SUMMARY OF SAFETY AND EFFECTIVENESS DATA (SSED)

I. GENERAL INFORMATION

Device Generic Name: PROGENSA PCA3 Assay

Device Trade Name: PROGENSA® PCA3 Assay

Applicant's Name and Address: Gen-Probe Incorporated
10210 Genetic Center Drive
San Diego, CA 92121

Date(s) of Panel Recommendation: None

Premarket Approval Application (PMA) Number: P100033

Date of FDA Notice of Approval: 02/13/2012

Expedited: Not Applicable

II. INDICATIONS FOR 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.

III. CONTRAINDICATIONS

None

IV. WARNINGS AND PRECAUTIONS

The Black box warning, Limitations, warnings and precautions can be found in the PROGENSA PCA3 Assay labeling.

V. DEVICE DESCRIPTION

A. Explanation of the Test:

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.

The PROGENSA PCA3 Assay is designed to quantify PCA3 and PSA RNA in first catch urine collected following a DRE consisting of three strokes per lobe. 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/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.

B. Device Configurations and Components:

The PROGENSA PCA3 Assay is comprised of two (PCA3 and PSA) quantitative nucleic acid amplification tests on the Gen-Probe DTS (Direct Tube Sampling) Systems. Relevant reagents from the PROGENSA PCA3 Assay Kit together with the three ancillary kits (the PROGENSA PCA3 Specimen Diluent Kit, the APTIMA Assay Fluids kit and the APTIMA Auto Detect Kit) are used to perform each test. The device also provides the PROGENSA PCA3 Urine Specimen Transport Kit.

1. PROGENSA PCA3 Assay Kit contains the following six (6) boxes:
   a. *Box 1*: PROGENSA PCA3 Refrigerated Box contains PCA3 Amplification Reagent [Non-infectious nucleic acids dried in HEPES buffered solution containing <10% bulking agent], PCA3/PSA Enzyme Reagent [Reverse transcriptase and RNA polymerase dried in HEPES buffered solution containing <10% bulking agent] and PCA3 Probe Reagent [Non-infectious chemiluminescent DNA probes dried in succinate buffered solution containing <5% bulking agent and <5% lithium lauryl sulfate (LLS)]. Box 1 is provided ready-to-use and is stored at 2-8°C until the labeled expiration date.

   b. *Box 2*: PROGENSA PCA3 Room Temperature Box contains PCA3 Amplification Reconstitution Solution [Aqueous solution containing preservatives (<1% parabens)], PCA3/PSA Enzyme Reconstitution Solution [HEPES buffered solution containing a surfactant (10% Triton X-100) and 20% glycerol], PCA3/PSA Probe Reconstitution Solution [Succinate buffered solution containing <5% LLS], PCA3/PSA Selection Reagent [Borate buffered solution containing surfactant (1% Triton X-100)] and PCA3 Target Capture Reagent [Non-infectious nucleic acid in HEPES buffered solution containing solid phase (<0.5 mg/mL)]. Box 2 is provided ready-to-use and is stored at 15-30°C until the labeled expiration date.

   c. *Box 3*: PROGENSA PCA3 Calibrator and Controls Kit contains PCA3 Calibrator 1 [Phosphate buffered solution containing <5% LLS], PCA3 Calibrators 2-5 [Non-infectious PCA3 nucleic acid in phosphate buffered solution containing <5% LLS] and PCA3 Positive Controls [Non-infectious PCA3 nucleic acid in phosphate buffered solution containing <5% LLS]. Box 3 is provided ready-to-use and is stored at 2-8°C until the labeled expiration date.

   d. *Box 4*: PROGENSA PSA Refrigerated Box contains PSA Amplification Reagent [Non-infectious nucleic acids dried in HEPES buffered solution containing <10% bulking agent], PCA3/PSA Enzyme Reagent [Reverse

transcriptase and RNA polymerase dried in HEPES buffered solution containing <10% bulking agent] and PSA Probe Reagent [Non-infectious chemiluminescent DNA probes dried in succinate buffered solution containing <5% bulking agent and <5% LLS]. Box 4 is provided ready-to-use and is stored at 2-8°C until the labeled expiration date.

e. *Box 5*: PROGENSA PSA Room Temperature Box contains PSA Amplification Reconstitution Solution [Aqueous solution containing preservatives (<1% parabens)], PCA3/PSA Enzyme Reconstitution Solution [HEPES buffered solution containing a surfactant (10% Triton X-100) and 20% glycerol], PCA3/PSA Probe Reconstitution Solution [Succinate buffered solution containing <5% LLS], PCA3/PSA Selection Reagent [Borate buffered solution containing surfactant (1% Triton X-100)] and PSA Target Capture Reagent [Non-infectious nucleic acid in HEPES buffered solution containing solid phase (<0.5 mg/mL)]. Box 5 is provided ready-to-use and is stored at 15-30°C until the labeled expiration date.

f. *Box 6*: PROGENSA PSA Calibrator and Controls Kit contains PSA Calibrator 1 [Phosphate buffered solution containing <5% LLS], PSA Calibrators 2-5 [Non-infectious PSA nucleic acid in phosphate buffered solution containing <5% LLS] and PSA Positive Controls [Non-infectious PSA nucleic acid in phosphate buffered solution containing <5% LLS]. Box 6 is provided ready-to-use and is stored at 2-8°C until the labeled expiration date.

2. PROGENSA PCA3 Ancillary Kits:.
   a. PROGENSA PCA3 Specimen Diluent Kit contains PCA3/PSA Specimen Transport Medium [Phosphate buffered solution containing 110 mM LLS].

   b. APTIMA Assay Fluids kit contains Wash Solution [HEPES buffered solution containing <2% sodium dodecyl sulfate], Buffer for Deactivation Fluid [Bicarbonate buffered solution] and Oil Reagent [Silicone oil].

   c. APTIMA Auto Detect Kit contains APTIMA Auto Detect 1 [Aqueous solution containing 0.1% hydrogen peroxide and 0.001 N nitric acid] and APTIMA Auto Detect 2 [1.6 N sodium hydroxide].

3. PROGENSA PCA3 Urine Specimen Transport Kit contains PROGENSA PCA3 Urine Specimen Transport Tubes [PROGENSA PCA3 urine transport medium] and Disposable Transfer Pipettes [for the transfer of 2.5 mL of urine from the primary collection container to the PROGENSA PCA3 urine specimen transport tube].

C. Specimen collection:

The PROGENSA PCA3 Assay utilizes first catch whole urine collected following a digital rectal examination (DRE), in which a urologist puts a gloved finger into the rectum to feel the prostate gland. The urologist applies three strokes for each lobe, using enough pressure each time to slightly depress the prostate surface, from the base to the apex and from the lateral to the median line. The DRE releases prostate cells through the prostate duct system into the urinary tract, where they can be

collected in the first catch urine (20 to 30 mL). The urine is collected in an appropriately labeled urine collection cup. This must be the first voided urine specimen following the DRE. After inverting the cup 5 times, 2.5 mL of urine is transferred to the PROGENSA Urine Specimen Transport Tube (immediately or within 4 hours maintained at 2-8°C) of the PROGENSA PCA3 Urine Specimen Transport Kit, using the disposable transfer pipette provided. The PROGENSA PCA3 Urine Specimen Transport Kit contains the Urine Transport Medium (UTM), which lyses the cells and stabilizes the RNA.

Processed urine specimens are transported to the laboratory in the urine specimen transport tube under ambient conditions (without temperature control) or frozen for arrival at the laboratory within 5 days of collection. 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, specimens can be stored at -35°C to -15°C for up to 11 months or, at or below -65°C for up to 36 months. Processed urine specimens may be subjected to up to 5 freeze-thaw cycles.

D. Principles of the Procedure:

The PROGENSA PCA3 Assay is comprised of two quantitative nucleic acid amplification tests. The assay combines the technologies of target capture, Transcription Mediated Amplification (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. Oligonucleotides ("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).

E. Test Calibrators and Controls:

Five PCA3 and PSA Calibrators and two external Controls are provided with the Assay Kit; their target concentrations are given in the Table 01 below:

Table 01: Calibrator and Control target concentration

Description	Target PCA3 Concentration (copies/mL)	Target PSA Concentration (copies/mL)
Calibrator 1	0	0
Calibrator 2	250	7,500
Calibrator 3	2,500	75,000
Calibrator 4	25,000	750,000
Calibrator 5	125,000	3,000,000
Control A	1,250	37,500
Control B	62,500	1,500,000

The Positive Calibrators and Controls are value assigned, so the actual copies/mL values for Calibrators 2 to 5 and Controls A and B will be slightly different than the target concentrations listed in the Table 01 (above), and will vary from lot to lot. The assigned values will be provided on a card in the package of calibrator and control vials and are used for calibration and determination of run validity. Three replicates of Calibrators containing known amounts of PCA3 or PSA RNA transcripts are included in every assay run and used to generate a standard curve. Two replicates of PCA3 and PSA controls are also included to verify the accuracy of results interpolated from the standard curve. Calibrators and controls are run on the same rack as specimens.

