# Progensa PCA3 Assay

For *in vitro* diagnostic use.

# General Information

## 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.*

## Limitations

A. 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.

B. Use of this assay is limited to personnel who have been trained in the procedure. Failure to follow the instructions given in this insert may result in erroneous results. Test results may be affected by improper specimen collection, technical error, or specimen mix-up.

C. Performance of the Progensa PCA3 Assay has not been established in men who undergo repeat biopsy less than 3 months or more than 7 years after their most recent negative biopsy (refer to *Clinical Performance*, Table 9).

D. Each laboratory must independently validate an LIS transfer process.

E. Reliable results are dependent on adequate urine specimen collection. Because the transport system used for this assay does not permit microscopic assessment of urine specimen adequacy, training of clinicians in proper urine specimen collection techniques is necessary. See *Specimen Collection, Transport, and Storage* for instructions.

F. 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.

G. 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.

H. 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.

I. Information from percent free PSA tests was not used in establishing the performance characteristics of the Progensa PCA3 Assay.

## Summary and Explanation of the Test

The use of the serum prostate-specific antigen (PSA) test for prostate cancer screening has resulted in the biopsy diagnosis of smaller, previously undetected tumors, thus creating a new diagnostic dilemma: Only a fraction of men with increased serum PSA levels have detectable prostate cancer. Men with at least one negative biopsy often have persistently increased serum PSA, due primarily to enlarged prostates and benign prostatic hyperplasia (BPH). Yet, a significant proportion of men with slightly increased serum PSA (2.5-4.0 µg/L) either have, or will develop, clinically significant prostate cancer (13).

PCA3 (also known as "PCA3$^{DD3}$" or "DD3$^{PCA3}$") is a non-coding prostate-specific RNA (1) that is highly over-expressed in prostate cancer cells, with a median 66-fold up-regulation compared to adjacent benign tissue (11). In contrast, PSA gene expression is similar in cancerous and benign prostate cells; PSA RNA levels may therefore be used to normalize for the amount of prostate-specific RNA in molecular test samples. The feasibility of quantitative PCA3-based molecular testing from urine sediments (11) and from whole urine (9) has been demonstrated. The clinical feasibility of a PCA3-based test from whole urine has been demonstrated in men undergoing repeat biopsy (10, 12).

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/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.

## 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).

PCA3 and PSA RNAs are quantified in separate tubes and the PCA3 Score is determined. Calibrators containing known amounts of PCA3 or PSA RNA transcripts 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.

# Reagents and Materials Provided

***Note:*** *For information on any hazard and precautionary statements that may be associated with reagents, refer to the Safety Data Sheet Library at www.hologic.com/sds.*

Reagents and materials provided in the Progensa PCA3 Assay Kit for the Progensa PCA3 Assay are listed below. Reagent Identification Symbols are also listed next to the reagent name.

**Progensa PCA3 Assay Kit, 2 x 100 Reactions (302355)**

**Progensa PCA3 100-Reaction Kit**

Progensa PCA3 Refrigerated Box — Store at 2°C to 8°C upon receipt until the labeled expiration date

| Symbol | Component | Quantity |
|---|---|---|
| **A** | **PCA3 Amplification Reagent**<br>*Non-infectious nucleic acids dried in HEPES buffered solution containing <10% bulking agent.* | 1 vial |
| **E** | **PCA3/PSA Enzyme Reagent**<br>*Reverse transcriptase and RNA polymerase dried in HEPES buffered solution containing <10% bulking agent.* | 1 vial |
| **P** | **PCA3 Probe Reagent**<br>*Non-infectious chemiluminescent DNA probes dried in succinate buffered solution containing <5% bulking agent and <5% lithium lauryl sulfate.* | 1 vial |

Progensa PCA3 Room Temperature Box — Store at 15°C to 30°C upon receipt until the labeled expiration date

| Symbol | Component | Quantity |
|---|---|---|
| **AR** | **PCA3 Amplification Reconstitution Solution**<br>*Aqueous solution containing preservatives (<1% parabens).* | 1 x 9.3 mL |
| **ER** | **PCA3/PSA Enzyme Reconstitution Solution**<br>*HEPES buffered solution containing a surfactant (10% Triton X-100) and 20% glycerol.* | 1 x 3.3 mL |
| **PR** | **PCA3/PSA Probe Reconstitution Solution**<br>*Succinate buffered solution containing <5% lithium lauryl sulfate.* | 1 x 12.4 mL |
| **S** | **PCA3/PSA Selection Reagent**<br>*Borate buffered solution containing surfactant (1% Triton X-100).* | 1 x 31mL |
| **TCR** | **PCA3 Target Capture Reagent**<br>*Non-infectious nucleic acid in HEPES buffered solution containing solid phase.* | 1 x 22 mL |
| | **Sealing Cards** | 1 package |
| | **Reconstitution Collars** | 1 package |

Progensa PCA3 Calibrator and Controls Kit — Store at 2°C to 8°C upon receipt until the labeled expiration date

| Symbol | Component | Quantity |
|---|---|---|
| CAL | **PCA3 Calibrator 1**<br>*Phosphate buffered solution containing <5% lithium lauryl sulfate.* | 1 x 2.0 mL |
| CAL | **PCA3 Calibrators 2-5**<br>*Non-infectious PCA3 nucleic acid in phosphate buffered solution containing <5% lithium lauryl sulfate.* | 4 x 1.7 mL |
| PC | **PCA3 Positive Controls**<br>*Non-infectious PCA3 nucleic acid in phosphate buffered solution containing <5% lithium lauryl sulfate.* | 2 x 1.7 mL |
| | **PCA3 Concentration Information Sheet** | 1 sheet |

### Progensa PSA 100-Reaction Kit

Progensa PSA Refrigerated Box — Store at 2°C to 8°C upon receipt until the labeled expiration date

| Symbol | Component | Quantity |
|---|---|---|
| A | **PSA Amplification Reagent**<br>*Non-infectious nucleic acids dried in HEPES buffered solution containing <10% bulking agent.* | 1 vial |
| E | **PCA3/PSA Enzyme Reagent**<br>*Reverse transcriptase and RNA polymerase dried in HEPES buffered solution containing <10% bulking agent.* | 1 vial |
| P | **PSA Probe Reagent**<br>*Non-infectious chemiluminescent DNA probes dried in succinate buffered solution containing <5% bulking agent and <5% lithium lauryl sulfate.* | 1 vial |

Progensa PSA Room Temperature Box — Store at 15°C to 30°C upon receipt until the labeled expiration date

| Symbol | Component | Quantity |
|---|---|---|
| AR | **PSA Amplification Reconstitution Solution**<br>*Aqueous solution containing preservatives (<1% parabens).* | 1 x 9.3 mL |
| ER | **PCA3/PSA Enzyme Reconstitution Solution**<br>*HEPES buffered solution containing a surfactant (10% Triton X-100) and 20% glycerol.* | 1 x 3.3 mL |
| PR | **PCA3/PSA Probe Reconstitution Solution**<br>*Succinate buffered solution containing <5% lithium lauryl sulfate.* | 1 x 12.4 mL |
| S | **PCA3/PSA Selection Reagent**<br>*Borate buffered solution containing surfactant (1% Triton X-100).* | 1 x 31 mL |
| TCR | **PSA Target Capture Reagent**<br>*Non-infectious nucleic acid in HEPES buffered solution containing solid phase.* | 1 x 22 mL |
| | **Sealing Cards** | 1 package |
| | **Reconstitution Collars** | 1 package |

Progensa PSA Calibrator and Controls Kit — Store at 2°C to 8°C upon receipt until the labeled expiration date

| Symbol | Component | Quantity |
|---|---|---|
| **CAL** | **PSA Calibrator 1**<br>*Phosphate buffered solution containing <5% lithium lauryl sulfate.* | 1 x 2.0 mL |
| **CAL** | **PSA Calibrators 2-5**<br>*Non-infectious PSA nucleic acid in phosphate buffered solution containing <5% lithium lauryl sulfate.* | 4 x 1.7 mL |
| **PC** | **PSA Positive Controls**<br>*Non-infectious PSA nucleic acid in phosphate buffered solution containing <5% lithium lauryl sulfate.* | 2 x 1.7 mL |
| | **PSA Concentration Information Sheet** | 1 sheet |

**Aptima® Assay Fluids — Store at 15°C to 30°C (2 boxes) upon receipt** until the labeled expiration date

| Symbol | Component | Quantity |
|---|---|---|
| **W** | **Wash Solution**<br>*HEPES buffered solution containing <2% sodium dodecyl sulfate.* | 1 x 402 mL |
| **DF** | **Buffer for Deactivation Fluid**<br>*Bicarbonate buffered solution.* | 1 x 402 mL |
| **O** | **Oil Reagent**<br>*Silicone oil.* | 1 x 24.6 mL |

# Materials

***Note:*** *For information on any hazard and precautionary statements that may be associated with reagents, refer to the Safety Data Sheet Library at www.hologic.com/sds.*

***Note:*** *Materials available from Hologic have catalog numbers listed.*

## Materials Required But Available Separately

| | Cat. No. |
|---|---|
| Progensa PCA3 Urine Specimen Transport Kit | 302352 |
| Hologic Leader® HC+ Luminometer | 104747 |
| Hologic Target Capture System (TCS) | 104555 |
| Aptima Auto Detect Kit | 301048 |
| 2 eppendorf Repeater Plus Pipettors | 105725 |
| Repeat pipettor tips (2.5 mL, 5.0 mL, 25.0 mL) | — |
| 2 to 4 SB100® Dry Heat Bath/Vortexers | 105524 |
| Micropipettor, 1000 µL RAININ PR1000 | 901715 |
| Tips, 1000 µL P1000 | 105049 |
| Bleach (minimum 5.25% or 0.7 M sodium hypochlorite solution) | — |
| Large-capped plastic container | — |
| Standard urine collection containers, without preservatives | — |
| Ten Tube Units (TTU) | TU0022 |
| Ten Tip Cassettes (TTC) | 104578 |
| SysCheck calibration standard | 301078 |

## Optional Materials

| | | Cat. No. |
|---|---|---|
| Progensa PCA3 100-Reaction Kit | | 302354 |
| Progensa PSA 100-Reaction Kit | | 302357 |
| Progensa PCA3 Calibrators and Controls Kit | | 302353 |
| Progensa PSA Calibrators and Controls Kit | | 302356 |
| Progensa PCA3/PSA Proficiency Panels | | 302350 |
| Progensa PCA3 Specimen Diluent Kit | | 302351 |
| Aptima Assay Fluids Kit | | 302002C |
| TECAN Freedom EVO 100/4 instrument | | 900932 |
| *PCA3 Deck Plate assembly, DTS® 800* | *902021* | |
| *Reagent reservoir (40 mL quarter module)* | *104765* | |
| *Split reagent reservoir (19 mL x 2 quarter module)* | *901172* | |
| *Disposable pipet tips with filter (1 mL)* | *10612513 (Tecan)* | |

| | Cat. No. |
|---|---|
| Transport tubes | 302521 |
| Pipettor, eppendorf 20 to 200 µL | 105726 |
| Tips, Pipette 20 to 200 µL | — |
| Replacement penetrable caps | 302520 |
| Replacement non-penetrable caps | 103036A |

# Warnings and Precautions

A. For *in vitro* diagnostic use.

**Laboratory Related**

B. Use only supplied or specified disposable laboratory ware.

C. Use routine laboratory precautions. Do not eat, drink, or smoke in designated work areas. Wear disposable, powderless gloves, protective eye wear, and laboratory coats when handling urine specimens and kit reagents. Wash hands thoroughly after handling urine specimens and kit reagents.

