDITION OF PROGENSA PCA3 ASSAY INFORMATION IMPROVES DIAGNOSTIC ACCURACY OVER EXISTING STANDARD OF CARE FACTORS

Multivariable logistic regression analysis was conducted to determine whether the addition of the PROGENSA PCA3 Assay information improved diagnostic accuracy over the standard of care information that is currently used for repeat biopsy decisions. The standard of care factors included the following: age, DRE result, family history, race, serum PSA test result and number of previous negative biopsies.

Table 4. Shows the results from the multivariable logistic regression analysis. In this analysis, the odds ratio (OR) for PCA3 Score (expressed as a binary categorical variable [positive or negative using a cutoff of 25]) was statistically significant ($P < .0001$). These results indicate that the PCA3 Score is a statistically significant predictor of repeat biopsy outcome in the presence of current standard of care factors used in the decision to perform a repeat biopsy.

TABLE 4. MULTIVARIABLE LOGISTIC REGRESSION RESULTS FOR THE OCCURRENCE OF PROSTATE CANCER ASSOCIATED WITH PCA3 SCORE USING A BINARY CUTOFF OF 25 AND OTHER CLINICAL FACTORS

FACTOR*	REGRESSION COEFFICIENT (SE)	ODDS RATIO (95% CI)	P VALUE
PCA3 SCORE (\geq 25 VS. <25)	1.5175 (0.2762)	4.5610 (2.6542, 7.8376)	<.0001
AGE IN YEARS (CONTINUOUS)	0.0073 (0.0158)	1.0073 (0.9766, 1.0389)	.6458
SUSPICIOUS DRE (YES VS. NO)	0.0251 (0.2801)	1.0254 (0.5928, 1.7753)	.9287
FAMILY HISTORY (ANY VS. NONE)	-0.0795 (0.3162)	0.9235 (0.4970, 1.7163)	.8014
FAMILY HISTORY (UNKNOWN/REFUSED VS. NONE)	0.3756 (0.5054)	1.4558 (0.5406, 3.9203)	.4574
RACE (BLACK VS. NON-BLACK)	0.5506 (0.4700)	0.5766 (0.2295, 1.4485)	.2414
SERUM PSA IN NG/ML (CONTINUOUS)	0.0669 (0.0215)	1.0691 (1.0250, 1.1152)	.0019
NUMBER OF PREVIOUS NEGATIVE BIOPSIES (2 VS. 1)	-0.7955 (0.3259)	0.4513 (0.2383, 0.8549)	.0146
NUMBER OF PREVIOUS NEGATIVE BIOPSIES (3+ VS. 1)	-0.8028 (0.4545)	0.4481 (0.1839, 1.0921)	.0774

SE = standard error, CI = confidence interval

Note: A total of N=464 subjects from the Full Analysis Set have complete data for all of the factors in the multivariable logistic regression analysis.

* Per the statistical analysis plan, prostate volume (continuous) was not included as a standard of care covariate, as the regression coefficient associated with prostate volume was not statistically significant at a .05 level ($P = .0583$) and the regression coefficient for PCA3 Score changed by less than 10% when prostate volume was removed from the model (actual observation 1.5%).

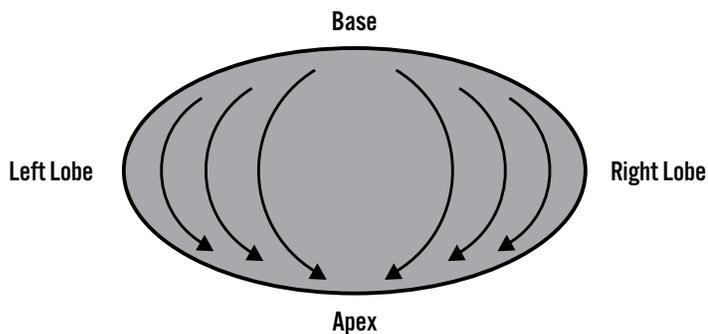
The PCA3 Score was also evaluated as a continuous measure. The OR for PCA3 Score (continuous) was 1.0099 (95% CI: 1.0058 – 1.0140; P < .0001). This indicates that each PCA3 Score unit increase represents a 0.99% increase in the likelihood of a positive repeat biopsy. Thus, PCA3 Score is also a statistically significant and independent predictor of the likelihood of positive repeat biopsy when measured incrementally.

SPECIMEN COLLECTION— IMPORTANT CONSIDERATIONS

Sample Preparation (Instructions For Urine Specimen Collection and Processing):

1. Conduct a DRE as described below immediately prior to urine collection: Apply pressure on the prostate, enough to depress the surface, from the base to the apex and from the lateral to the median line for each lobe as shown in Figure 2. Perform exactly three strokes for each lobe. This is NOT intended to be a prostatic massage.