F. Validation of Test runs and PCA3 Score:

1. PCA3 and PSA RNAs are quantified in separate tubes. Specimens are run in duplicate to generate RLU and the concentration of the two analytes as copies/mL is assigned from the calibration curve. Subsequently, the software uses duplicate values and calculates an average (Mean) value. Quality control procedures generate a QC Report and Run Report as described below.

    a. *QC Report*: The QC Report lists assay run validity criteria, assigned and interpolated concentrations, and recoveries of calibrators and controls. The report also lists the parameters that define the four-parameter logistic dose response calibration curve.

    b. *Run Validity*: Based on Quality Control Procedures, the Raw Run Report provides information on run validity (PASS or FAIL) and on the individual reaction tubes tested with the PROGENSA PCA3 Assay. If a run is invalid (FAIL), all tubes in that run will be labeled invalid. However, individual tubes may be deemed invalid within a valid run (PASS). For back-to-back runs (i.e., both PCA3 and PSA analytes are tested in the same assay run), it is possible that one analyte run may be invalid while the other analyte run is valid; in this case, failed analyte will be re-run in a separate run.

c. *PCA3 Score*: In back-to-back runs where both analyte runs are valid, the software automatically matches the individual PCA3 and PSA analyte results for specimens and determines the PCA3 Score (if calculable). The software calculates and lists the PCA3 Score of specimens in the Ratio Report. When PCA3 and PSA analytes are tested in different runs, the software cannot automatically determine the PCA3 Score. Manual matching of the analyte results is necessary to determine the PCA3 Score or PCA3 Score range.

G. Interpretation of Test Results:

The PCA3 Score is calculated as the ratio of PCA3 RNA copies to PSA RNA copies, multiplied by 1000. If the reported PCA3 Score is below the cut-off of 25, the result is interpreted as NEGATIVE. If the PCA3 Score is above or equal to the cut-off of 25, the result is interpreted as POSITIVE. A NEGATIVE result is associated with a decreased likelihood of a positive biopsy. As the PCA3 score decreases, the likelihood of a positive biopsy decreases. The PCA3 Score should be interpreted in conjunction with other laboratory and clinical data available to the clinician and relevant guidelines in the decision for repeat biopsy. Caution is required in the following conditions:

1. 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.

2. For specimens with PCA3 analyte levels outside the calibrator range and PSA analyte levels inside the calibrator range, the PCA3 Score may be reported as a range (>[(125,000/B)*1000] or <[(250/B)*1000] where B is the PSA analyte level).

3. For specimens with PSA analyte levels above the calibrator range and PCA3 analyte levels inside the calibrator range, the PCA3 Score may be reported as a range <[(A/3,000,000)*1000] where A is the PCA3 analyte level.

4. Specimens with PSA analyte levels below the calibrator range have insufficient RNA for accurate analysis and a new specimen must be collected.

5. PCA3 Scores are only calculated using results from valid runs and specimens. Invalid runs and invalid specimens must be retested for that analyte.

6. 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. For example, if the PCA3 Score obtained is "<100", an overall interpretation relative to the cutoff of 25 cannot be made. If a numerical value is required for interpretation relative to the cutoff of 25, specimen dilution and retesting may generate a PCA3 Score instead of a PCA3 Score range. If dilution and retesting still cannot provide a PCA3 Score which can be used for interpretation relative to the cut-off of 25, another specimen collection must be requested.

Retesting is optional if a sample has an out-of-range high result for one analyte and an in-range result for the other analyte. However, for the clinical study, the operator was instructed to retest samples having results meeting these conditions, as a PCA3 Score was preferred for the study analyses. Of the 480 samples tested in the clinical study, 73 sample results (73/480=15.2%) required further testing or further testing was considered optional.

VI. ALTERNATIVE PRACTICES AND PROCEDURES

Prostate biopsy with an appropriate number of cores is considered the "gold standard" and is required for a diagnosis of prostate cancer. A prostate biopsy is an invasive procedure in which a number of samples (cores) of prostate tissue are removed and examined under a microscope. Early detection of prostate cancer has historically relied on serum PSA testing and/or DRE. An abnormal result from either of these tests will most likely lead to a recommendation for prostate biopsy. There are no other FDA-cleared or approved alternatives for the tests that would/would not recommend repeat biopsy.

VII. MARKETING HISTORY

The PROGENSA PCA3 Assay was CE-Marked on September 30, 2006 and is currently marketed to the following countries:

• Australia	• Hong Kong	• Pakistan
• Austria	• Hungary	• Philippines
• Bahamas	• Iceland	• Portugal
• Belgium	• Indonesia	• Romania
• Bermuda	• Ireland	• Russia
• Bulgaria	• Italy	• Saudi Arabia
• Cambodia	• Japan	• South Africa
• Chile	• Kenya	• Spain
• Czech Republic	• Libya	• Sweden
• Denmark	• Luxembourg	• Switzerland
• Dominican Republic	• Madagascar	• Tunisia
• Finland	• Malaysia	• UAE
• France	• Mali	• United Kingdom
• Germany	• Namibia	• Uruguay
• Greece	• Netherlands	• Vietnam
• Honduras	• Norway	

There have been no instances where the product has been withdrawn from any country for any reason related to safety and effectiveness.

VIII. POTENTIAL ADVERSE EFFECTS OF THE DEVICE ON HEALTH

The risk associated with the PROGENSA PCA3 Assay is a false assay result (i.e., a false positive or false negative result). A false negative result from the PROGENSA PCA3 Assay may defer necessary follow-up procedures (e.g., delay a follow-up prostate

biopsy). The associated risk of delaying clinical action is that the cancer may continue to spread leading to an irreversible adverse condition. Although the test is indicated for use in men for whom a repeat biopsy would be recommended by a urologist based on current standard of care, a false positive result from the PROGENSA PCA3 Assay may lead to more aggressive follow-up procedures (e.g., increased number of cores taken at a subsequent biopsy), which may expose the patient to increased risk.

The PROGENSA PCA3 Assay is intended to be used in conjunction with other clinical information; it is not meant to be used as the sole determinant for follow-up procedures. Therefore, the decision in determining appropriate patient management (e.g., the decision for repeat biopsy) must be based on an assessment of multiple risk indicators and not solely on the PROGENSA PCA3 Assay result.

IX. SUMMARY OF PRECLINICAL STUDIES

The nonclinical and analytical studies were conducted in accordance with 21CFR 58 "Good Laboratory Practices for Nonclinical Laboratory Studies".

A. Precision Studies:

Precision measurements were conducted to evaluate precision (reproducibility and repeatability) according to CLSI document EP5-A2.

1. Reproducibility:

   PROGENSA PCA3 Assay reproducibility was evaluated on DTS Systems at 3 external clinical testing sites using a 3-member reproducibility panel. Testing was performed using 3 reagent lots and 3 calibrator and control lots. Two operators at each of the 3 testing sites independently performed, over 15 days, five (5) PROGENSA PCA3 Assay runs per each of the 3 reagent lots (1 lot per day). Each run contained 4 sets of the 3 reproducibility panel members. The total number of results for each panel member was 360.

   Reproducibility panel members were created by spiking PCA3 and PSA *in vitro* transcripts into a urine matrix composed of negative (female) urine specimens and PROGENSA PCA3 Urine Transport Medium. The use of spiked female urine (transcripts in female urine) as the sample source in the precision studies was validated by direct comparison of spiked female urine vs. clinical specimens with a target PCA3 Score of 25 (at cut-off). Specimens were tested in duplicate in 16 runs (patient sample volume limitations did not permit testing in replicates comparable to the precision studies) by multiple operators, and total standard deviation (SD) and coefficient of variation (CV) were calculated. The sample precision in clinical specimens was comparable to sample precision in spiked female urine samples (Table 02).

Table 02: Comparison of spiked female urine vs. clinical specimens

Sample Source	PCA3 Analyte	PSA Analyte	PCA3 Score
Clinical Specimens	6.8% - 12.5%	8.0% - 15.9%	8.3% - 16.0%
Spiked Female Urine	5.1% - 18.2%	9.2% - 17.9%	13.3% - 20.6%

The analyte concentrations and targeted PCA3 Scores for each panel member are shown in Table 03. Panel members 2 and 3 had RNA concentrations representative of the copy levels found in post-DRE urine specimens; Panel member 1 had RNA concentrations near the low end of the PCA3 and PSA dynamic ranges.

Table 03: Reproducibility Panel Composition.

Panel Member	n*	PCA3 RNA Concentration	PSA RNA Concentration	Targeted PCA3 Score
1	359	Low	Low	35
2	359	Mid	High	10
3	357	High	Mid	86

*Five samples (one sample of Panel Member 1, one sample of Panel Member 2, and three samples of Panel Member 3) had invalid or out-of-range PCA3 and/or PSA analyte results leading to invalid or non-evaluable PCA3 Scores and were not included in the analyses.