D. **Warning: Irritants, Corrosives.** Avoid contact of Auto Detect 1 and Auto Detect 2 with skin, eyes and mucous membranes. If these fluids come into contact with skin or eyes, wash with water. If these fluids spill, dilute the spill with water before wiping dry.

E. Work surfaces, pipettors, and other equipment must be regularly decontaminated with 2.5% to 3.5% (0.35 M to 0.5 M) sodium hypochlorite solution (see *Procedural Notes*).

F. A separate area for post-amplification is strongly recommended to minimize amplicon contamination in the assay. This dedicated area should be away from the pre-amplification area, where reagent preparation, target capture, and amplification take place.

G. To help prevent lab areas from becoming contaminated with amplicon, the laboratory area should be arranged with a unidirectional workflow from reagent preparation through post-amplification. Specimens, equipment, and reagents should not be returned to the area where a previous step was performed. Personnel should not move back into previous work areas without proper contamination safeguards. Only one run per shift should be performed.

**Specimen Related**

H. After urine has been added in the urine specimen transport tube, the liquid level must initially fall between the two black indicator lines on the tube label (at least 2.5 mL of urine is required). Otherwise, the specimen must be rejected.

I. Maintain proper storage conditions during specimen shipping to ensure the integrity of the specimen. Specimen stability under shipping conditions other than those recommended has not been evaluated.

J. Expiration dates listed on the collection kits pertain to the collection site and not the testing facility. Samples collected any time prior to the expiration date of the collection kit, and transported and stored in accordance with the package insert, are valid for testing even if the expiration date of the collection tube has passed.

K. Store all specimens at specified temperatures. The performance of the assay may be affected by use of improperly stored specimens. See *Specimen Collection, Transport, and Storage* for specific instructions.

L. Urine specimens may be infectious. Use Universal Precautions when performing this assay. Proper handling and disposal methods should be established by the laboratory director. Only personnel adequately qualified as proficient in the use of the Progensa PCA3 Assay and adequately trained in handling infectious materials should perform this procedure.

M. Avoid cross-contamination during the specimen handling steps. Urine specimens can contain high levels of RNA target. Ensure specimen containers do not contact one another, and discard used materials without passing them over any containers. If gloves come in contact with a specimen, change gloves to avoid cross-contamination.

**Assay Related**

N. Do not use this kit after its expiration date.

O. **For the Progensa PCA3 Assay kit, do not interchange, mix, or combine assay reagents with different lot numbers** (i.e., for each analyte, the assay reagents in the "refrigerated" box and "room temperature" box must come from the same lot).

P. Store all assay reagents at specified temperatures. The performance of the assay may be affected by use of improperly stored assay reagents. See *Storage and Handling Requirements* for specific instructions.

Q. For assay deactivation (see *DTS Systems Test Procedure*), the sodium hypochlorite concentration must be at least 2.6% (0.35 M) **after** 1:1 dilution with Buffer for Deactivation Fluid. Therefore, the starting concentration must be a minimum 5.25% (0.7 M) sodium hypochlorite to achieve the final concentration required for deactivation.

R. Tips with hydrophobic plugs must be used. A minimum of two repeat pipettors must be dedicated for use with this assay: one for use in the pre-amplification steps, and one for use in the post-amplification steps. One micropipettor must be dedicated for use in specimen transfer unless a TECAN Freedom EVO 100/4 instrument is used. All pipettors must be cleaned regularly as described in *Procedural Notes*.

S. When using repeat pipettors for reagent addition, do not touch the reaction tube with the pipettor tip to prevent carryover from one tube to another.

T. Separate SB100® instruments must be dedicated to the pre-amplification area for target capture and TMA and the post-amplification area for HPA.

U. Safety Data Sheets can be viewed online at www.hologic.com/sds and are available upon request.

# Storage and Handling Requirements

A. Consult Table 1 for reagent storage information.

*Table 1: Reagent Storage*

| Reagent/Fluid | Unopened Storage | Opened/Reconstituted Stability (up to expiration date) |
|---|---|---|
| **Amplification Reagents** | 2°C to 8°C until the expiration date | 30 days at 2°C to 8°C* |
| **Probe Reagents** | 2°C to 8°C until the expiration date | 30 days at 2°C to 8°C* |
| **Enzyme Reagent** | 2°C to 8°C until the expiration date | 30 days at 2°C to 8°C* |
| **Target Capture Reagents** | 15°C to 30°C until the expiration date | 30 days at 15°C to 30°C |
| **Amplification Reconstitution Solution** | 2°C to 30°C until the expiration date | N/A (single-use) |
| **Probe Reconstitution Solution** | 2°C to 30°C until the expiration date | N/A (single-use) |
| **Enzyme Reconstitution Solution** | 2°C to 30°C until the expiration date | N/A (single-use) |
| **Selection Reagent** | 2°C to 30°C until the expiration date | 30 days at 15°C to 30°C |
| **Calibrators** | 2°C to 8°C until the expiration date | N/A (single-run) |
| **Controls** | 2°C to 8°C until the expiration date | N/A (single-run) |
| **Oil Reagent** | 15°C to 30°C until the expiration date | 30 days at 15°C to 30°C |
| **Wash Solution** | 15°C to 30°C until the expiration date | 30 days at 15°C to 30°C |
| **Buffer for Deactivation Fluid** | 15°C to 30°C until the expiration date | 28 days at 15°C to 30°C |

*May use again for other assay runs up to four times, provided that the total amount of time at room temperature is no greater than 24 hours.

B. **Do not store the Target Capture Reagent at temperatures below 15°C.**

C. The Probe Reagent and Reconstituted Probe Reagent are photosensitive. Protect these reagents from extended exposure to light during storage and preparation for use.

D. **Do not freeze the reagents.**

E. Do not use reagents or fluids after the expiration date.

F. Progensa PCA3 and PSA Calibrators and Controls are single-run vials and must be discarded after use.

G. Changes in the physical appearance of the reagent supplied may indicate instability or deterioration of these materials. If changes in the physical appearance of the reagents are observed once resuspended (e.g., obvious changes in reagent color or cloudiness indicative of microbial contamination), contact Hologic Technical Support before use.

H. Discard reconstituted reagent after 30 days or by the expiration date, whichever comes first.

I. Leftover opened or reconstituted reagents may be used in subsequent assays if they have been stored properly after the initial use. The leftover reagent may be pooled with freshly prepared or other leftover reagent of the same lot. **Do not interchange, mix, or combine assay reagents with different lot numbers** (see *Warnings and Precautions*). No components of the pooled reagent may exceed the opened or reconstituted reagent storage limits. Ensure that the pooled reagent has been thoroughly mixed and that sufficient volume has been prepared to provide enough reagent for an entire assay run.

# Specimen Collection, Transport, and Storage

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. Urine is processed using the Progensa PCA3 Urine Specimen Transport Kit. Stability of PCA3 and PSA RNA in urine and processed urine was established by monitoring RNA copy levels in urine specimens collected per the instructions below.

A. Instructions for urine specimen collection and processing (for additional details, see "Progensa Physician Instructions" and "Progensa PCA3 Urine Specimen Transport Kit" instructions label):

1. Conduct a DRE as described below immediately prior to urine collection:

   Apply enough pressure to slightly depress the prostate surface, from the base to the apex and from the lateral to the median line for each lobe as shown in Figure 1. Perform exactly three strokes for each lobe. This is **NOT** intended to be a prostatic massage.

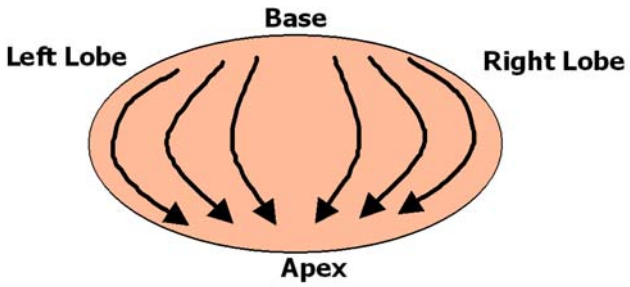



***Figure 1. Proper Direction of Applied Prostate Pressure***

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. Otherwise, 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 4 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 5 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 5 times to mix. This is now known as the processed urine specimen.

B. Specimen transport and storage before testing (for details, see “Progensa Physician Instructions” and “Progensa PCA3 Urine Specimen Transport Kit” instructions label):

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 5 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 5 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 2 for the allowable storage times at different temperatures.

*Table 2: Processed Urine Specimen Storage Durations*

| Storage Temperature | Time |
|---|---|
| Processed specimen storage and shipment: | 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 5 freeze–thaw cycles.

C. Specimen storage after testing

1. Specimens that have been assayed must be stored upright in a rack.
2. The urine specimen transport tubes, if not recapped with an intact cap, should be covered with a new, clean plastic or foil barrier.
3. If assayed specimens need to be frozen or shipped, remove the penetrable cap and place new, non-penetrable caps on the urine specimen transport tubes. If specimens need to be shipped for testing at another facility, recommended temperatures must be maintained. **Avoid splashing and cross-contamination.**

***Note:*** *Specimens must be shipped in accordance with applicable national and international transportation regulations.*

# DTS Systems Test Procedure

A. Work Area Preparation

1. Prior to starting the assay, wipe down work surfaces and pipettors with 2.5% to 3.5% (0.35 M to 0.5 M) sodium hypochlorite solution. Allow sodium hypochlorite solution to contact surfaces and pipettors for at least 1 minute and then follow with a water rinse. Do not allow the sodium hypochlorite solution to dry. Cover the bench surface on which the reaction will be performed with clean, plastic-backed absorbent laboratory bench covers.
2. Place a sufficient number of Ten Tip Cassettes into the Target Capture System (TCS). Ensure that the TCS wash bottle is filled with Wash Solution and the aspirator is connected to the vacuum pump. (Refer to the *Target Capture System Operator's Manual*.)

