GEN-PROBE®

PROGENSA® PCA3

PROGENSA® PCA3 Assay

For *in vitro* diagnostic use.

For U.S. export only.

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Intended Use

The PROGENSA PCA3 Assay is an *in vitro* nucleic acid amplification test (NAAT) that detects Prostate Cancer Gene 3 (PCA3) messenger ribonucleic acid (mRNA) in male urine specimens to generate a PCA3 Score. The PCA3 Score is intended for use in conjunction with standard-of-care diagnostic algorithms as an aid in the diagnosis of prostate cancer.

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 (1), 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 (1). While biopsy remains the gold standard for prostate cancer detection, more accurate tests with better specificity are needed to help guide decisions to biopsy the prostate.

PCA3 (also known as "PCA3^{DD3}" or "DD3^{PCA3}") is a non-coding prostate-specific mRNA that is highly over-expressed in prostate cancer cells, with a median 66-fold up-regulation compared to adjacent benign tissue (2). In contrast, PSA gene expression is similar in cancerous and benign cells; PSA mRNA levels may therefore be used to normalize for the amount of prostate-specific ribonucleic acid (RNA) in molecular test samples. The feasibility of quantitative PCA3-based molecular testing from urine sediments (2) and from whole urine (3) has been demonstrated.

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 mRNAs are quantified, and the PCA3 Score is determined based on the ratio of PCA3/PSA mRNA. In addition to normalizing PCA3 signal, measurement of PSA mRNA also serves to confirm that the yield of prostate-specific RNA is sufficient to generate a valid result. Higher PCA3 Scores correlate with higher probability of a positive prostate biopsy.

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 mRNA, and detect amplicon, respectively.

When the PROGENSA PCA3 Assay is performed in the laboratory, the target mRNA 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 mRNAs are quantified in separate tubes and the PCA3 Score is determined. Calibrators containing known amounts of PCA3 or PSA RNA transcript are included in every assay run and used to generate a standard curve. PCA3 and PSA controls are also included to verify the accuracy of results interpolated from the standard curve.

Reagents

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

PROGENSA® PCA3/PSA Assay Kit, 2 x 100 Reactions, Cat. No. 302355 (8 boxes)

PROGENSA PCA3 100-Reaction Kit

PROGENSA PCA3 Refrigerated Box - Storage at 2°C to 8°C			
Symbol	**Component**	**Quantity**	**Description**
A	PCA3 Amplification Reagent	1 X 100 reactions	*Non-infectious nucleic acids dried in HEPES buffered solution containing <10% bulking agent.*
E	PCA3/PSA Enzyme Reagent	1 X 100 reactions	*Reverse transcriptase and RNA polymerase dried in HEPES buffered solution containing <10% bulking agent.*
P	PCA3 Probe Reagent	1 X 100 reactions	*Non-infectious chemiluminescent DNA probes dried in succinate buffered solution containing <5% bulking agent and <5% lithium lauryl sulfate.*

PROGENSA PCA3 Room Temperature Box - Storage at 15°C to 30°C			
Symbol	**Component**	**Quantity**	**Description**
AR	PCA3 Amplification Reconstitution Solution	1 X 9.3 mL	*Aqueous solution containing preservatives (<1% parabens).*
ER	PCA3/PSA Enzyme Reconstitution Solution	1 X 3.3 mL	*HEPES buffered solution containing a surfactant (10% Triton X-100) and 20% glycerol.*
PR	PCA3/PSA Probe Reconstitution Solution	1 X 12.4 mL	*Succinate buffered solution containing <5% lithium lauryl sulfate.*
S	PCA3/PSA Selection Reagent	1 X 31 mL	*Borate buffered solution containing surfactant (1% Triton X-100).*
TCR	PCA3 Target Capture Reagent	1 X 22 mL	*Non-infectious nucleic acid in HEPES buffered solution containing solid phase (<0.5 mg/mL).*
	Sealing Cards	1 package	
	Reconstitution Collars	1 package	

PROGENSA PCA3 Calibrator and Controls Kit - Storage at 2°C to 8°C

Symbol	Component	Quantity	Description
CAL	PCA3 Calibrator 1	1 X 2.0 mL	*Phosphate buffered solution containing <5% lithium lauryl sulfate.*
CAL	PCA3 Calibrators 2-5	4 X 1.7 mL	*Non-infectious PCA3 nucleic acid in phosphate buffered solution containing <5% lithium lauryl sulfate.*
PC	PCA3 Positive Controls	2 X 1.7 mL	*Non-infectious PCA3 nucleic acid in phosphate buffered solution containing <5% lithium lauryl sulfate.*