Table 04 summarizes the variability of the PROGENSA PCA3 Assay within runs, between runs, between operators, between reagent lots, and between sites/instruments for each panel member for PCA3 and PSA analyte copies/mL and for PCA3 Score.

Table 04: Assay Reproducibility.

Parameter Panel Member	Mean Value	Within-Run CV%	Between-Run CV%	Between-Operator CV%	Between-Lot CV%	Between-Site CV%	Total CV%
PCA3 copies/mL							
1	678	12.2	10.7	0.0	4.9	2.6	17.2
2	18,969	5.1	4.3	0.0	0.0	1.4	6.8
3	97,006	4.8	3.9	2.3	3.3	3.0	8.0
PSA copies/mL							
1	16,747	16.2	9.7	3.4	1.9	0.0	19.3
2	1,638,117	7.8	7.1	0.0	4.2	2.8	11.7
3	994,851	6.6	7.0	0.0	2.6	3.4	10.5
PCA3 Score							
1	41	17.0	16.1	4.3	7.4	0.0	25.0
2	11	10.7	9.1	0.0	4.3	2.9	15.0
3	98	8.6	8.1	3.0	1.9	0.0	12.3

For calculating SD, use: SD = mean*CV%

The reproducibility (within-run, between-run, between-operator, between-lot, and between-site components of imprecision) was determined to be adequate.

2. Within-Laboratory Precision:
PROGENSA PCA3 Assay within-laboratory precision was evaluated at the internal site using a 4-member reproducibility panel. Three panel members (1 to 3) comprised PCA3 and PSA *in vitro* transcripts in processed female urine, similar to the reproducibility panels (see above). The fourth panel member was comprised of PCA3 and PSA *in vitro* transcripts in processed female urine diluted in specimen diluent (Mid/Diln).

Testing was performed using one reagent lot and one calibrator and control lot. One operator performed twenty PROGENSA PCA3 Assay runs on DTS Systems; each run contained 4 sets of the 4 panel members. Table 05 summarizes the variability of the PROGENSA PCA3 Assay within-run, between-run and between-day components of imprecision for each panel member for PCA3 and PSA analyte copies/mL and for PCA3 Score.

Table 05: PROGENSA PCA3 Assay Repeatability.

Parameter Panel Member	PCA3 Conc	PSA Conc	n[1]	Mean Value	Within-Run CV%	Between-Run CV%	Between-Day CV%	Total CV%
PCA3 copies/mL								
1	Low	Low	80	661	12.9	8.1	10.1	18.3
2	Mid	High	80	18,626	5.5	4.0	0.8	6.9
3	High	Mid	80	99,846	3.8	1.1	3.3	5.2
4	Mid/Diln	Mid/Diln	80	24,482	4.8	4.3	0.0	6.4
PSA copies/mL								
1	Low	Low	80	18,298	15.6	4.6	1.5	16.4
2	Mid	High	77	2,017,466	9.4	1.4	0.0	9.5
3	High	Mid	80	1,247,896	18.3	0.0	3.6	18.7
4	Mid/Diln	Mid/Diln	80	603,427	17.9	5.3	0.0	18.7
PCA3 Score								
1	Low	Low	80	36	19.0	7.7	2.3	20.7
2	Mid	High	77	9	12.0	6.2	0.6	13.6
3	High	Mid	80	81	13.6	0.0	3.3	14.0
4	Mid/Diln	Mid/Diln	80	41	14.6	9.3	0.0	17.3

[1]Three samples of Panel Member 2 had out-of-range PSA analyte results leading to non-evaluable PCA3 Scores and were not included in the analyses.
For calculating SD, use: SD = mean*CV%

The within-laboratory precision (within-run, between-run and between-day components of imprecision) was determined to be adequate.

3. Additional Statistical Modeling:
In addition to the precision studies for the PCA3 Score described above, a statistical modeling of possible values of %CV for the PCA3 Score imprecision based on the precision profiles of each individual analyte, PCA3 and PSA, was performed. There are many possible combinations of the amounts of individual analyte that give the same value of the PCA3 Score and the additional statistical modeling provided information about precision profile of the PCA3 Score for different combinations of individual analyte values. The precision data from the precision studies described above were used for building precision profiles of each individual analyte, PCA3 and PSA. The precision profiles were constructed by performing linear interpolation using the known precision data from the reproducibility studies with actual samples. For each possible combination of the values of the individual analytes, the value of the PCA3 Score corresponding to this combination of values of analytes and %CV of the PCA3 Score were estimated. Because the PCA3 Score is based on separate measures of individual analytes in a sample, random measurement errors of each analyte can be considered as uncorrelated. The data of the clinical study were used for consideration of which combinations of PCA3 and PSA were clinically possible; for these combinations, PCA3 Score and %CV were estimated. The additional statistical modeling showed that 94% of subjects in the clinical study with PCA3 Score close to the cutoff=25 had total imprecision of 14%-18% (6% of subjects had total imprecision of 18%-25%).

B. Analytical Sensitivity:

1. Limit of Blank, Limit of Detection and Limit of Quantitation:
Limit of blank, detection, and quantitation were determined per Clinical and Laboratory Standards Institute approved guideline EP17-A, "Protocols for Determination of Limits of Detection and Limits of Quantitation". Limit of blank (LoB) was determined using percentile method.

The limit of quantitation (LoQ) of the PROGENSA PCA3 Assay was determined using an 8-member panel. The panel was comprised of 4 blank specimens (processed female urine that contains no detectable prostate-specific PCA3 or PSA RNA) and the blank specimens each spiked with PCA3 and PSA *in vitro* transcripts at Calibrator 2 concentrations. One operator performed ten PROGENSA PCA3 Assay runs on DTS Systems; each run contained 2 sets of the 8 panel members. Limit of detection (LoD) was calculated by LoB + 1.65 SD. The LoD of the PCA3 analyte was 239 copies/mL (CV 31.2%); and for the PSA analyte it was 3,338 copies/mL (CV 24.2%) (Table 06). The LoQs of both analytes were the same as the corresponding LoD. The lower limit of the dynamic range of the PROGENSA PCA3 Assay is defined by the lowest positive calibrator.

Table 06: Analytical sensitivity

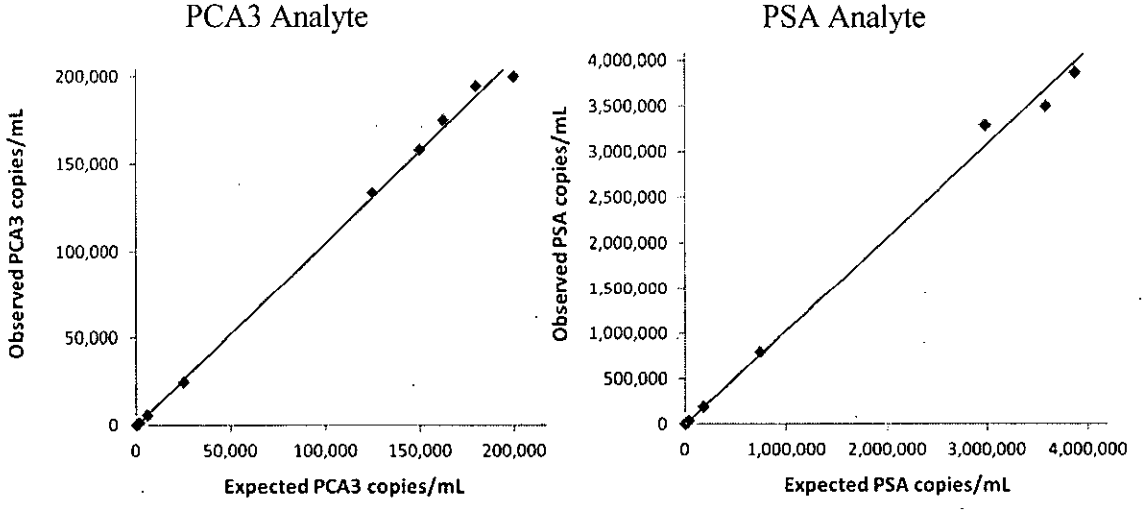
Analyte	Limit of Blank copies/mL	Limit of Detection copies/mL	CV at Limit of Detection	Limit of Quantitation copies/mL
PCA3	90	239	31.2%	239
PSA	254	3,338	24.2%	3,338

C. Linearity and Measuring Interval:

1. Linearity:
The linear range was determined according to CLSI EP06-A, Evaluation of the Linearity of Quantitative Measurement Procedures: A Statistical Approach. Based on the results of the Limit of Detection Study, linearity testing for the two analytes PCA3 and PSA was done on a range 20-30% wider than the anticipated measuring interval.

a. *Linearity studies using PCA3 and PSA in vitro transcripts in processed female urine*: The linear range of the PROGENSA PCA3 Assay was determined using an 11-member linearity panel. A dilution series was prepared from PCA3 and PSA *in vitro* transcripts in processed female urine due to unavailability of very high concentration clinical material. Dilutions from a single concentration spanned beyond the assay range for each analyte. One operator performed four PROGENSA PCA3 Assay runs on DTS Systems; each run contained 2 sets of the 11-member linearity panel. Results were analyzed using regression analysis according to CLSI EP6-A (4). Results of weighted linear regression analysis are presented in Figure 1. For PCA3 analyte, the PROGENSA PCA3 Assay demonstrated linearity from 135 to 200,032 copies/mL with deviation from linearity less than 9% in this interval; the dynamic range of the assay for PCA3 analyte is 250 to 125,000 copies/mL. For PSA analyte, linearity was demonstrated from 4,670 to 3,874,323 copies/mL with deviation from linearity less than 7%; the dynamic range is 7,500 to 3,000,000 copies/mL.