B. Reagent Reconstitution and Preparation

Reagent reconstitution should be performed prior to beginning specimen transfer.

1. To reconstitute Amplification, Enzyme, and Probe Reagents, combine the bottles of lyophilized reagent with the reconstitution solution. If refrigerated, allow the Reconstitution Solutions to reach room temperature before use.

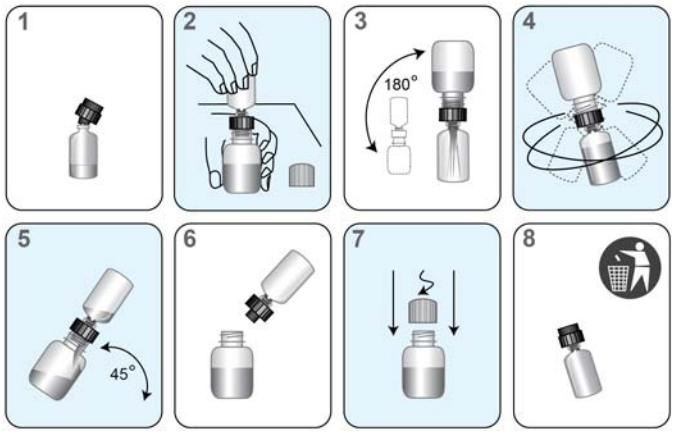



***Figure 2. Reconstitution Process***

a. Pair the appropriate reconstitution solution with its dried reagent. Verify that the vials have matching label colors to ensure they are paired properly.
b. Open the dried reagent vial and firmly insert the notched end of the reconstitution collar into the vial opening (Figure 2, Step 1).
c. Open the matching reconstitution solution, and set the cap on a clean, covered work surface. While holding the solution bottle on the bench, firmly insert the other end of the reconstitution collar into the bottle opening (Figure 2, Step 2).
d. Slowly invert the assembled bottles. Allow the solution to drain from the bottle into the glass vial (Figure 2, Step 3). Wait for the lyophilized reagent to go into solution, then gently swirl the solution in the glass vial to mix. Avoid creating foam while swirling the bottle (Figure 2, Step 4).
e. Invert the assembly and tilt at a 45° angle to minimize foaming (Figure 2, Step 5). Allow all of the liquid to drain back into the plastic bottle.
f. Remove the reconstitution collar and glass vial (Figure 2, Step 6).

g. Re-cap the plastic bottle (Figure 2, Step 7). Record operator initials and reconstitution date on all reconstituted reagent vials. Be sure to record the analyte (PCA3 or PSA) on the Probe Reagent vials.

h. Discard both the reconstitution collar and vial (Figure 2, Step 8).

2. Previously reconstituted Amplification, Enzyme, and Probe Reagents must reach room temperature (15°C to 30°C) prior to the start of the assay. Refer to *Storage and Handling Requirements* if pooling leftover reagents. If reconstituted Amplification Reagent contains precipitate that does not return to solution at room temperature, heat at 62°C ± 1°C for 1 to 2 minutes in the pre-amplification area. If reconstituted Probe Reagent contains precipitate that does not return to solution at room temperature, heat at 62°C ± 1°C for 1 to 2 minutes in the post-amplification area. After these heat steps, the reconstituted reagents may be used even if residual precipitate remains. After resuspension, mix the vials by gentle inversion.

C. Rack Setup

The repeat pipettor used in target capture, specimen transfer and amplification should be dedicated for use in these steps only (see *Warnings and Precautions*).

1. Set up one rack for the PCA3 analyte and another rack for the PSA analyte.

   ***Note:*** *If the number of specimens is low enough, both analytes may be tested in a single rack. If using the TECAN Freedom EVO 100/4 instrument, separate racks must be maintained for each analyte. No more than two full racks (20 TTUs) may be tested at a time.*

2. In the Ten Tube Unit (TTU) rack(s), place enough TTUs to accommodate the calibrators, controls, and specimens for each analyte.

3. Label the TTUs with the sample/specimen IDs. Table 3 describes the addition of the calibrators, controls, and specimens. Start PSA calibrators on a new TTU.

   ***Note:*** *Calibrators are to be run in three replicates and controls in two replicates each, and must be run on the same rack as specimens. Specimens must be run in duplicate. Do not leave empty reaction tubes between calibrators, controls, and specimens. If using the TECAN Freedom EVO 100/4 instrument, refer to the TECAN Freedom EVO 100/4 Application Sheet for the Progensa PCA3 Assay (TECAN Freedom EVO Application Sheet) for further instructions.*

*Table 3: Example Rack Setup*

| Rack Position | Sample Description | *Target PCA3 Concentration (copies/mL) | *Target PSA Concentration (copies/mL) |
|---|---|---|---|
| 1 to 3 | Calibrator 1 | 0 | 0 |
| 4 to 6 | Calibrator 2 | 250 | 7,500 |
| 7 to 9 | Calibrator 3 | 2,500 | 75,000 |
| 10 to 12 | Calibrator 4 | 25,000 | 750,000 |
| 13 to 15 | Calibrator 5 | 125,000 | 3,000,000 |
| 16 to 17 | Control A | 1,250 | 37,500 |
| 18 to 19 | Control B | 62,500 | 1,500,000 |
| 20 to n | Specimen | unknown | unknown |

*PCA3 and PSA 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, and will vary from lot to lot. The concentration information is provided on a card in the package of calibrator and control vials and is used for calibration and determination of run validity.

D. Concentration Information Verification

Verify with the Progensa PCA3 Assay Software system administrator that the concentration information for the lots of Progensa PCA3 and PSA Calibrators and Controls Kits tested has been entered. For more information, see the *Progensa PCA3 Assay Software System Administrator's Manual*.

***Note:*** *Entry of concentration information is required* ***before the first use*** *of each new calibrators and controls kit lot. Subsequent runs using calibrators and controls from the same kit lot do not require further action.*

E. Worklist Editor Setup

Generate an assay run worklist using the Hologic Worklist Editor on a computer located in the pre-amplification area. For use of the Worklist Editor, refer to the *Hologic Worklist Editor Operator's Manual*. If using the TECAN Freedom EVO 100/4 instrument, see also the *TECAN Freedom EVO Application Sheet* for further instructions.

F. Sample Preparation

1. Allow the calibrators and controls to reach room temperature prior to testing. Mix the vials by gentle inversion.
2. Allow specimens to reach room temperature prior to testing. **Do not vortex specimens.** The specimens should be mixed by occasional, gentle inversion during the warming period. See *Procedural Notes* for information about precipitate that will not go into solution and handling frozen specimens.

G. Pre-Amplification

The pre-amplification environment must be 15°C to 30°C. Run both racks in parallel. Refer to the *SB100 Dry Heat Bath/Vortexer Application Shee*t for the Progensa PCA3 Assay (*SB100 Application Shee*t) if necessary. If using the TECAN Freedom EVO 100/4 instrument, refer to the *TECAN Freedom EVO Application Sheet* for further instructions.

1. Prepare the pre-amp SB100 instrument for use.
   a. Press the "|" symbol on the power switch on the back of the pre-amp SB100 instrument(s). After successful initialization, the first screen displays the serial number and SB100 software/firmware version number.
   b. The **Main Menu** displays after 5 seconds:
   c. Press the ◄ key to select the **Select Run Mode** menu.
   d. Press the ▲ key to select the **Run Protocol** menu.
   e. Select the **PCA3 PREAMP V1.1** protocol on the pre-amp SB100 instrument by pressing the ► key and scrolling up or down through the name list using the ▲ or ▼ keys.
   f. Press the ↵ key to start the protocol. The SB100 instrument automatically preheats the sample block to 62°C.
2. Thoroughly mix by swirling or inverting the Target Capture Reagent (TCR). Using the repeat pipettor, add 100 µL of the analyte-specific TCR to the appropriate reaction tube.
3. Uncap the Calibrator vial or pierce the cap with the micropipettor and add 400 µL of the Calibrator to the properly labeled reaction tube. Use the same pipette tip to withdraw replicate additions from the vial. Use new pipette tips for each Calibrator vial. Repeat for the addition of Controls and specimens. Cover and save any leftover specimen and store at or below 8°C (see *Specimen Collection, Transport, and Storage* for more information) in case retesting is necessary.
4. Cover the TTUs with the sealing card(s). Cover the sealing card(s) with the SB100 frame.
5. When the sample block has reached 62°C, the SB100 instrument beeps. Load the rack in the SB100 sample block as indicated in the message on the screen. When finished, press the ► key to continue.

   Holding the frame and rack together, ease the rack into the sample block. Take care not to splash contents onto the sealing card(s). Rotate the black knobs until the bearings lock into the holes on the frame.

   ***Note:*** *When loading and unloading the rack in the sample block, hold the frame and rack assembly together to ensure TTUs are locked in position in the rack.*

   ***Note:*** *Ease the rack in or out of the sample block to avoid splashing contents on the sealing card(s). DO NOT JERK THE RACK.*
6. Press the ► key to continue. The SB100 instrument will:
   a. Vortex the rack for 10 seconds.
   b. Incubate the rack at 62°C for 35 minutes.
   c. Vortex the rack for 60 seconds.
   d. In the next 30 minutes, ramp down from 62°C to 23°C and incubate at 23°C until the end of the incubation.
7. When the SB100 instrument has completed the last incubation, the message on the screen indicates that target capture steps such as magnetic separation, aspiration, and wash will be performed next.
8. Gently remove the rack from the sample block. Take care not to splash contents onto the sealing card(s).
9. Place the rack with the Front tab forward on the TCS magnetic base for 5 to 10 minutes. Load the TTC rack with TTCs.

10. Prime the dispense station pump lines by pumping Wash Solution through the dispense manifold. Pump enough liquid through the system so that there are no air bubbles in the line and all ten nozzles are delivering a steady stream of liquid.
11. Turn on the vacuum pump and disconnect the aspiration manifold at the first connector between the aspiration manifold and the trap bottle. Ensure that the vacuum gauge meets the leak test specification. It may take 15 seconds to achieve this reading. Reconnect the manifold, and ensure that the vacuum gauge meets the vacuum level specification. Leave the vacuum pump on until all target capture steps are completed and the aspiration manifold tubing is dry.