PROGENSA PSA 100-Reaction Kit

PROGENSA PSA Refrigerated Box - Storage at 2°C to 8°C			
Symbol	**Component**	**Quantity**	**Description**
A	PSA Amplification Reagent	1 X 100 reactions	*Non-infectious nucleic acids dried in HEPES buffered solution containing <10% bulking agent.*
E	PCA3/PSA Enzyme Reagent	1 X 100 reactions	*Reverse transcriptase and RNA polymerase dried in HEPES buffered solution containing <10% bulking agent.*
P	PSA Probe Reagent	1 X 100 reactions	*Non-infectious chemiluminescent DNA probes dried in succinate buffered solution containing <5% bulking agent and <5% lithium lauryl sulfate.*

PROGENSA PSA Room Temperature Box - Storage at 15°C to 30°C			
Symbol	**Component**	**Quantity**	**Description**
AR	PSA Amplification Reconstitution Solution	1 X 9.3 mL	*Aqueous solution containing preservatives (<1% parabens).*
ER	PCA3/PSA Enzyme Reconstitution Solution	1 X 3.3 mL	*HEPES buffered solution containing a surfactant (10% Triton X-100) and 20% glycerol.*
PR	PCA3/PSA Probe Reconstitution Solution	1 X 12.4 mL	*Succinate buffered solution containing <5% lithium lauryl sulfate.*
S	PCA3/PSA Selection Reagent	1 X 31 mL	*Borate buffered solution containing surfactant (1% Triton X-100).*
TCR	PSA Target Capture Reagent	1 X 22 mL	*Non-infectious nucleic acid in HEPES buffered solution containing solid phase (<0.5 mg/mL).*
	Sealing Cards	1 package	
	Reconstitution Collars	1 package	

PROGENSA PSA Calibrator and Controls Kit - Storage at 2°C to 8°C

Symbol	Component	Quantity	Description
CAL	PSA Calibrator 1	1 X 2.0 mL	*Phosphate buffered solution containing <5% lithium lauryl sulfate.*
CAL	PSA Calibrators 2-5	4 X 1.7 mL	*Non-infectious PSA nucleic acid in phosphate buffered solution containing <5% lithium lauryl sulfate.*
PC	PSA Positive Controls	2 X 1.7 mL	*Non-infectious PSA nucleic acid in phosphate buffered solution containing <5% lithium lauryl sulfate.*

APTIMA® Assay Fluids - Storage at 15°C to 30°C (2 boxes)

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

Note: All of the materials included in the PROGENSA PCA3/PSA Assay Kit may also be purchased separately (see the *Materials* section for details).

Materials

Note: Materials available from Gen-Probe have catalog numbers listed.

Materials Required But Not Provided

PROGENSA® PCA3 Urine Specimen Transport Kit (Cat. No. 302352)

GEN-PROBE® LEADER® HC+ Luminometer (Cat. No. 104747)

GEN-PROBE® Target Capture System (TCS) (Cat. No. 104555)

APTIMA® Auto Detect Kit (Cat. No. 301048)

2 eppendorf Repeater Plus Pipettors (Cat. No. 105725)

Repeat pipettor tips (2.5 mL, 5.0 mL, 25.0 mL)

Either:

2 Multi-tube vortex mixers (Cat. No. 102160F)

3 Circulating water baths (62°C ± 1°C, 42°C ± 1°C, 62°C ± 1°C) (Cat. No. 104586F)

3 Water bath spacers (Cat. No. 104627)

Or:

2 SB100® Dry Heat Bath/Vortexers (Cat. No. 105524F) (Additional SB100 instruments may be required depending on required throughput)

Micropipettor, 1000 µL RAININ PR1000 (Cat. No. 901715)

Tips, 1000 µL P1000 (Cat. No. 105049)

Pipettor, eppendorf 20 to 200 µL (Cat. No. 105726)

Tips, Pipette 20 to 200 µL

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) (Cat. No. TU0022)

Ten Tip Cassettes (TTC) (Cat. No. 104578)

SysCheck calibration standard (Cat. No. 301078)

Optional Materials

PROGENSA® PCA3 100-Reaction Kit (Cat. No. 302354)

PROGENSA® PSA 100-Reaction Kit (Cat. No. 302357)

PROGENSA® PCA3 Calibrators and Controls Kit (Cat. No. 302353)