Figure 1: PROGENSA PCA3 Assay Linearity for PCA3 and PSA Analytes, Transcript Samples.

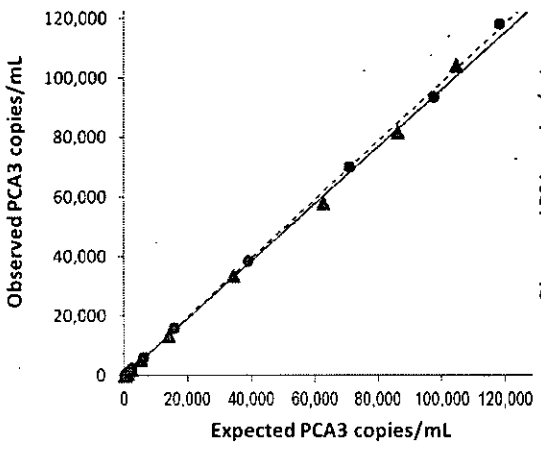


b. *Linearity studies using clinical specimens in specimen diluent or processed female urine*: Linearity was verified through 85% of the dynamic range in a dilution series of clinical materials. For each analyte, two sets of a 10-member linearity panel were tested and analyzed as above. Each set consisted of a dilution series prepared in PCA3 Specimen Diluent and another one in processed female urine independently. For PCA3 analyte, the PROGENSA PCA3 Assay demonstrated linearity from 130 to 104,564 copies/mL in processed female urine with deviation from linearity less than 6%. In PCA3 Specimen Diluent, the

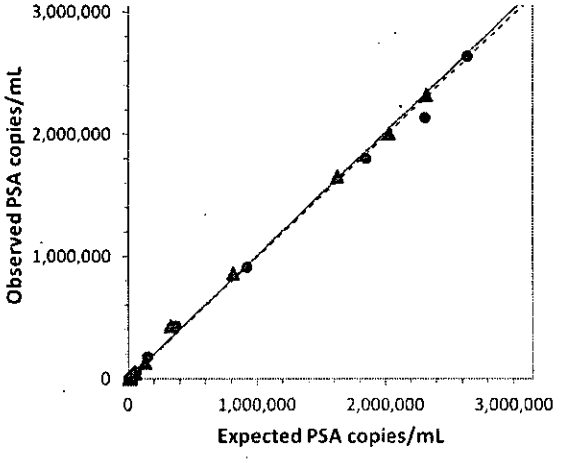
PCA3 analyte demonstrated linearity from 162 to 118,237 copies/mL with deviation less than 6% in this interval. For PSA analyte, linearity was demonstrated from 4,243 to 2,324,179 copies/mL with deviation from linearity less than 30% in processed female urine. In PCA3 Specimen Diluent, the PSA analyte demonstrated linearity from 4,890 to 2,640,820 copies/mL with deviation less than 23% in this interval. Although the deviation from linearity for the PSA analyte was within study acceptance criteria, the higher-than-expected deviation may have been caused by variation during linearity panel preparation. In summary, there was no significant diluent matrix effect. See Figure 2 (the solid line with triangles represents panel members diluted in processed female urine, and the dashed line with circles represents panel members diluted in PCA3 Specimen Diluent).

Figure 2: PROGENSA PCA3 Assay Linearity for PCA3 and PSA Analytes, Clinical Specimens.

PCA3 Analyte



PSA Analyte



The measuring interval for PCA3 analyte is 250 – 125,000 copies/mL and the measuring interval for PSA analyte is 7,500 – 3,000,000 copies/mL. The interval of possible numerical values of the PCA3 Score is 0 to 16,667. In the clinical study, the range of the PCA3 Scores of 466 patients was 0 – 462.

2. Recovery Studies:
PROGENSA PCA3 Assay analyte quantitation was compared to an independent method (trueness could not be evaluated as no reference method yet exists). PCA3 and PSA *in vitro* transcripts were quantified by UV-vis spectrophotometry (assuming 1 optical density unit at 260 nm is equal to 40 µg/mL RNA) at a much higher concentration than tested with PROGENSA PCA3 Assay. An 8-member test panel was prepared by dilution of the UV-quantified transcripts into processed female urine (107 to 1010 fold). Two operators each performed four (4) PROGENSA PCA3 Assay runs; each run contained 4 sets/replicates of the 8-member test panels. Percent recovery was calculated as the ratio of PCA3 Assay measured copies/mL to UV-determined copies/mL, multiplied by 100 (Table 07).

Table 07: Copy Recovery of the PROGENSA PCA3 Assay.

Analyte	Panel Member	n[1]	UV-Calculated Concentration, copies/mL	Measured Concentration, copies/mL	Recovery
PCA3	1	32	1,250	1,377	110%
	2	32	12,500	12,452	100%
	3	32	62,500	56,501	90%
	4	32	6,250	7,244	116%
	5	32	250	294	118%
	6	32	500	590	118%
	7	32	95,000	89,963	95%
	8	31	125,000	124,337	100%
PSA	1	32	37,500	36,110	96%
	2	32	375,000	372,237	99%
	3	32	1,500,000	1,309,999	87%
	4	32	150,000	171,612	114%
	5	32	7,500	9,025	120%
	6	32	15,000	18,199	121%
	7	31	3,000,000	2,554,682	85%
	8	31	2,280,000	2,198,033	96%

[1]Three samples (one sample in Panel Member 7 and two samples in Panel Member 8) had invalid PCA3 and/or PSA analyte results and were not included in the analyses.

From the clinical study, a total of 480 subjects out of 495 subjects who were eligible for analysis (97.0%) had a valid PCA3 Score: 4.0% (19/480) subjects had PCA3 analyte copies/mL in the range 250 copies/mL to 500 copies/mL and 1.3% (6/480) subjects had PSA analyte copies/mL in the range 7,500 copies/mL to 15,000 copies/mL. There were 0.8% (4/480) subjects having both PCA3 analyte and PSA analyte concentrations in these specified ranges, thus there were 4.4% (21/480) unique subjects with specimen results for either or both analytes in the specified ranges.

D. Analytical Specificity:

1. Unspliced Transcript:
   PROGENSA PCA3 Assay was designed to detect only the prostate cancer-specific exon 3-exon 4 spliced PCA3 RNA (Verhaegh G.W. et al, 2003). The assay did not detect 1.25×10^6 copies/mL of unspliced PCA3 RNA in processed female urine significantly above background.

2. Interfering Substances:
   Interfering substances were tested with PROGENSA PCA3 Assay according to CLSI EP7-A2, Interference Testing in Clinical Chemistry, using pooled clinical (post-digital rectal examination, male) urine specimens. To specimen aliquots, common over-the-counter medications, prescription medications, dietary supplements, endogenous substances, blood components, and microorganisms were added. Concentrations of some interferents were based on CLSI EP7-A2, and for medications not listed in EP7-A2, the test concentration was set at three times the maximum therapeutic dose per liter. For other substances, the test concentration was

five times the dosage per liter or five times the typical amount found in urine. Interference panels were tested in randomized order, four sets per run; two operators conducted testing. The control was always tested in the same run as interferent samples.

a. *Endogenous Substance Interference*:
PROGENSA PCA3 Assay results from interference testing of ten endogenous substances (listed in the Table 08 below) in the pooled clinical specimens showed no interference (no significant change in PCA3 Score) from these endogenous substances.

Table 08: Endogenous Substance Interference

Substance	Test Concentration
Albumin	60 g/L
Bilirubin (unconjugated)	0.342 mmol/L
Calcium	5 mmol/L
Cholesterol	13 mmol/L
Glucose	55 mmol/L
Hemoglobin	2 g/L
Immunoglobulin G	32 mg/L
Triglycerides	37 mmol/L
Uric acid	1.4 mmol/L
Red blood cells	5.10×10^7 cells/L
White blood cells	7.60×10^7 cells/L

b. *Exogenous Substance Interference*:
PROGENSA PCA3 Assay results from interference testing of twenty seven exogenous substances in the pooled clinical specimens showed that of all substances tested, only selenium (Table 09) and saw palmetto (Table 10) interfere with quantitation of PCA3 and PSA analytes by reducing the analyte quantitation similarly for both; however, they do not have a high effect on the PCA3 Score.