    See the Target Capture System Vacuum Specifications Sheet located at the back of the *Target Capture System Operator's Manual* or contact Hologic Technical Support for further information.
12. Firmly attach the aspiration manifold to the first set of tips. Lower the tips into the first TTU until the tips contact the top of the liquid. Maintain tip contact with the top of the liquid as the tips move downward until the tips come into brief contact with the bottoms of the tubes. Gently tap the tips against the bottoms of the tubes until all remaining liquid is removed. Do not hold the tips in prolonged contact with the bottoms of the tubes or tap the tips rapidly because excess foam may be created in the vacuum trap.
13. After the aspiration is complete, eject the tips into their original tip cassette. Repeat the aspiration steps for the remaining TTUs, using a dedicated tip for each reaction tube.
14. Place the dispense manifold over each TTU and, using the dispense station pump, deliver 1.0 mL of Wash Solution into each tube of the TTU.
15. Cover tubes with the sealing card(s) and remove the rack from the TCS.
16. Press the ▶ key to continue. Attach the frame, load the rack in the sample block, and lock the knobs into place over the frame.
17. Press the ▶ key to continue. The SB100 instrument will vortex the rack for 10 seconds.
18. After vortexing, the message on the screen indicates that target capture steps will be performed next.

    Remove the rack, and then press the ▶ key. The SB100 instrument will preheat the sample block to 62°C and will beep when 62°C is reached.
19. Place rack on the TCS magnetic base for 5 to 10 minutes.
20. The next SB100 message indicates the next steps are to aspirate Wash Solution, and then to add Amplification Reagent and Oil Reagent.
21. Aspirate all liquid as in Step 12 and Step 13.
22. After the final aspiration, remove the rack from the TCS base and visually inspect the tubes to ensure that all liquid has been aspirated, and all tubes contain magnetic particle pellets. If any liquid is visible, place the rack back onto the TCS base for 2 minutes, and repeat the aspiration for that TTU using the same tips used previously for each reaction tube. If ANY magnetic particle pellet is visible after aspiration is completed, the tube may be accepted. If no pellet is visible, the specimen should be retested. If the same specimen does not contain a magnetic particle pellet at this step in a subsequent run, this may indicate a specimen-specific problem. Re-collection of the urine specimen is recommended in this situation.

H. Amplification

Perform Step 5 on one rack before repeating on the second rack. If necessary, refer to the *SB100 Application Sheet*. If using the TECAN Freedom EVO 100/4 instrument, refer to the *TECAN Freedom EVO Application Sheet* for further instructions.

1. Using the repeat pipettor, add 75 µL of the reconstituted analyte-specific Amplification Reagent to each reaction tube. All reaction mixtures in the rack should be red in color.
2. Using the repeat pipettor, add 200 µL of Oil Reagent. Cover the tubes with the sealing card(s).
3. Press the ▶ key to continue. Load the rack in the SB100 sample block as indicated on the screen.

   Attach the frame, load the rack in the sample block, and then lock the knobs over the frame.
4. Press the ▶ key to continue. The SB100 instrument will:
   a. Vortex the rack for 10 seconds.
   b. Incubate the rack at 62°C for 10 minutes.
   c. Ramp down from 62°C to 42°C and incubate at 42°C until the end of the 5 minute incubation.
5. After the sample block has reached 42°C, the SB100 instrument will beep. The message on the screen indicates that Enzyme Reagent should be added.

   Remove the frame and discard the sealing card(s). Using the repeat pipettor, add 25 µL reconstituted Enzyme Reagent to each tube while the rack is in the sample block at 42°C. Immediately cover the tubes with fresh sealing card(s), attach the frame, and lock the knobs into place. Press the ▶ key.

   ***Note: The addition of enzyme must be completed within 90 seconds or less.*** *Complete this step on one rack before repeating on the second rack.*

   The SB100 instrument will:

   a. Gently vortex the rack for 5 seconds.
   b. Incubate the rack at 42°C for 60 minutes.
6. When the Enzyme incubation is complete, the message on the screen indicates that the rack should be removed for HPA.
7. Remove the rack and remove the frame, but leave the sealing card(s) on the tubes. The message on the screen indicates that the Pre-Amp protocol is complete.

   ***Note:*** *The rack must be removed within 5 minutes of completion of amplification. Otherwise, a Suspend Time error will occur. The error message indicates how much time has elapsed since it has been in error mode. Either continue or abort the protocol.*
8. Press any key to return to the **Main Menu**.
9. Turn off the pre-amp SB100 instrument(s) if there are no further tests to run. Proceed to the post-amplification area with the racks, covered with sealing card(s).

I. Post-Amplification

The repeat pipettor used in hybridization and selection should be dedicated for these steps only (see *Warnings and Precautions*). The post-amplification environment, including detection, must be 15°C to 30°C. If necessary, refer to the *SB100 Application Sheet*.

1. Prepare the post-amp SB100 instrument for use.
   a. Power up the post-amp SB100 instrument(s). The **Main Menu** displays after 5 seconds.
   b. Press the ◄ key to select the **Select Run Mode** menu.
   c. Press the ▲ key to select the **Run Protocol** menu.
   d. Select the **PCA3 PSTAMP V1.1** protocol on the post-amp SB100 instrument by pressing the ► key and scrolling up or down through the name list using the ▲ or ▼ keys.
   e. Press the ↵ key to start the protocol. The SB100 instrument will preheat the sample block to 62°C.
2. Hybridization
   a. When the sample block has reached 62°C, the SB100 instrument will beep. The message on the screen indicates to add Probe and load the rack in the SB100 sample block. Remove the sealing card(s), and use the repeat pipettor to add 100 µL reconstituted analyte-specific Probe Reagent to each tube. All reaction mixtures should be yellow in color.
   b. Cover the tubes with the sealing card(s) and frame. Place the rack in the sample block and lock the knobs over the frame.
   c. Press the ► key to continue. The SB100 instrument will:
      i. Vortex the rack for 10 seconds.
      ii. Incubate the rack at 62°C for 20 minutes.
   d. When the incubation is over, the message on the screen indicates that the rack should be removed to cool.
      i. Remove the rack from the sample block and incubate at room temperature for 5 minutes.
      ii. Press the ► key to start the timer.

      ***Note:*** *The rack must be removed and the ► key pressed within 5 minutes of completion of the Probe incubation. Otherwise a Suspend Time error will occur. The error message indicates how much time has elapsed since it has been in error mode. Either continue or abort the protocol.*
   e. After 5 minutes the SB100 instrument will beep. The message on the screen indicates that Selection Reagent will be added next.
3. Selection
   a. Remove the sealing card(s) and using the repeat pipettor, add 250 µL Selection Reagent to each tube. All reaction mixtures should be pink in color.
   b. Cover the tubes with the sealing card(s), attach the frame, load the rack in the sample block, and lock the knobs over the frame.
   c. Press the ► key to continue. The SB100 instrument will:
      i. Vortex the rack for 10 seconds.
      ii. Incubate the rack at 62°C for 10 minutes.
      iii. Cool the rack to 23°C.
   d. When the Selection incubation is complete, the message on the screen indicates that the rack should be removed.
   e. Remove the rack and remove the frame, but leave the sealing card(s) on the tubes. The message on the screen indicates that the Post-Amp protocol is complete.
   f. Press any key to return to the **Main Menu**.

g. Turn off the post-amp SB100 instrument(s) if there are no further tests to be run.
h. Proceed with the steps under *Detection*.

J. Detection

For use of the Leader HC+ Luminometer, refer to the *Leader HC+ Luminometer Operator's Manual*. For use of the Progensa PCA3 Assay Software, refer to the *Progensa PCA3 Assay Software System Administrator's Manual* and *Operator's Manual*.

1. Ensure there are sufficient volumes of Auto Detect 1 and 2 to complete the reactions.
2. Prepare the Leader HC+ Luminometer by placing one empty TTU in cassette position number 1 and perform the WASH protocol once.
3. Load the TTUs into the luminometer using the diagram in the luminometer as a guide. If testing both analytes (back-to-back run), load all PCA3 TTUs first, immediately followed by all PSA TTUs.
4. Log on to the computer. Click **NEW RUN** and select the appropriate assay protocol and concentrations. Click **NEXT** to begin the run.

   ***Note:*** *The run must be completed within 2 hours of the end of the 62°C Selection incubation.*
5. Prepare Deactivation Fluid by mixing equal volumes of 5.25% (0.7 M) sodium hypochlorite solution and Buffer for Deactivation Fluid in a large-capped plastic container. Label and write the expiration date on the plastic container. This Deactivation Fluid is stable for 4 weeks at room temperature.
6. When the run is finished, the assay software will generate two run reports, a Raw Run Report and a Ratio Report, if the runs are back-to-back (see *Quality Control Procedures* and *Interpretation of Results*).
7. When the run is finished, remove the used TTUs from the luminometer and place the TTUs into the container of Deactivation Fluid. Allow the TTUs to sit in the container for at least 15 minutes before disposal. Proper handling and disposal methods should be established by the laboratory director.

## Procedural Notes

A. Specimen Preparation

1. If specimens contain suspended precipitates, heat at 37°C for up to 5 minutes followed by gentle inversion. In the event that the precipitate does not go back into solution, ensure that the precipitate does not prevent delivery of specimen.
2. Frozen specimens must be thawed at room temperature (15°C to 30°C, may use a water bath) with occasional inversion during the thaw to prevent formation of an insoluble plug. Mix the vials by gentle inversion once the ice inside the vial has thawed enough to become loose and can move freely. Continue warming until the specimen is completely thawed and again mix the vials by gentle inversion.
   a. If a plug forms and specimens will be pipetted with the TECAN Freedom EVO 100/4 instrument, refreeze the specimen, repeat the thawing instructions and ensure that no plug forms. If unable to eliminate the plug, specimen must be hand-pipetted.
   b. If a plug forms and specimens will be hand-pipetted with a micropipettor, no further actions are necessary but ensure the plug does not prevent delivery of specimen.

B. Control, Calibrator, and Specimen Pipetting

1. The volume of calibrator, control, or specimen added to the TTU should be 400 µL. Visual inspection of the volume pipetted into the TTU is recommended to ensure proper volume transfer. Proper volume is needed to provide accurate results.
2. Ensure the pipette tip is seated correctly on the pipettor and check that the volume setting is correct. It is recommended to visually check the volume setting at the end of each TTU (every 10 tubes). Release the pipette plunger slowly at a steady rate when drawing the sample, to avoid generation of foam and bubbles.