PROGENSA® PSA Calibrators and Controls Kit (Cat. No. 302356)

PROGENSA® PCA3/PSA Proficiency Panels (Cat. No. 302350)

PROGENSA® PCA3 Specimen Diluent Kit (Cat. No. 302351)

APTIMA® Assay Fluids Kit (Cat. No. 302002C)

TECAN Freedom EVO 100/4 (Cat. No. 900932)

PCA3 Deck Plate assembly, DTS® 800 (Cat. No. 902021)

Reagent reservoir (40 mL quarter module) (Cat. No. 104765)

Split reagent reservoir (19 mL x 2 quarter module) (Cat. No. 901172)

Transport tubes (Cat. No. 302521)

Disposable pipet tips with filter (1 mL) (Tecan No. 10612513)

Replacement penetrable caps (Cat. No. 302520)

Replacement non-penetrable caps (Cat. No. 103036A)

Warnings and Precautions

A. For *in vitro* diagnostic use.

B. For U.S. export only.

Laboratory Related

C. Use only supplied or specified disposable laboratory ware.

D. 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.

E. **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 the affected area with water. If these fluids spill, dilute the spill with water before wiping it dry.

F. 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 (bleach solution) (see *Procedural Notes*).

G. 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.

H. 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.

Specimen Related

I. After urine has been added, the liquid level in the urine specimen transport tube must initially fall between the two black indicator lines on the tube label. Otherwise, the specimen must be rejected.

J. 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.

K. 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.

L. 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.

M. 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.

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

Assay Related

O. Do not use this kit after its expiration date. **Do not interchange, mix, or combine reagents from kits with different lot numbers.**

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* and *Procedural Notes* for specific instructions.

Q. For assay deactivation (see *Test Procedure*), the minimum bleach concentration must be 2.6% (0.35 M) sodium hypochlorite **after** 1:1 dilution with deactivation buffer. Therefore, the starting bleach 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. Adequate mixing is necessary to achieve accurate assay results. For complete details, see *Procedural Notes*.

U. Separate water baths must be dedicated for the pre-amplification, amplification, and post-amplification steps in the assay.

V. Some reagents of this kit are labeled with risk and safety symbols according to the European Directive 1999/45/EC and should be handled accordingly. Material Safety Data Sheets can be viewed at www.gen-probe.com 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 4 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 Gen-Probe Technical Support before use.

H. 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 reagents from kits with different lot numbers.** 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 mRNA 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 mRNA in urine and processed urine was established by monitoring mRNA copy levels in urine specimens collected per the instructions below.

A. Instructions for urine specimen collection and processing:

1. It may be helpful to request that the patient consume a large volume of water (approximately 500 mL) to ensure sufficient urine for collection.
2. Conduct a DRE as described below immediately prior to urine collection:

   Apply pressure on the prostate, enough to depress the surface approximately 1 cm, 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.**

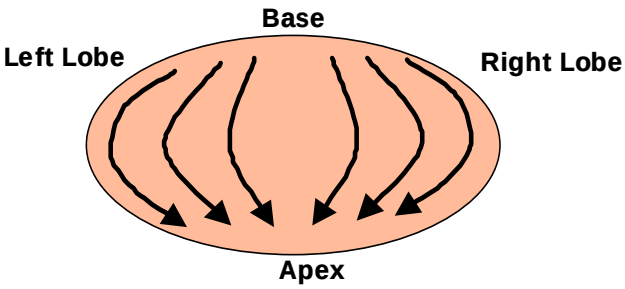

Figure 1. Proper Direction of Applied Prostate Pressure

3. 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. 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.
4. **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. Do not freeze unprocessed urine specimens.**
5. 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.
6. 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:

1. Processed urine specimens must be transported to the laboratory in the urine specimen transport tube at or below 30°C (may be 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 may store specimens at 2°C to 8°C for up to 14 days before disposal is required. If longer time periods are needed, specimens can be stored at or below -20°C for up to 90 days. 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:	
At or below 30°C	up to 5 days*
After receipt at testing site:	
2°C to 8°C	up to 14 days
-20°C or below	up to 90 days

*Time allowed for shipment at 30°C or below.

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: Follow International Air Transportation Association (IATA) PI650 requirements for packaging when urine specimens are transported by common land and air carriers.