Table 09: Dose-Response Testing of Selenium Interference.

Parameter	Test Concentration	Mean of 4 Replicates copies/mL	Percent
PCA3 copies/mL	0 mg/L	51,760	100.0%
	0.25 mg/L	45,004	86.9%
	0.5 mg/L	39,134	75.6%
	0.75 mg/L	35,181	68.0%
	1 mg/L	30,200	58.3%
PSA copies/mL	0 mg/L	636,100	100.0%
	0.25 mg/L	556,813	87.5%
	0.5 mg/L	521,505	82.0%
	0.75 mg/L	447,109	70.3%

Parameter	Test Concentration	Mean of 4 Replicates copies/mL	Percent
	1 mg/L	409,142	64.3%
PCA3 Score	0 mg/L	81	100.0%
	0.25 mg/L	81	100.0%
	0.5 mg/L	75	92.6%
	0.75 mg/L	79	97.5%
	1 mg/L	73	90.1%

Table 10: Dose-Response Testing of Saw Palmetto Interference.

Parameter	Test Concentration	Mean of 4 Replicates copies/mL	Percent
PCA3 copies/mL	0 g/L	30,595	100%
	2.81 g/L	29,142	95.3%
	5.63 g/L	23,829	77.9%
	8.44 g/L	19,526	63.8%
	11.25 g/L	15,054	49.2%
PSA copies/mL	0 g/L	399,919	100.0%
	2.81 g/L	312,425	78.1%
	5.63 g/L	290,529	72.6%
	8.44 g/L	214,874	53.7%
	11.25 g/L	154,401	38.6%
PCA3 Score	0 g/L	76	100.0%
	2.81 g/L	98	128.9%
	5.63 g/L	83	109.2%
	8.44 g/L	90	118.4%
	11.25 g/L	97	127.6%

No interference (no significant change in PCA3 Score) from other exogenous substances was observed (Table 11). However, 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. This information is included in the Limitation section of the Package Insert.

Table 11: Exogenous Substance Interference.

Substance	Test Concentration
Acetaminophen	1.324 mmol/L
Acetylsalicylic acid	3.62 mmol/L
Alfuzosin	30 mg/L
Allopurinol	0.294 mmol/L
Amlodipine	0.245 µmol/L
Atenolol	37.6 µmol/L
Atorvastatin	25 mg/L
Ciprofloxacin	30.2 µmol/L

Substance	Test Concentration
Diphenhydramine	19.6 µmol/L
Doxazosin	1.33 µmol/L
Doxycycline	67.5 µmol/L
Dutasteride	1.5 mg/L
Esomeprazole	0.12 g/L
Finasteride	15 mg/L
Fluoxetine	11.2 µmol/L
Flutamide	2.25 g/L
Furosemide	0.181 mmol/L
Ibuprofen	2.425 mmol/L
Levofloxacin	48.6 µmol/L
Lisinopril	0.74 µmol/L
Metformin	0.31 mmol/L
Selenium	1 mg/L
Saw palmetto	11.25 g/L
Sildenafil	12.9 nmol/L
Sulfasalazine	0.754 mmol/L
Tamsulosin	1.2 µg/L
Terazosin	7.8 µmol/L

c. *Microorganisms Interference*:
PROGENSA PCA3 Assay results from interference testing of six microorganisms (listed in Table 12 below) in pooled clinical specimens showed no interference (no significant change in PCA3 Score) resulting from contamination with these microorganisms.

Table 12: Microorganism Interference.

Organism	Test Concentration
Candida albicans	5 x 10^6 CFU*/L
Escherichia coli	5 x 10^6 CFU/L
Klebsiella pneumoniae	5 x 10^6 CFU/L
Proteus mirabilis	5 x 10^6 CFU/L
Pseudomonas aeruginosa	5 x 10^6 CFU/L
Staphylococcus aureus	5 x 10^6 CFU/L

*CFU = colony-forming units

3. Assay Carryover/Cross-Contamination:
To determine the rate of false positive analyte results (carryover or cross-contamination) obtained with the PROGENSA PCA3 Assay using DTS Systems, samples containing high titer PCA3 and PSA *in vitro* transcripts were interspersed throughout specimen processing racks containing negative samples. High titer

positive samples for this study were created by spiking PCA3 and PSA *in vitro* transcripts into processed female urine to concentrations of 1.5×10^5 copies/mL PCA3 and 3.6×10^6 copies/mL PSA. Negative samples were composed of processed female urine (female urine contains no prostate-specific PCA3 or PSA RNA), where equal volumes of female urine and PROGENSA PCA3 UTM were mixed. Five specimen rack configurations were generated, where eight high titer samples were interspersed among 32 negative samples. The overall false positive rate was calculated, where a false positive was defined as quantifiable analyte (greater than Calibrator 2, which defines the lower limit of the assay's dynamic range) in a valid negative sample. The observed false positive rate for the PROGENSA PCA3 Assay with DTS Systems, when high titer samples are interspersed throughout a rack of negative samples, was 0% for both analytes. These results (Table 13) demonstrate that the risk of sample carryover or cross-contamination on DTS Systems is very low.

Table 13: PROGENSA PCA3 Assay Crossover

Analyte tested	Parameter	Specimen Type	
		Negative	Positive
PCA3 Analyte	Tested (n)	224	56
	Valid (n)	210	55
	Positive (n)	0	55
	Positive (%)	0	100
PSA Analyte	Tested (n)	160	40
	Valid (n)	160	40
	Positive (%)	0	40
	Positive	0	100

E. Stability studies:

Studies supporting stability claims were conducted in accordance to EP25-A: Evaluation of Stability of *In Vitro* Diagnostic Reagents; Approved Guideline.

1. Specimen stability:
   a. *Urine specimen before processing*:
   Twelve unprocessed clinical specimens were incubated at 5°C or 30°C for 0, 1, 4 or 24 hrs and analyzed in a single run of PROGENSA PCA3 Assay using one reagent lot. The data for each urine specimen analyzed according to EP25-A, showed an acceptable drift in PCA3 analyte, PSA analyte and PCA3 Score for 4 hours (Table 14).

   Table 14: Unprocessed Urine Stability at 30°C and 2-8°C for 0 hours vs. 4 hours - Deming Regression Analysis

Temperature tested	Parameter	Intercept	Slope	% Drift
at 30°C	PCA3 Score	1.95	0.95	5%
	PCA3 Analyte	241	0.93	7%

Temperature tested	Parameter	Intercept	Slope	% Drift
	PSA Analyte	-10,599	0.92	8%
at 2-8°C	PCA3 Score	2.01	0.98	2%
	PCA3 Analyte	-491	1.12	12%
	PSA Analyte	-31,567	1.03	3%

Based on these results, 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 PROGENSA Urine Specimen Transport Tube within 4 hours of collection. Otherwise, the specimen must be rejected and the urologist must collect a new specimen.

b. *Processed urine specimen during transportation*:
Processed urine specimens are transported to the laboratory in the PROGENSA Urine Specimen Transport Tube. To provide support for the label claim that specimens may be shipped under ambient conditions, a Deming regression analysis was performed on the 12 processed specimens that were subjected to temperature fluctuations that could possibly occur during transportation, tested at Day 0 vs. after 6 days of cycling; from 30°C to -70°C Freeze-thaw and from 30°C to 55°C High-Temperature cycling. The data for each urine specimen analyzed according to EP25-A, showed an acceptable drift in PCA3 analyte, PSA analyte and PCA3 Score (Table 15).

Table 15: Processed Urine Shipping Stability during 30°C to -70°C (Freeze-thaw) and 30°C to 55°C (High-Temperature) cycling - Deming Regression.

Temperature cycle tested	Parameter	Intercept	Slope	% Drift
30°C to -70°C	PCA3 Score	-0.60	1.08	8%
	PCA3 Analyte	109	0.95	5%
	PSA Analyte	4,243	0.98	2%
30°C to 55°C	PCA3 Score	0.34	0.92	8%
	PCA3 Analyte	357	0.91	9%
	PSA Analyte	-8,086	1.10	10%

Based on these results, the specimen 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 5 days of collection. Upon receipt of the shipment, the laboratory should verify that the date of specimen collection is on the tube. If specimens were shipped under ambient conditions and are received greater than 5 days after specimen collection, the specimen must be rejected and a request for a new specimen should be made.

c. *Processed urine specimen at the testing site*:
Twelve processed clinical specimen were tested at refrigerated and frozen conditions, and freeze-thaw cycles after receipt at the testing site. The data for

each urine specimen analyzed according to EP25-A, showed an acceptable drift in PCA3 analyte, PSA analyte and PCA3 Score (Table 16).