C. Reagents

1. Probe Reconstitution Solution may precipitate during storage. Warm the solution at 62°C ± 1°C for 1 to 2 minutes. After this heat step, the Probe Reconstitution Solution may be used even if residual precipitate remains. After resuspension, mix the vial by gentle inversion.
2. When pipetting reagents other than Enzyme Reagent, aim slightly to the side of the bottom of the reaction tube (where the bottom curves up to meet the sides). When pipetting Enzyme Reagent, aim directly for the center of the reaction tube. Visually confirm that reagents are being dispensed correctly (no excessive amount of reagent on the sides of the tubes and proper color change).

D. Temperature

1. The target capture, amplification, hybridization, and selection steps are temperature dependent.
2. Room temperature is defined as 15°C to 30°C.

E. Time

The target capture, amplification, hybridization, and selection reactions are all time dependent. Adhere to specific times in the *DTS Systems Test Procedure*.

F. Decontamination

1. Surfaces and Pipettors

   Laboratory bench surfaces and pipettors must be decontaminated regularly with 2.5% to 3.5% (0.35 M to 0.5 M) sodium hypochlorite solution. Allow the solution to contact surfaces for at least 1 minute and then follow with a water rinse. **Do not allow the sodium hypochlorite solution to dry.** Chlorine solutions may pit equipment and metal. Thoroughly rinse equipment with water to avoid pitting.

2. TCS Aspiration Manifold

   After each use:

   a. Move the dispense manifold out of the way.
   b. Place a new TTC into the TTC rack. Turn on the vacuum pump. Attach the aspiration manifold to the tips in the TTC. Aspirate all Wash Solution remaining in the priming trough of the Wash Solution dispense station.
   c. Pour at least 100 mL of 0.5% to 0.7% (0.07 M to 0.1 M), or if preferred 2.5% to 3.5% (0.35 M to 0.5 M), sodium hypochlorite solution into the priming trough. Aspirate all of the solution through the aspiration manifold.
   d. Pour at least 100 mL of deionized water into the priming trough. Aspirate all of the water through the aspiration manifold.
   e. Eject the tips into their original TTC.

f. Leave the vacuum pump on until the manifold tubing is dry to prevent back flow (about 3 minutes).
g. Decontaminate the aspiration manifold surfaces as described in *TCS Unit*.

3. TCS Waste Container

   Clean the waste bottle at least once a week or when the waste bottle is 25% full, whichever comes first.

   a. Turn off the vacuum pump and allow the vacuum pressure to equalize.
   b. Release the quick disconnect fittings between the waste bottle and overflow bottle, and the waste bottle and aspiration manifold.
   c. Remove the waste bottle from the vacuum trap enclosure.
   d. Remove the cap and carefully add 400 mL of 5% to 7% (0.7 M to 1.0 M) sodium hypochlorite solution to the bottle (or 1 L if using a 10 L waste bottle).

      ***Note:*** *This may be done in a fume hood to avoid the release of fumes into the laboratory.*

   e. Cap the waste bottle and gently swirl the contents until fully mixed.
   f. Let the waste bottle sit for 15 minutes and then dispose of the contents (waste).
   g. Rinse the waste bottle with water to remove any remaining waste.
   h. Cap the empty waste bottle and place it in the vacuum trap enclosure. Attach the quick disconnect fitting to the TCS unit. Carefully discard both gloves.

4. TCS Unit

   Wipe the surfaces of the TCS unit, aspiration manifold, and surface of the Wash Buffer ejector tips with paper towels moistened with 2.5% to 3.5% (0.35 M to 0.5 M) sodium hypochlorite solution. Follow with a water rinse and then dry the surfaces completely with paper towels.

5. SB100 Instruments

   Do not spray any fluid directly on the instrument. There is wiring under the deck that will be damaged if sodium hypochlorite solution drips onto it. Do not pour or squirt sodium hypochlorite solution or water directly onto the SB100 instrument.

   Wipe the surfaces of the instrument (or frame) with paper towels moistened with 2.5% to 3.5% (0.35 M to 0.5 M) sodium hypochlorite solution. Allow the solution to contact surfaces for at least 1 minute. After a minute, thoroughly wipe the surfaces with water-moistened paper towels to avoid pitting. Dry the surface completely with a paper towel.

6. Racks

   Submerge the racks in 2.5% to 3.5% (0.35 M to 0.5 M) sodium hypochlorite solution, ensuring that they are covered by the solution. Keep the racks submerged for 10 minutes. Longer exposure will damage the racks. Rinse the racks thoroughly with water and then dry completely with paper towels.

G. Assay Contamination

1. The introduction of contaminating materials may occur if sufficient care is not taken during the assay procedure.
2. TTUs must be decontaminated in Deactivation Fluid as described in the *DTS Systems Test Procedure*. Do not reuse the TTUs.
3. Perform regular decontamination of equipment and work surfaces as described in *Decontamination*.
4. As in any reagent system, excess powder on some gloves may cause contamination of opened tubes. It is recommended that operators use powderless gloves.

# Quality Control Procedures

A. Run Validity

1. Calibrators and controls must be run with all assays and on the same rack as test specimens. The following criteria must be met in order for a run to be considered valid:

   Average RLU of Calibrator 2 > RLU Cutoff

   Where RLU Cutoff = Average RLU of Calibrator 1

   + 1.645 standard deviations of Calibrator 1 RLU replicates

   + 1.645 standard deviations of Calibrator 2 RLU replicates.

   Average interpolated Calibrator 5 recovery = 100 ± 30%

   Average interpolated Control A recovery = 100 ± 60%

   Average interpolated Control B recovery = 100 ± 35%

2. The Progensa PCA3 Assay Software automatically evaluates the results against the above criteria and will report the Run Status as PASS if the validity criteria are met, and FAIL if the validity criteria are not met.
3. If the Run Status is FAIL, all test results in the same run are invalid for that analyte and must not be reported.
4. If a run is invalid, the run must be repeated for that analyte (see *Interpretation of Results*). If the run is valid for the other analyte, those results may be used in data analysis with the repeat, valid run of the first analyte.

B. Specimen Validity

Within a valid run, individual specimen results may be deemed INVALID and will be indicated in the Raw Run Report (see *Interpretation of Results*). Although individual replicates for a specimen may be valid, a specimen will be invalidated if the interpolated copies/mL difference between the replicates exceeds 5.8-fold (the second replicate is 5.8 times more than the first replicate or the second replicate is 5.8 times less than the first replicate or coefficient of variation of the two replicates is more than 100%). Testing of the specimen for that analyte must be repeated.

C. External Controls

Each laboratory under their normal operating conditions should establish their own external controls to monitor test system components, environment, and operator performance. Previously tested patient specimens may be used, provided the laboratory determines the acceptable performance level for the patient specimens. The laboratory will establish the frequency of testing controls that detect immediate errors and monitor test performance over time and maintain records according to standard laboratory quality control practices (2, 3, 8).

# Interpretation of Results

A. Types of Reports

1. Raw Run Report

   The Raw Run Report provides information on run validity (PASS or FAIL; see *Quality Control Procedures*) 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), one analyte run may be invalid while the other analyte run is valid.

   The Exceptions Summary is found at the end of the Raw Run Report. For back-to-back runs where both analyte runs are valid, specimens listed in the Exceptions Summary may require retesting of one analyte. Although a PCA3 Score result may be listed in the Exceptions Summary, this result is not considered reportable until manual matching has been performed and the result is listed in a Ratio Report. If only one analyte was tested or if one analyte run is invalid, all specimens tested will be listed in the Exceptions Summary.

2. Ratio Report

   The assay software automatically generates a Ratio Report for a back-to-back run where both analyte runs are valid. The software calculates and lists the PCA3 Score of specimens in the Ratio Report. Specimens listed in the Ratio Report either require no further testing or both analytes must be retested. Specimens not listed in the Ratio Report will be found in the Exceptions Summary section of the Raw Run Report.

   A Ratio Report can also be generated after manual matching (see *Manual Matching* for more information).

3. 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 (9). For more information, refer to the *Progensa PCA3 Assay Software Operator's Manual*.

B. Matching

1. Automatic Matching

   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 results are listed in the Ratio Report or Exceptions Summary of the Raw Run Report.

2. Manual Matching

   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 (refer to the *Progensa PCA3 Assay Software Operator's Manual*). Manual matching may also be required for results that are listed in the Exceptions Summary of the Raw Run Report. After manual matching, the PCA3 Score(s) for the matched specimen(s) will be listed in a new Ratio Report.

C. Interpreting Reports

1. PCA3 Score

***Note: Only PCA3 Scores and PCA3 Score ranges listed in the Ratio Report are reportable.*** *Results that appear in the Exceptions Summary may require further action and are not reportable.*

The PCA3 Score is calculated as the ratio of PCA3 RNA copies to PSA RNA copies, multiplied by 1000. PCA3 Scores may only be calculated using results from valid runs and specimens. Invalid runs and invalid specimens must be retested for that analyte (see *Retesting* for more information).

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 cut-off 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.

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).

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.

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

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 cut-off of 25 cannot be made. If a numerical value is required for interpretation relative to the cut-off 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.

2. Interpreting Status and Analysis Codes

The Status column in both the Raw Run Report and Ratio Report lists information in "s:a" format. Run-specific status codes ("s") are listed before (to the left of) the colon and analyte-specific analysis codes ("a") are listed after (to the right of) the colon. Analyte-specific codes are listed in lowercase for PCA3 results and uppercase for PSA results. Each report contains descriptions of the status and analysis codes that appear in that report. For example, codes may indicate if a specimen or replicate result is valid or out-of-range. Refer to the *Progensa PCA3 Assay Software Operator's Manual* for a full listing of status and analysis codes and more details.

If a PCA3 Score is reported in the Ratio Report and no status or analysis codes appear in the PCA3 or PSA Status columns, this indicates both analytes tested valid and "in range." The specimen result is reportable and no further actions are necessary.

If a status or analysis code appears in the Exceptions Summary or in the Ratio Report, retesting may be necessary (see *Interpreting the Results in the Exceptions Summary* and *Interpreting Results in the Ratio Report*). If analyte results come from separate runs and have an analysis code(s), find the combination for both analytes in Table 4 or Table 5 to determine if further action is necessary.