Figure 2. Proper Direction of Applied Prostrate Pressure



2. Following the DRE, direct the patient to provide first-catch urine (approximately 20 to 30 mL of the initial urine stream) in an appropriately labeled urine collection cup. This must be the first voided urine specimen following the DRE. Use a collection cup free of any preservatives. If a patient cannot stop his urine flow and provides more urine than the requested first 20 to 30 mL, keep the entire volume. Very high urine volumes can lower PCA3 and PSA analyte concentrations and may infrequently result in an invalid specimen. Thus, the patient should try to avoid filling the urine collection cup. If the patient is unable to provide the requested volume of urine (at least 2.5 mL is required to run the PROGENSA PCA3 Assay) then the specimen must be rejected.

3. **Unprocessed urine specimens, if not immediately processed, must be maintained at 2°C to 8°C or kept on ice. The chilled, unprocessed urine specimen must be transferred into the urine specimen transport tube within four hours of collection. Otherwise, the specimen must be rejected and the urologist must collect a new specimen. Do not freeze unprocessed urine specimens.**

4. To process urine specimens, tightly cap and invert the urine specimen five times to resuspend cells. Remove the cap of the

urine specimen transport tube and transfer 2.5 mL of the collected urine into the tube using the disposable transfer pipette provided. The correct volume of urine has been added when the fluid level is between the black fill lines on the urine specimen transport tube label.

5. Re-cap the urine specimen transport tube tightly and invert the urine specimen five times to mix. This is now known as the processed urine specimen.
6. Avoid cross-contamination during the specimen handling steps. Urine specimens can contain high levels of RNA target. Ensure specimen containers do not contact each other and discard used materials without passing them over any containers. If gloves come in contact with a specimen, change gloves to avoid cross-contamination.
7. Ship or transport processed urine specimens according to instructions provided by your testing laboratory. Shipping arrangements must be made to ensure specimens are received by the testing site within five days of collection.

PROCESSED SAMPLE TRANSPORT AND STORAGE BEFORE TESTING:

1. Processed urine specimens must be transported to the laboratory in the urine specimen transport tube. They may be shipped under ambient conditions (without temperature control) or frozen. Shipping arrangements must be made to ensure specimens are received by the testing site within five days of collection. Upon receipt of the shipment, the laboratory should verify the date of specimen collection on the tube. If specimens were shipped under ambient conditions and are received greater than five days after specimen collection, the specimen must be rejected and a request for a new specimen should be made. The laboratory may store specimens at 2°C to 8°C for up to 14 days before disposal is required. If longer time periods are needed, refer to Table 6 for the allowable storage times at different temperatures.

TABLE 6. PROCESSED URINE SPECIMEN STORAGE DURATIONS

STORAGE TEMPERATURE	TIME
PROCESSED SPECIMEN STORAGE AND SHIPMENT:	
AT OR BELOW 30°C	UP TO 5 DAYS*
AFTER RECEIPT AT TESTING SITE:	
2°C TO 8°C	UP TO 14 DAYS
-35°C TO -15°C	UP TO 11 MONTHS**
AT OR BELOW -65°C	UP TO 36 MONTHS**
*Time allowed for shipment under ambient conditions or frozen. **Time allowed after refrigerated storage.	

2. Processed urine specimens may be subjected to up to five freeze-thaw cycles.

INTERPRETATION OF PROGENSA PCA3 ASSAY RESULTS

The PCA3 Score is calculated as the ratio of PCA3 RNA copies to PSA RNA copies, multiplied by 1000. As the PCA3 Score increases, the likelihood for a positive biopsy increases. As the PCA3 Score decreases, the likelihood for a positive biopsy decreases.

If the reported PCA3 Score is below the cut-off of 25, the result should be interpreted as NEGATIVE. If the PCA3 Score is above or equal to the cut-off of 25, the result should be interpreted as POSITIVE. A NEGATIVE result is associated with decreased likelihood of a positive biopsy.

Due to normal assay variability, specimens with PCA3 Scores near the cutoff of 25 (i.e., 18 to 31) could yield a different overall interpretation of POSITIVE or NEGATIVE upon repeat testing. PCA3 Scores in the range from 18 to 31 should therefore be interpreted with caution. The PCA3 Score should be used in conjunction with other patient information to aid in the decision for repeat biopsy.

Sometimes a <[Calculated Score] or >[Calculated Score] is reported. This occurs if the PCA3 and/or PSA analyte concentration is outside the quantitative range. If <[Calculated Score] is below the cut-off of 25, the result should be interpreted as NEGATIVE. If >[Calculated Score] is above the cut-off of 25, the result should be interpreted as POSITIVE. In some cases, it may not be possible to determine if a specimen is POSITIVE or NEGATIVE. If the PCA3 Score is indeterminate relative to the cutoff of 25, another specimen must be collected from the patient and retested.

The laboratory may request an additional urine specimen if the laboratory cannot provide a PCA3 Score that can be used for interpretation relative to the cutoff of 25.

Footnotes

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