Table 16: Processed Urine stability at specific temperature, Before vs. After the cycle - Deming Regression

Temperature cycles	Parameter	Intercept	Slope	% Drift
2°C to 8°C / 0-days vs. 18 days (After Freeze-Thaw Cycling)	PCA3 Score	0.02	1.04	4%
	PCA3 Analyte	49	0.90	10%
	PSA Analyte	27,394	0.73	27%
2°C to 8°C / 0-days vs.18 days (After High-Temperature Cycling)	PCA3 Score	-0.57	0.86	14%
	PCA3 Analyte	772	0.89	11%
	PSA Analyte	-89,840	1.24	24%
-35°C to -15°C / 0 months vs.12 months	PCA3 Score	-2.14	1.13	13%
	PCA3 Analyte	-1,134	1.16	16%
	PSA Analyte	-28,697	1.07	7%
At or Below -65°C / 0 months vs. 38 months	PCA3 Score	1.28	0.96	4%
	PCA3 Analyte	-87	1.16	16%
	PSA Analyte	20,976	1.06	6%
Freeze-Thaw / Before vs. After 6 Freeze-Thaws	PCA3 Score	-0.60	1.08	8%
	PCA3 Analyte	109	0.95	5%
	PSA Analyte	4,243	0.98	2%

Based on these results, the laboratory may store specimens at (1) 2°C to 8°C for up to 14 days, (2) -35°C to -15°C for up to 11 months, (3) at or below -65°C for 36 months, and (4) five freeze-thaw cycles, before disposal is required.

2. PROGENSA PCA3 Kit Reagent Stability:
The stability test design of the PROGENSA PCA3 Assay Kit, Calibrators and Controls for unopened and opened/reconstituted reagents included testing of three independent lots of the assay reagent kit with three independent lots of calibrators and controls throughout the study. The room temperature reagents (Amplification Reconstitution Solutions, Enzyme Reconstitution Solution, Probe Reconstitution Solution, Selection Reagent, and Target Capture Reagents) were stored at 28°±2°C for the duration of the study. The refrigerated reagents (lyophilized Amplification Reagents, Enzyme Reagent, and Probe Reagents) and Calibrators and Controls were stored at 5°±3°C for the duration of the study. Additionally, vials of Calibrator 2 and Control B from the same set were frozen (≤-65°C) in the beginning of the study and were tested as samples at every time point throughout the study. Reagent stability data were analyzed for drift based on the recommendations in EP25-A.

Expiration dating for the unopened reagents of the device was established at:

a. 18 months for the Amplification Reagents (PCA3 and PSA), Probe Reagents (PCA3 and PSA), PCA3/PSA Enzyme Reagent, Calibrators (PCA3 and PSA), and Controls (PCA3 and PSA), when stored at 2-8°C.

b. 18 months for the PCA3/PSA Amplification Reconstitution Solution, PCA3/PSA Probe Reconstitution Solution, PCA3/PSA Enzyme Reconstitution Solution, and PCA3/PSA Selection Reagent, when stored at 2-30°C.

c. 16 months for the Target Capture Reagents (PCA3 and PSA), when stored at 15-30°C.

d. 24 months for the APTIMA Assay Fluids (Oil Reagent, Wash Solution and Buffer for Deactivation Fluid), when stored at 15-30°C.

Expiration dating for the Opened/Reconstituted reagents of the device was established at:

a. 30 days for the Amplification Reagents (PCA3 and PSA), Probe Reagents (PCA3 and PSA), PCA3/PSA Enzyme Reagent, when stored at 2-8°C.

b. 30 days for the PCA3/PSA Selection Reagent, Target Capture Reagents (PCA3 and PSA), APTIMA Assay Fluids (Oil Reagent, Wash Solution and Buffer for Deactivation Fluid, when stored at 15-30°C.

c. Single-run use for Calibrators (PCA3 and PSA), Controls (PCA3 and PSA), PCA3/PSA Amplification Reconstitution Solution, PCA3/PSA Probe Reconstitution Solution, and PCA3/PSA Enzyme Reconstitution Solution.

3. PROGENSA PCA3 Urine Transport Kit Stability:
Three independent lots of PROGENSA PCA3 Urine Specimen Transport Tubes were incubated at 28°±2°C for the duration of the study. At each stability time point, tubes were spiked with female urine and PCA3 and PSA *in vitro* transcripts to mimic clinical specimen processing. Stability data (PCA3 RNA copies/mL and PSA RNA copies/mL) were analyzed for drift based on the recommendations in EP25-A. The results of these analyses support the labeled expiry date of 20 months, when stored at 15-30°C.

F. Animal Studies
Not Applicable.

G. Additional Studies
None.

X. SUMMARY OF PRIMARY CLINICAL STUDIES

A clinical study was performed to establish a reasonable assurance of safety and effectiveness of sparing unnecessary repeat biopsies with the PROGENSA PCA3 Assay. The assay is 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 in the US. Data from this clinical study were the basis for the PMA approval decision. A summary of the clinical study is presented below.

A. Study Design

Five hundred and seven (507) 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. 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 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).

1. Clinical Inclusion and Exclusion Criteria:
   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. Performance of the PROGENSA PCA3 Assay required information about repeat biopsies, therefore, clinical study analysis included only men who had been recommended for a repeat biopsy by their urologists.

   Subject Inclusion Criteria:
   Men 40+ years of age 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 clinician were eligible for study participation.

   - The most recent previous negative biopsy must have been performed at least 42 days before the DRE and post-DRE urine specimen collection (for PROGENSA PCA3 Assay testing).

   - The most recent previous negative biopsy must have consisted of at least 8 cores and the pathology report must have been available.

   - The subject and/or legally authorized representative must have been willing to undergo the informed consent process prior to study participation.

   Subject Exclusion Criteria:
   Patients were ineligible for study enrollment if the subject, clinician, or medical record reported:

- Use of finasteride (Proscar, Propecia), leuprolide acetate (Lupron Depot), or other medications or hormones known to affect serum PSA levels within 90 days of study enrollment.

- Use of dutasteride (Avodart) within 6 months (180 days) of study enrollment.

- Clinical symptoms of urinary tract infection (including prostatitis) at the time of enrollment.

- History of prostate cancer.

- History of invasive treatments for benign prostatic hyperplasia (BPH) or lower urinary tract symptoms (LUTS; e.g., transurethral resection of the prostate [TURP], heat, laser, or ultrasound treatments) within 180 days of study enrollment.

- Medical history or concurrent illness that the investigator considered sufficiently serious to interfere with the conduct, completion, or results of the trial, or constitute an unacceptable risk to the subject.

- Participation in pharmaceutical or treatment related clinical study within 6 months of study enrollment. Trials for non-prostate conditions may have been considered acceptable with approval by the investigator and sponsor.

2. Difference Between Subjects Recommended for Biopsy (Enrolled) and Subjects Not Recommended for Biopsy:
Information regarding prostate cancer risk factors was compared between the populations of men who completed questionnaires and not enrolled into study and subjects who enrolled into the study. A multivariable logistic regression analysis was first performed using data from men included in the Target Analysis Set (enrolled [n = 489] and not enrolled [n = 605]) to determine the association of each risk factor with recommendation for biopsy. Following this analysis, the magnitude and significance of differences in each risk factor between the populations of enrolled subjects (n = 489) and men who completed questionnaires and were not recommended for a repeat biopsy (n = 505) were determined. Standard of care factors including age, suspicious DRE, family history, race, most recent serum PSA, prostate volume, number of previous negative biopsies, time since most recent negative biopsy (continuous), and medical practice setting were evaluated. The outcome variable was recommendation for repeat biopsy. Suspicious DRE result (OR = 2.1339; 95% CI: 1.4049 – 3.2411; p = 0.0004), the most recent serum PSA test result (OR = 1.1841; 95% CI: 1.1201 – 1.2516; p < 0.0001), time since most recent negative biopsy (OR = 1.0429, 95% CI: 1.0313 – 1.0545; p < 0.0001), and medical practice setting (OR = 2.5169; 95% CI: 1.6625 – 3.8105; p < 0.0001) were significant and independent predictors for recommendation for repeat biopsy.

The difference between subjects recommended for biopsy and not recommended for biopsy determined that the performance of the PROGENSA PCA3 Assay has

not been established in men for whom a repeat biopsy was not already recommended (not enrolled subjects). This issue was addressed in device labeling that 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*", which complements the unboxed 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*". In addition, differences in patients recommended vs. not recommended for biopsy are presented in device labeling.