3. Interpreting the Results in the Exceptions Summary

   The Exceptions Summary may not list any exceptions. In these cases, no further actions are necessary.

   If the Exceptions Summary lists a specimen(s) for back-to-back runs where both analyte runs are valid, refer to Table 4 for instructions.

   For individual analyte runs, refer to *Interpreting Status and Analysis Codes*. In back-to-back runs where one analyte run is invalid, retest the invalid run (see *Retesting* for more information), and treat the results as though individual analyte runs had been performed. Manual matching will be required.

   A specimen may be labeled as invalid although the individual tubes (replicates) may be labeled as valid. It is the combined result of the replicates that determines specimen validity, and a large difference between replicates will invalidate a specimen (see *Quality Control Procedures* for more information).

*Table 4: Progensa PCA3 Assay Exceptions Summary Conditions*

| PCA3 Result (Analysis Code*) | PSA Result (Analysis Code*) | Listed PCA3 Score | Further Testing? | Action/Comment |
|---|---|---|---|---|
| In range (no code) | Invalid** (A, B, E, H, or I) | -- | Yes | Retest PSA (see *Retesting*) and manually match results. |
| Out-of-range low (g) | Invalid (A, B, E, H, or I) | -- | Yes | Retest PSA (see *Retesting*) and manually match results. |
| Invalid (a, b, e, h, or i) | In range (no code) | -- | Yes | Retest PCA3 (see *Retesting*) and manually match results. |
| In range (no code) | Out-of-range high (F) | <[Calculated PCA3 Score]*** | Optional | 1. Manually match to get <[Calculated PCA3 Score] OR<br>2. Dilute specimen in specimen diluent (see *Dilution of Out-of-Range High Specimens*), retest PSA, and manually match results if a PCA3 Score is required. |
| Out-of-range high (f) | In range (no code) | >[Calculated PCA3 Score] | Optional | 1. Manually match to get >[Calculated PCA3 Score] OR<br>2. Dilute specimen in specimen diluent, retest PCA3, and manually match results if a PCA3 Score is required. |
| Out-of-range low (g) | In range (no code) | <[Calculated PCA3 Score] | No | Manually match to get <[Calculated PCA3 Score]. |
| Out-of-range low (g) | Out-of-range high (F) | <[Calculated PCA3 Score] | No | Manually match to get <[Calculated PCA3 Score]. |

*Refer to the *Progensa PCA3 Assay Software Operator's Manual* for a full listing of analysis codes.

**Applies only to invalid specimens within a valid run.

***For out-of-range values, the Calculated PCA3 Score is computed using the copy level for the nearest positive calibrator.

4. Interpreting Results in the Ratio Report

   If a specimen is listed in the Ratio Report with a PCA3 Score, the result is a reportable PCA3 Score and no further actions are necessary. If no PCA3 Score is listed, expressed as "--" in the PCA3 Score column, refer to Table 5 for instructions.

*Table 5: Progensa PCA3 Assay Ratio Report Conditions*

| PCA3 Result (Analysis Code*) | PSA Result (Analysis Code*) | Listed PCA3 Score | Further Testing? | Action/Comment |
|---|---|---|---|---|
| In range (no code) | In range (no code) | PCA3 Score | No | No further actions; result is reportable. |
| Invalid** (a, b, e, h, or i) | Invalid (A, B, E, H, or I) | -- | Yes | Retest both analytes (see *Retesting*). |
| Invalid (a, b, e, h, or i) | Out-of-range high (F) | -- | Yes | Dilute specimen in specimen diluent (see *Dilution of Out-of-Range High Specimens*), retest both analytes. |
| Out-of-range high (f) | Invalid (A, B, E, H, or I) | -- | Yes | Dilute specimen in specimen diluent, retest both analytes. |
| Out-of-range high (f) | Out-of-range high (F) | -- | Yes | Dilute specimen in specimen diluent, retest both analytes. |
| Invalid (a, b, e, h, or i) | Out-of-range low (G) | -- | No | Sample has insufficient RNA for accurate analysis. A new specimen must be collected from the patient. |
| In range (no code) | Out-of-range low (G) | -- | No | Sample has insufficient RNA for accurate analysis. A new specimen must be collected from the patient. |
| Out-of-range high (f) | Out-of-range low (G) | -- | No | Sample has insufficient RNA for accurate analysis. A new specimen must be collected from the patient. |
| Out-of-range low (g) | Out-of-range low (G) | -- | No | Sample has insufficient RNA for accurate analysis. A new specimen must be collected from the patient. |

*Refer to the *Progensa PCA3 Assay Software Operator's Manual* for a full listing of analysis codes.

**Applies only to invalid specimens within a valid run. If specimens were invalid because the run was invalid, results will be listed in the Exceptions Summary (see *Interpreting the Results in the Exceptions Summary* for more information).

D. Retesting

1. Guidelines for Retesting

   a. Although it is not imperative that both analytes be tested in the same run, **both analyte results must come from the same sample vial for a reportable PCA3 Score**. In case the analytes are not run in the same run but 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 (refer to the Progensa PCA3 Assay Software Operator's Manual)

   b. All invalid runs must be repeated and all invalid specimens from valid runs must be retested.

   c. Retest the specimen(s) using a new set of calibrators and controls.

   d. Proper storage of the leftover specimen prior to retesting is essential (see *Specimen Collection, Transport, and Storage* for more information).

   e. A manual match of PCA3 and PSA analytes may be necessary to determine the PCA3 Score (see *Manual Matching* for more information).

2. Dilution of Out-of-Range High Specimens
    a. If a specimen concentration extrapolates above Calibrator 5 within a valid run, the result is "out-of-range high" and the result will be labeled with an "f" or "F" analysis code in the run report(s). The concentration will be expressed as >[Calibrator 5 concentration].
    b. Invert the processed urine specimen to mix it prior to dilution of the specimen. A recommended, but not required, dilution is 1:10 using the Progensa PCA3 Specimen Diluent Kit. In an appropriate vial, add 1800 µL specimen diluent and 200 µL specimen; cap tube and invert five times to mix completely. The dilution factor will be "10" in the run worklist. If both analytes are to be retested, double the volumes (use 3600 µL specimen diluent and 400 µL specimen). Test the diluted specimen with the assay.
    c. If, upon retesting, the specimen result is again out-of-range high, further dilution until the specimen result interpolates within range of the calibrators is required. Further dilution of the initial 1:10 dilution is permissible, provided the initial 1:10 dilution was stored properly (see *Specimen Collection, Transport, and Storage* for more information).

# Performance Characteristics

## Clinical Performance

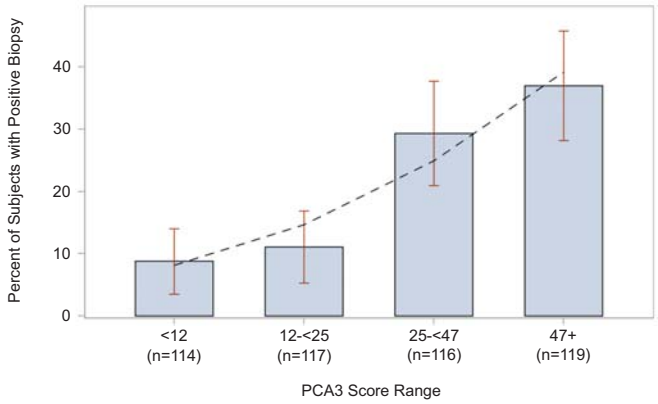
Four hundred ninety five (495) eligible male subjects were enrolled from a total of 14 clinical sites, including academic institutions, community-based urology clinics, and group health organizations. Men who had at least one previous negative prostatic biopsy, who had never had a positive prostatic 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 enrolled in the study. 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%); 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 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 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 (with 95% confidence limits).




Note. Dashed line represents the predicted probability of positive biopsy from a logistic regression model. Ranges represent quartiles of the PCA3 Score distribution.

***Figure 3. Positive Biopsy Results by PCA3 Score with 95% Confidence Limits***

Table 6 shows the performance characteristics of the Progensa PCA3 Assay relative to prostatic biopsy outcome at a PCA3 Score cut-off value of 25.

*Table 6: Performance Characteristics of the Progensa PCA3 Assay*

| | Biopsy Result | | Total | Performance Characteristic | Estimate | 95% CI |
|---|---|---|---|---|---|---|
| | Biopsy Positive | Biopsy Negative | | | | |
| **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 |
| | | | | **PLR** | 1.81 | 1.53-2.11 |
| **Positive Biopsy 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.

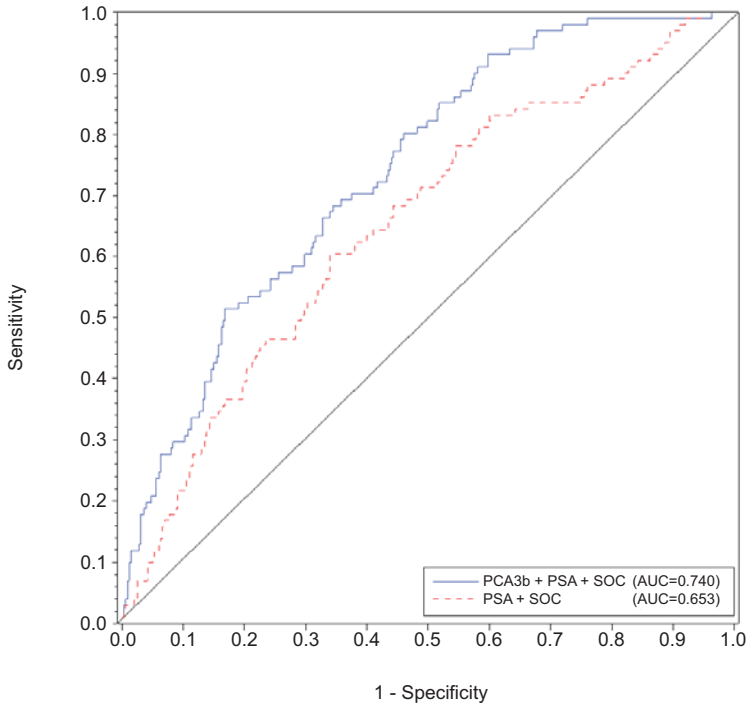
Table 7 shows the area under the curve (AUC) of the receiver operating characteristic (ROC) curves for the Progensa PCA3 Assay. The ROC AUC for PCA3 Score was 0.707 (95% CI: 0.649–0.746). The ROC AUC for total serum PSA test combined with standard of care (SOC) covariates (including age, DRE result, 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 7: 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 using a binary test with cut-off 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 4 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 cut-off.