B. Study Population Demographics and Baseline Parameters

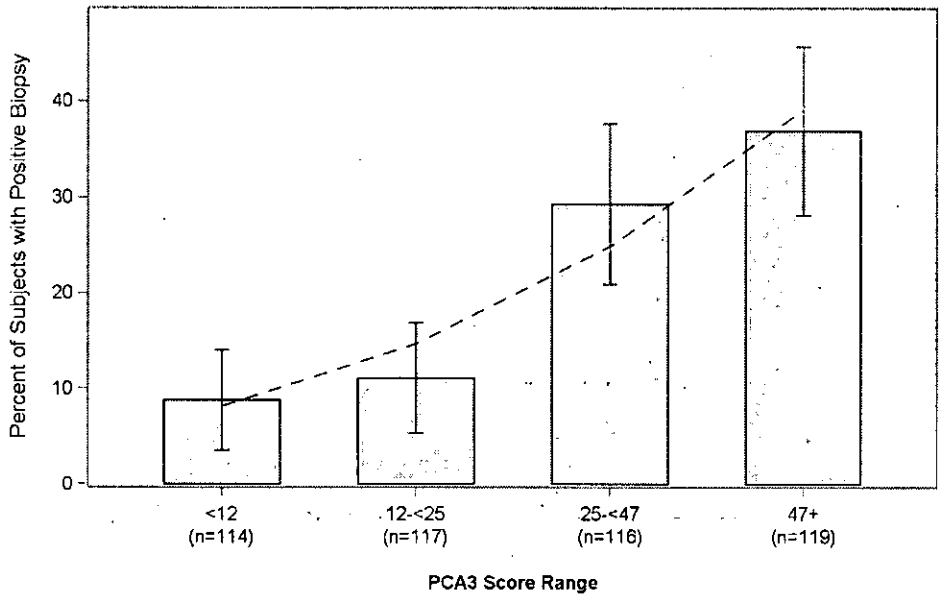
A total of 507 subjects were enrolled and informed consent was obtained by the clinical sites. Prior to database lock, and after source documentation at the clinical sites were verified, a total of 12 subjects were found to be ineligible for the study and were subsequently withdrawn. The reasons for withdrawal from study were: 7 subjects did not meet inclusion/exclusion criteria, 3 subjects were previously enrolled, 1 subject later enrolled and completed all study procedures and 1 subject had missing Informed Consent Form (ICF)/source documentation. For the remaining 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 two subjects (0.4%), and unknown for five subjects (1.0%); one subject reported both White and American Indian/Alaska Native. Four hundred and 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 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.

C. Safety and Effectiveness Results

Four hundred and 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 3 shows the percentage of subjects with positive prostatic biopsy results by PCA3 Score interval (95% CI).

Figure 3: Positive Biopsy Results by PCA3 Score With 95% Confidence Limits



Note: The dashed line represents the predicated probability of positive biopsy from a logistic regression model. Ranges represent quartiles of the PCA3 Score distribution.

Table 17 shows the performance characteristics of the PROGENSA PCA3 Assay relative to prostatic biopsy outcome at a PCA3 Score cut-off value of 25.

Table 17: Performance Characteristics of the PROGENSA PCA3 Assay:

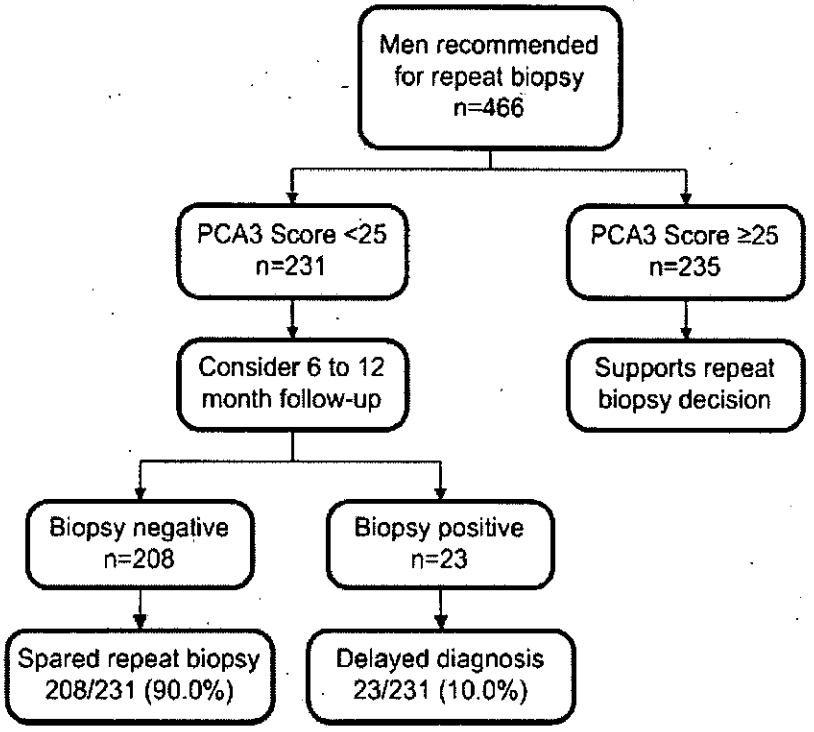
	Biopsy Result					
	Biopsy Positive	Biopsy Negative	Total	Performance Characteristic	Estimate	95% CI
PCA3 Score ≥ 25	79	156	235	Sensitivity %	77.5 (79/102)	68.4-84.5
PCA3 Score <25	23	208	231	Specificity %	57.1 (208/364)	52.0-62.1
Total	102	364	466	PPV %	33.6 (79/235)	30.0-37.2
				NPV %	90.0 (208/231)	86.5-93.1
Positive Biopsy				PLR	1.81	1.53-2.11
Prevalence %	21.9 (102/466)			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

Figure 4 (below) summarizes the potential impact of PROGENSA PCA3 Assay use. In the clinical study, 231 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 (79/235, or 34%, of these men had positive biopsies).

The potential clinical benefit is that 208 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 benefit versus risk, 9 men may have avoided an unnecessary repeat biopsy for every 1 man whose diagnosis may be delayed. In the context of the entire study population (466 total subjects), 231/466 (49.6%) of prostate biopsies would have been avoided, and 23/466 (4.9%) of men who harbored biopsy-detectable prostate cancer would have been monitored instead of receiving an immediate repeat biopsy.

Figure 4: Potential Impact of PROGENSA PCA3 Assay Use



1. The Contribution of PROGENSA PCA3 Assay Information Beyond 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 18 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. 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 18: 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 (≥ 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 (0.5923, 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

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%).

Table 19 shows the area under the curve (AUC) of the receiver operating characteristic (ROC) curves for the PROGENSA PCA3 Assay. The ROC AUC for PCA3 Score was 0.707 (95% CI: 0.649 – 0.746). The ROC AUC for total serum PSA test combined with standard of care (SOC) covariates (including age, DRE result, family history of prostate cancer, race, and number of previous negative biopsy procedures; multivariable logistic regression model "PSA + SOC") was 0.653 (95% CI: 0.593 – 0.713). When PCA3 Score of 25 as a binary test point was added to PSA + SOC, the ROC AUC was 0.740 (95%CI: 0.689 – 0.791); an increase of 0.087 (95% CI: 0.037 – 0.137).

Table 19: Receiver Operating Characteristics of the PROGENSA PCA3 Assay, Total Serum PSA Test, and Standard of Care Covariates.

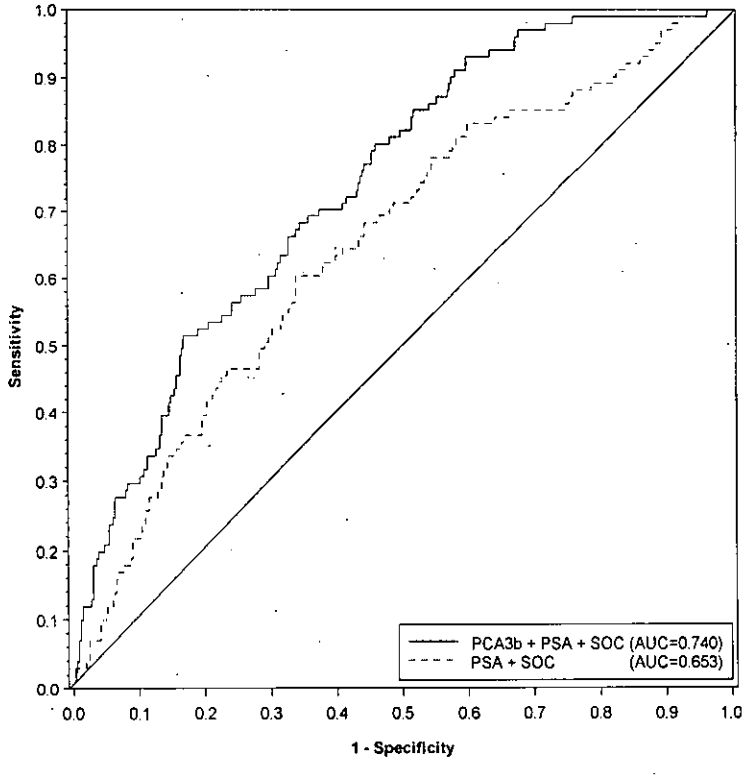
Model	ROC AUC (95% CI)	ROC AUC Comparison	ROC AUC Difference (95% CI)[1]
PCA3c	0.707 (0.649 - 0.764)	N/A	N/A
PSA + SOC	0.653 (0.593 – 0.713)	N/A	N/A
PCA3b + PSA + SOC	0.740 (0.689 - 0.791)	(PCA3b + PSA + SOC) - (PSA + SOC)	0.087 (0.037 - 0.137)
PCA3c + PSA + SOC	0.733 (0.679 - 0.786)	(PCA3c + PSA + SOC) - (PSA + SOC)	0.080 (0.033 - 0.126)
PCA3c + PSA	0.710 (0.653 - 0.766)	(PCA3c + PSA) – PSA	0.105 (0.042 - 0.168)

PCA3c=PCA3 Score (continuous), PCA3b=PCA3 Score as a binary test with cutoff of 25, PSA=total serum PSA test, SOC=standard of care covariates, AUC=Area Under the ROC curve, N/A=not applicable, CI=confidence interval.

[1] AUC for the PROGENSA PCA3 Assay minus the AUC for the comparator.

Figure 5 shows the ROC curves for total serum PSA test results and standard of care covariates with and without PROGENSA PCA3 Assay results using a PCA3 Score of 25 as a cutoff.

Figure 5: ROC Curves for Total Serum PSA Test Results and Standard of Care Covariates, With and Without PCA3 Score With Cutoff of 25.



PCA3b=PCA3 Score with a cutoff of 25, PSA=total serum PSA test, SOC=standard of care covariates.

Table 20 shows the results of adding PCA3 Score (at a cutoff of 25) to a model including current standard of care factors (serum PSA level, age, DRE result, family history of prostate cancer, race and number of previous negative biopsies). At a fixed sensitivity of 90%, the addition of PCA3 Score resulted in statistically significant increases in specificity, PPV, and NPV of 22.6%, 6.4%, and 7.1%, respectively. All lower bounds of the 90% CIs were above 0%. Most importantly, addition of PCA3 Score increased the NPV from 86.9% to 94.0%. The results of these analyses demonstrated that the addition of the PCA3 Score provided a significant and potentially clinically useful improvement in clinical accuracy when used in combination with these standard of care factors.

Table 20: Improvement in Performance Characteristics When PCA3 Score is Added to a Multivariable Logistic Regression Model.

					Improvement by Adding PCA3 Score		
Test Description	Se	Sp% (95% CI)	PPV% (95% CI)	NPV% (95% CI)	Sp (90% CI)	PPV (90% CI)	NPV (90% CI)
Current standard of care model[1]	90% Fixed	18.9 (10.3, 36.9)	23.8 (21.9, 28.7)	86.9 (79.2, 93.5)	N/A	N/A	N/A
PCA3(25) + Current standard of care model	90% Fixed	41.5 (32.5, 49.9)	30.2 (27.1, 33.5)	94.0 (92.3, 95.4)	22.6 (9.0, 33.1)	6.4 (2.8, 9.6)	7.1 (1.7, 13.4)

Se=sensitivity, Sp=specificity, PPV (NPV) = positive (negative) predictive value, N/A = not applicable

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.

[1]Includes the current standard of care factors used in the decision to perform repeat biopsy (without PCA3 Score).

2. Safety Results:
The major clinical concerns involved poor performance in patients with atypical small acinar proliferation (ASAP) as described in Subgroup analyses below and verification bias resulting in associated unknown performance (potential off-label use) in patients not recommended for repeat biopsy. These concerns were addressed through Labeling and Black box and non-Black box warnings.

3. Subgroup Analyses
The study was not designed to evaluate subgroups; therefore performance results for individual subgroups may not be conclusive. PROGENSA PCA3 Assay performance in men with ASAP on their most recent negative biopsy was assessed in the clinical study and results indicate that the PROGENSA PCA3

Assay is not informative of biopsy outcome in this subgroup, as summarized in the Table 21 below.

Table 21: Performance Characteristics of the PROGENSA PCA3 Assay in Men with ASAP on their Most Recent Negative Biopsy.

	Biopsy Result					
	Biopsy Positive	Biopsy Negative	Total	Performance Characteristic	Estimate	95% CI
PCA3 Score ≥ 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				PLR	0.94	0.54-1.40
Prevalence %	30.6% (15/49)			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

Poor performance in ASAP is addressed through the boxed warning, which states that "*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*". In addition, the poor performance in ASAP is presented in device labeling.

Table 22 shows the performance characteristics of the PROGENSA PCA3 Assay relative to prostatic biopsy outcome for other subgroups of the study population. In addition to poor performance in ASAP above, poor performance was also apparent in the subgroup with previous biopsy < 3 months, but this is attributed to the fact that 84.6% (11/13) of the men in this subgroup had ASAP on their most recent biopsy.

Table 22: Performance Characteristics of the PROGENSA PCA3 Assay by Subgroups.

Sub group	n	TP	FP	TN	FN	Se %	Sp %	PPV %	NPV %	PLR	NLR	OR
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)	0.1 (0.02-0.33)	16.5 (3.84-70.94)

Sub group	n	TP	FP	TN	FN	Se %	Sp %	PPV %	NPV %	PLR	NLR	OR
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)	1.6 (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)	5.8 (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 (75.0-97.8)	56 (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 (11.8-76.9)	25 (7.1-59.1)	25 (3.2-49.9)	40 (6.9-76.2)	0.53 (0.05-1.59)	2.4 (0.50-21.76)	0.22 (0.02-2.45)
3 mo - <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)	1.6 (0.28-4.85)	0.57 (0.02-2.01)	2.8 (0.20-40.06)
Race												
Black	39	6	16	16	1	85.7 (48.7-97.4)	50 (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 (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)	0.4 (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 (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)

Sub group	n	TP	FP	TN	FN	Se %	Sp %	PPV %	NPV %	PLR	NLR	OR
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.

XI. PANEL MEETING RECOMMENDATION AND FDA'S POST-PANEL ACTION

In accordance with the provisions of section 515(c)(2) of the act as amended by the Safe Medical Devices Act of 1990, this PMA was not referred to the Immunology panel, an FDA advisory committee, for review and recommendation.

XII. CONCLUSIONS DRAWN FROM PRECLINICAL AND CLINICAL STUDIES

A. Safety Conclusions

The adverse effects of the device are based on data collected in a clinical study conducted to support PMA approval as described above. The results of the preclinical and clinical studies demonstrate the acceptable safety of the PROGENSA PCA3 Assay when used in accordance with its instructions for use, warnings and precautions, and limitations specified in the product labeling; together with the urologist's interpretation of biopsy results, other risk factors, and professional guidelines, should be safe and pose minimal risk to the patient due to false test results.

B. Effectiveness Conclusions

The effectiveness of the PROGENSA PCA3 Assay has been demonstrated 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.

C. **Overall Conclusions**

The data in this application support the reasonable assurance of safety and effectiveness of this device when used in accordance with the indications for use. The data from the analytical studies demonstrated acceptable precision, analytical sensitivity, and analytical specificity of the PROGENSA PCA3 Assay when used according to the instructions for use, the warnings and precautions, and limitations sections of the labeling. The statistical and benefit-risk analyses of the clinical data in this application have shown that the assay is safe and effective for its approved indications when used according to the directions for use in the labeling.

XIII. CDRH DECISION

CDRH issued an approval order on 02/13/2012. The final conditions of approval are described in the approval order are described below.

All advertisements and other descriptive printed material issued by the applicant or distributor with respect to the device shall include a statement of the intended uses of the device and relevant warnings shown below.

Black Box 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.

The applicant's manufacturing facilities were inspected and found to be in compliance with the device Quality System (QS) regulation (21 CFR 820).

XIV. APPROVAL SPECIFICATIONS

Directions for use: See Device labeling.

Hazards to Health from Use of the Device: See Indications, Contraindications, Warnings, Precautions, and Adverse Events in the device label.

Post-approval Requirements and Restrictions: See approval order.

XV. REFERENCES

A. CLSI EP5-A2: Evaluation of Precision Performance of Quantitative Methods.

B. CLSI EP6-A, Evaluation of the Linearity of Quantitative Measurement Procedures: A Statistical Approach.

C. CLSI EP7-A2, Interference Testing in Clinical Chemistry.

D. CLSI EP12-A2, User Protocol for Evaluation of Qualitative Test Performance.

E. CLSI EP17-A, Protocols for Determination of Limits of Detection and Limits of Quantitation.

F. CLSI C24-A3, Statistical Quality Control for Quantitative Measurement Procedures; Principles and Definitions.

G. CLSI EP25-A, Evaluation of Stability of *In Vitro* Diagnostic Reagents.

H. Verhaegh G.W., J.B. de Kok, D. Hessels, F. Smit, and J.A. Schalken. 2003. Cancer Res. 63:4748-4749.