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

***Figure 4. ROC Curves for Total Serum PSA Test Results and Standard of Care Covariates, with and without PCA3 Score with Cut-off of 25***

Table 8 shows the performance characteristics of the Progensa PCA3 Assay relative to prostatic biopsy outcome at selected cut-offs.

*Table 8: Performance Characteristics of the Progensa PCA3 Assay at Selected Cut-offs*

| PCA3 Score Cut-off | n | TP | FP | TN | FN | Se % (95% CI) | Sp % (95% CI) | PPV % (95% CI) | NPV % (95% CI) | PLR (95% CI) | NLR (95% CI) | OR (95% CI) |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 5 | 466 | 100 | 343 | 21 | 2 | 98.0 (93.1-99.5) | 5.8 (3.8-8.7) | 22.6 (21.7-23.2) | 91.3 (74.3-99.1) | 1.04 (0.99-1.08) | 0.34 (0.03-1.23) | 3.06 (0.71-13.27) |
| 10 | 466 | 94 | 279 | 85 | 8 | 92.2 (85.3-96.0) | 23.4 (19.3-28.0) | 25.2 (23.6-26.7) | 91.4 (84.8-95.9) | 1.20 (1.10-1.30) | 0.34 (0.15-0.64) | 3.58 (1.67-7.66) |
| 15 | 466 | 87 | 233 | 131 | 15 | 85.3 (77.1-90.9) | 36.0 (31.2-41.0) | 27.2 (24.9-29.4) | 89.7 (84.7-93.7) | 1.33 (1.18-1.49) | 0.41 (0.24-0.64) | 3.26 (1.81-5.87) |
| 20 | 466 | 80 | 188 | 176 | 22 | 78.4 (69.5-85.3) | 48.4 (43.3-53.5) | 29.9 (26.8-32.8) | 88.9 (84.8-92.4) | 1.52 (1.31-1.75) | 0.45 (0.29-0.64) | 3.40 (2.04-5.70) |
| **25** | **466** | **79** | **156** | **208** | **23** | **77.5 (68.4-84.5)** | **57.1 (52.0-62.1)** | **33.6 (30.0-37.2)** | **90.0 (86.5-93.1)** | **1.81 (1.53-2.11)** | **0.40 (0.26-0.56)** | **4.58 (2.75-7.62)** |
| 35 | 466 | 64 | 112 | 252 | 38 | 62.7 (53.1-71.5) | 69.2 (64.3-73.8) | 36.4 (31.3-41.4) | 86.9 (83.9-89.8) | 2.04 (1.63-2.52) | 0.54 (0.41-0.69) | 3.79 (2.40-6.00) |
| 45 | 466 | 46 | 79 | 285 | 56 | 45.1 (35.8-54.8) | 78.3 (73.8-82.2) | 36.8 (30.1-43.6) | 83.6 (81.1-86.2) | 2.08 (1.54-2.76) | 0.70 (0.57-0.83) | 2.96 (1.87-4.71) |
| 55 | 466 | 38 | 63 | 301 | 64 | 37.3 (28.5-46.9) | 82.7 (78.5-86.2) | 37.6 (29.8-45.7) | 82.5 (80.3-84.8) | 2.15 (1.52-3.00) | 0.76 (0.64-0.88) | 2.84 (1.75-4.61) |
| 65 | 466 | 36 | 39 | 325 | 66 | 35.3 (26.7-44.9) | 89.3 (85.7-92.1) | 48.0 (38.1-57.9) | 83.1 (81.2-85.3) | 3.29 (2.20-4.91) | 0.73 (0.61-0.83) | 4.55 (2.69-7.68) |
| 75 | 466 | 34 | 34 | 330 | 68 | 33.3 (24.9-42.9) | 90.7 (87.2-93.2) | 50.0 (39.4-60.5) | 82.9 (81.0-85.1) | 3.57 (2.32-5.47) | 0.74 (0.63-0.84) | 4.85 (2.82-8.35) |
| 100 | 466 | 24 | 22 | 342 | 78 | 23.5 (16.4-32.6) | 94.0 (91.0-96.0) | 52.2 (38.7-65.4) | 81.4 (79.9-83.3) | 3.89 (2.25-6.75) | 0.81 (0.72-0.90) | 4.78 (2.55-8.97) |

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. For calculations in this table, PCA3 Score values greater than or equal to the cut-off are considered positive and PCA3 Score values less than the cut-off are considered negative.

Table 9 shows the performance characteristics of the Progensa PCA3 Assay relative to prostatic biopsy outcome for subgroups of the study population. Table 10 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 10).

*Table 9: Performance Characteristics of the Progensa PCA3 Assay by Subgroups*

| Subgroup | n | TP | FP | TN | FN | Se % (95% CI) | 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) | 2.80 (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 to <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 (3.14-9.36) |
| 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 (0.01-0.76) | 15.55 (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 (2.40-6.87) |

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 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 10).

*Table 10: Performance Characteristics of the Progensa PCA3 Assay in Men with ASAP on Their Most Recent Negative Biopsy*[1]

| | Biopsy Result | | Total | Performance Characteristic | Estimate | 95% CI |
|---|---|---|---|---|---|---|
| | Biopsy Positive | Biopsy Negative | | | | |
| **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 |
| | | | | **PLR** | 0.94 | 0.54-1.40 |
| **Positive Biopsy 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.

[1]The ~~PROGENSA~~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.

## Precision: Reproducibility

Progensa PCA3 Assay reproducibility (5) 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 performed, over 15 days, 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 analyte concentrations and targeted PCA3 Scores for each panel member are shown in Table 11. 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 11: Reproducibility Panel Composition*

| Panel Member | PCA3 RNA Concentration | PSA RNA Concentration | Targeted PCA3 Score |
|---|---|---|---|
| 1 | Low | Low | 35 |
| 2 | Mid | High | 10 |
| 3 | High | Mid | 86 |

Table 12 summarizes the variability of the Progensa PCA3 Assay within runs, between runs, between sites/instruments, between operators, and between reagent lots for each panel member for PCA3 and PSA analyte copies/mL and for PCA3 Score.

*Table 12: Reproducibility of the Progensa PCA3 Assay PCA3 Copies/mL, PSA Copies/mL, and PCA3 Score Results on DTS Systems by Panel Member*

| Parameter Panel Member | PCA3 Conc | PSA Conc | n[1] | Mean Value | Within Run | | Between Run | | Between Site | | Between Operator | | Between Lot | | Total | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | SD | CV% | SD | CV% | SD | CV% | SD | CV% | SD | CV% | SD | CV% |
| **PCA3 copies/mL** | | | | | | | | | | | | | | | | |
| **1** | Low | Low | 359 | 678 | 83 | 12.2 | 73 | 10.7 | 18 | 2.6 | 0 | 0.0 | 33 | 4.9 | 116 | 17.2 |
| **2** | Mid | High | 359 | 18,969 | 959 | 5.1 | 820 | 4.3 | 261 | 1.4 | 0 | 0.0 | 0 | 0.0 | 1,289 | 6.8 |
| **3** | High | Mid | 357 | 97,006 | 4,620 | 4.8 | 3,797 | 3.9 | 2886 | 3.0 | 2,246 | 2.3 | 3,237 | 3.3 | 7,721 | 8.0 |
| **PSA copies/mL** | | | | | | | | | | | | | | | | |
| **1** | Low | Low | 359 | 16,747 | 2,713 | 16.2 | 1,621 | 9.7 | 0 | 0.0 | 568 | 3.4 | 318 | 1.9 | 3,226 | 19.3 |
| **2** | Mid | High | 359 | 1,638,117 | 127,184 | 7.8 | 117,100 | 7.1 | 45,603 | 2.8 | 0 | 0.0 | 68,132 | 4.2 | 191,337 | 11.7 |
| **3** | High | Mid | 357 | 994,851 | 65,724 | 6.6 | 69,177 | 7.0 | 33,705 | 3.4 | 0 | 0.0 | 26,336 | 2.6 | 104,569 | 10.5 |
| **PCA3 Score** | | | | | | | | | | | | | | | | |
| **1** | Low | Low | 359 | 41 | 7.0 | 17.0 | 6.6 | 16.1 | 0.0 | 0.0 | 1.8 | 4.3 | 3.0 | 7.4 | 10.3 | 25.0 |
| **2** | Mid | High | 359 | 11 | 1.2 | 10.7 | 1.0 | 9.1 | 0.3 | 2.9 | 0.0 | 0.0 | 0.5 | 4.3 | 1.7 | 15.0 |
| **3** | High | Mid | 357 | 98 | 8.5 | 8.6 | 7.9 | 8.1 | 0.0 | 0.0 | 2.9 | 3.0 | 1.9 | 1.9 | 12.1 | 12.3 |

Conc = concentration.
[1]Five samples (1 sample of Panel Member 1, 1 sample of Panel Member 2, and 3 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.

## Precision: Repeatability

Progensa PCA3 Assay repeatability was evaluated at Hologic, Inc. using a 4-member repeatability 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 comprised PCA3 and PSA *in vitro* transcripts in processed female urine diluted in specimen diluent.

Testing was performed using 1 reagent lot and 1 calibrators and controls lot. One operator performed 20 Progensa PCA3 Assay runs on DTS Systems; each run contained 4 sets of the 4 repeatability panel members. Table 13 summarizes the variability of the Progensa PCA3 Assay within runs, between runs and between days for each panel member for PCA3 and PSA analyte copies/mL and for PCA3 Score.

*Table 13: Progensa PCA3 Assay Repeatability*

| Parameter Panel Member | PCA3 Conc | PSA Conc | n[1] | Mean | Within Run | | Between Run | | Between Day | | Total | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | SD | CV% | SD | CV% | SD | CV% | SD | CV% |
| **PCA3 copies/mL** | | | | | | | | | | | | |
| **1** | Low | Low | 80 | 661 | 85 | 12.9 | 54 | 8.1 | 67 | 10.1 | 121 | 18.3 |
| **2** | Mid | High | 80 | 18,626 | 1,033 | 5.5 | 752 | 4.0 | 156 | 0.8 | 1,287 | 6.9 |
| **3** | High | Mid | 80 | 99,846 | 3,820 | 3.8 | 1,111 | 1.1 | 3,260 | 3.3 | 5,143 | 5.2 |
| **4** | Mid/Diln | Mid/Diln | 80 | 24,482 | 1,169 | 4.8 | 1,047 | 4.3 | 0 | 0 | 1,569 | 6.4 |
| **PSA copies/mL** | | | | | | | | | | | | |
| **1** | Low | Low | 80 | 18,298 | 2,862 | 15.6 | 837 | 4.6 | 275 | 1.5 | 2,995 | 16.4 |
| **2** | Mid | High | 77 | 2,017,466 | 190,359 | 9.4 | 27,935 | 1.4 | 0 | 0 | 192,398 | 9.5 |
| **3** | High | Mid | 80 | 1,247,896 | 228,984 | 18.3 | 0 | 0 | 44,626 | 3.6 | 233,292 | 18.7 |
| **4** | Mid/Diln | Mid/Diln | 80 | 603,427 | 108,192 | 17.9 | 32,253 | 5.3 | 0 | 0 | 112,897 | 18.7 |
| **PCA3 Score** | | | | | | | | | | | | |
| **1** | Low | Low | 80 | 36 | 6.9 | 19.0 | 2.8 | 7.7 | 0.9 | 2.3 | 7.5 | 20.7 |
| **2** | Mid | High | 77 | 9 | 1.1 | 12.0 | 0.6 | 6.2 | 0.1 | 0.6 | 1.2 | 13.6 |
| **3** | High | Mid | 80 | 81 | 11.1 | 13.6 | 0 | 0 | 2.7 | 3.3 | 11.4 | 14.0 |
| **4** | Mid/Diln | Mid/Diln | 80 | 41 | 6.0 | 14.6 | 3.8 | 9.3 | 0 | 0 | 7.1 | 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.

## Analytical Specificity

A. Unspliced Transcript

Progensa PCA3 Assay was designed to detect only the prostate cancer-specific exon 3-exon 4 spliced PCA3 RNA (14). The assay did not detect 1.25 x $10^6$ copies/mL of unspliced PCA3 RNA in processed female urine significantly above background.

B. Interfering Substances

The substances listed in Table 14 were added to aliquots of pooled clinical specimens. The specimens were tested with Progensa PCA3 Assay according to CLSI EP7-A2 (2005) (7). At the concentrations listed, no assay interference (no significant change in PCA3 Score) was observed.

*Table 14: Substances Tested for Progensa PCA3 Assay Interference*

| Medications and Supplements | | Endogenous Substances | |
|---|---|---|---|
| **Substance** | **Test Concentration** | **Substance** | **Test Concentration** |
| Acetaminophen | 1.324 mmol/L | Albumin | 60 g/L |
| Acetylsalicylic acid | 3.62 mmol/L | Bilirubin (unconjugated) | 0.342 mmol/L |
| Alfuzosin | 30 mg/L | Calcium | 5 mmol/L |
| Allopurinol | 0.294 mmol/L | Cholesterol | 13 mmol/L |
| Amlodipine | 0.245 µmol/L | Glucose | 55 mmol/L |
| Atenolol | 37.6 µmol/L | Hemoglobin | 2 g/L |
| Atorvastatin | 25 mg/L | Immunoglobulin G | 32 mg/L |
| Ciprofloxacin | 30.2 µmol/L | Triglycerides | 37 mmol/L |
| Diphenhydramine | 19.6 µmol/L | Uric acid | 1.4 mmol/L |
| Doxazosin | 1.33 µmol/L | Red blood cells | $5.10 \times 10^7$ cells/L |
| Doxycycline | 67.5 µmol/L | White blood cells | $7.60 \times 10^7$ cells/L |
| Dutasteride | 1.5 mg/L | | |
| Esomeprazole | 0.12 g/L | **Microorganisms** | |
| Finasteride | 15 mg/L | **Organism** | **Test Concentration** |
| Fluoxetine | 11.2 µmol/L | *Candida albicans* | $5 \times 10^6$ CFU*/L |
| Flutamide | 2.25 g/L | *Escherichia coli* | $5 \times 10^6$ CFU/L |
| Furosemide | 0.181 mmol/L | *Klebsiella pneumoniae* | $5 \times 10^6$ CFU/L |
| Ibuprofen | 2.425 mmol/L | *Proteus mirabilis* | $5 \times 10^6$ CFU/L |
| Levofloxacin | 48.6 µmol/L | *Pseudomonas aeruginosa* | $5 \times 10^6$ CFU/L |
| Lisinopril | 0.74 µmol/L | *Staphylococcus aureus* | $5 \times 10^6$ CFU/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 | | |

*CFU = colony-forming units.

## Analytical Sensitivity

The limit of quantitation of Progensa PCA3 Assay was determined using an 8-member analytical sensitivity panel. The panel comprised 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 10 Progensa PCA3 Assay runs on DTS Systems; each run contained 2 sets of the 8 analytical sensitivity panel members. The limit of detection and limit of quantitation were calculated according to CLSI EP17-A (6). The limit of detection 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%). The limits of quantitation of both analytes were the same as the corresponding limits of detection. The lower limit of the dynamic range of the Progensa PCA3 Assay is defined by the lowest positive calibrator.

## Linearity and Measuring Intervals

### 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 spanned beyond the assay range for each analyte.

One operator performed 4 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 5 .

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

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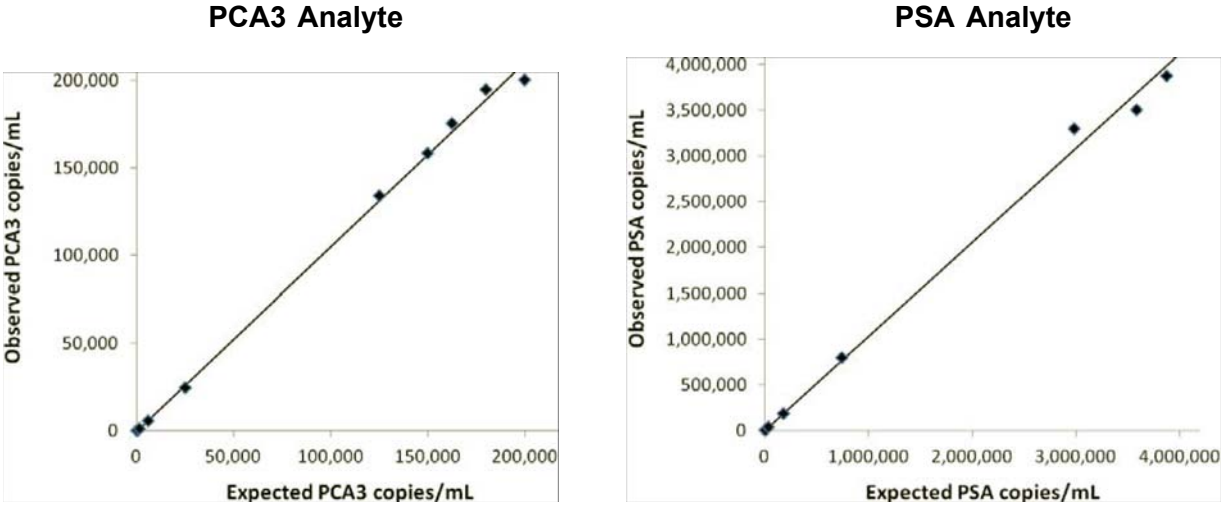


***Figure 5. Progensa PCA3 Assay Linearity for PCA3 and PSA Analytes, Transcript Samples***

### 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. Two 10-member linearity panels, one each prepared in PCA3 Specimen Diluent or processed female urine independently for each analyte, were tested and analyzed as above. 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%[1] in processed female urine. In PCA3 Specimen Diluent, the PSA analyte demonstrated linearity from 4,890 to 2,640,820 copies/mL in PCA3 Specimen Diluent with deviation less than 23%[1] in this

1 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.

interval. There was no significant diluent matrix effect. See Figure 6 (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).

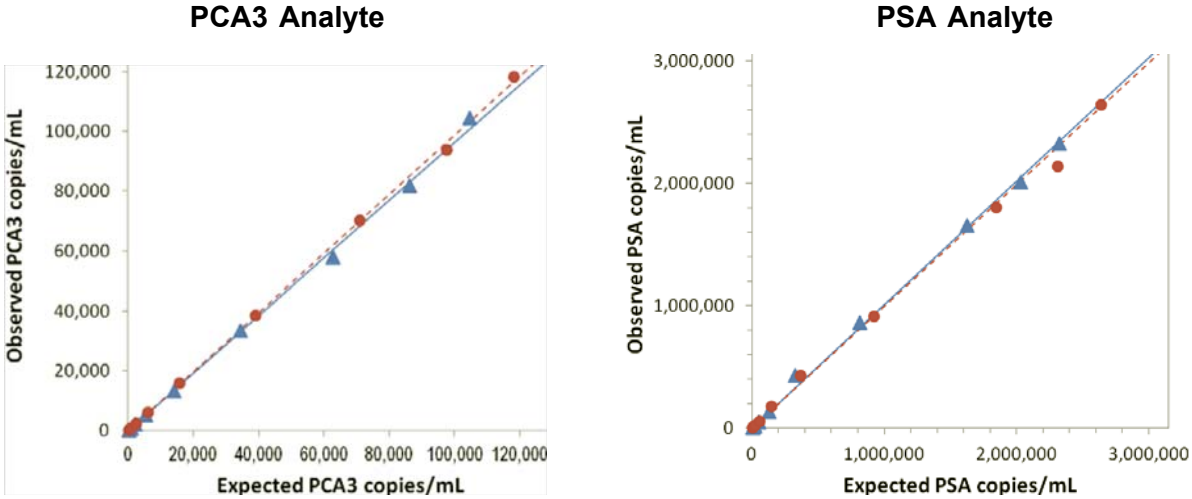


***Figure 6. Progensa PCA3 Assay Linearity for PCA3 and PSA Analytes, Clinical Specimens***

The measuring interval for PCA3 analyte is 250 to 125,000 copies/mL and the measuring interval for PSA analyte is 7,500 to 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 to 462.

## Recovery

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 260nm 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 ($10^7$- to $10^{10}$-fold). Two operators each performed 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 Progensa PCA3 Assay measured copies/mL to UV-determined copies/mL, multiplied by 100 (Table 15).

*Table 15: 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 that 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.

# Web Access to Progensa PCA3 Assay Labeling

The following labeling is available on the Hologic website: www.hologic.com/package-inserts

- Progensa PCA3 Assay Package Insert
- Physician Brochure for the Progensa PCA3 Assay
- Physician Instructions for the Progensa PCA3 Assay

For a paper copy of any of these package inserts, please contact your sales representative or call:

+1 888 484 4747 or e-mail: molecularsupport@hologic.com

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