Test Procedure

A. Work Area Preparation

1. Adjust one water bath to 62°C ± 1°C for pre-amplification, a second water bath to 42°C ± 1°C for amplification, and a third water bath to 62°C ± 1°C for post-amplification. Ensure water baths contain sufficient water (see *Procedural Notes*). If using the SB100 Dry Heat Bath/Vortexer, refer to the *SB100 Dry Heat Bath/Vortexer Application Sheet for the PROGENSA PCA3 Assay* (*SB100 application sheet*).
2. 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 (bleach solution). Allow bleach solution to contact surfaces and pipettors for at least 1 minute and then follow with a water rinse. Do not allow the bleach solution to dry. Cover the bench surface on which the reaction will be performed with clean, plastic-backed absorbent laboratory bench covers.
3. 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.

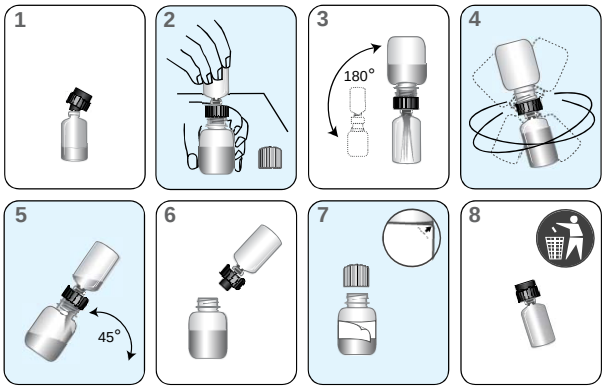

Figure 2. Reconstitution Process

   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 (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 and peel away and discard the top label (Figure 2, Step 7). On the remaining bottle label, record operator initials, reconstitution date, and lyophilized reagent lot number 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).

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

2. Previously reconstituted Probe, Amplification, and Enzyme 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 (c/mL)	*Target PSA Concentration (c/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 c/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 assigned values will be provided on a card in the package of calibrator and control vials and are used for calibration and determination of run validity.

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 *Quick Reference Guide for PROGENSA PCA3 Assay* (*Quick Reference Guide*) or *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 GEN-PROBE Worklist Editor on a computer located in the pre-amplification area. For use of the Worklist Editor, refer to the *Quick Reference Guide* or *GEN-PROBE 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. If using the SB100 Dry Heat Bath/Vortexer, 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. Thoroughly mix by swirling or inversion the Target Capture Reagent (TCR). Using the repeat pipettor, add 100 µL of the analyte-specific TCR to the appropriate reaction tube.
2. Pierce the cap of the Calibrator vial with the micropipettor and add 400 µL of the Calibrator to the properly labeled reaction tube. Using the same pipette tip, withdraw replicate additions from the vial through the pierced cap. 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.
3. Cover the TTUs with the sealing card(s) and shake the rack gently by hand. **Do not vortex.** Incubate the rack at 62°C ± 1°C in a water bath for 30 ± 5 minutes.
4. Remove the rack from the water bath and blot bottoms of tubes dry on absorbent material.
5. Ensure the sealing cards are firmly seated. If necessary, replace with new sealing cards and seal the TTUs tightly.
6. Vortex the rack for 60 seconds on the multi-tube vortex mixer (see *Procedural Notes*). Begin vortexing within 2 minutes of removal of rack from water bath.
7. Without removing sealing cards, incubate the rack at room temperature for 30 ± 5 minutes.
8. Place the rack with the Front tab forward on the TCS magnetic base for 5 to 10 minutes. Load the TTC rack with TTCs.
9. 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.
10. 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 Gen-Probe Technical Support for further information.
11. Firmly attach the aspiration manifold to the first set of tips. Aspirate all liquid by lowering the tips into the first TTU until the tips come into brief contact with the bottoms of the tubes. Do not hold the tips in contact with the bottoms of the tubes.
12. 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.
13. 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.
14. Cover tubes with a sealing card and remove the rack from the TCS. Vortex once on the multi-tube vortex mixer. See *Procedural Notes* for more information.
15. Place rack on the TCS magnetic base for 5 to 10 minutes.
16. Aspirate all liquid as in Steps 11 and 12.
17. 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

Note: Enzyme addition to a reaction rack (Steps 6 and 7 below) must be performed in 90 seconds or less.

Perform Steps 6 and 7 on one rack before repeating on the second rack. If using the SB100 Dry Heat Bath/Vortexer, 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 now be red in color.
2. Using the repeat pipettor, add 200 µL of Oil Reagent.
3. Cover the tubes with a sealing card and vortex on the multi-tube vortex mixer.
4. Incubate the rack in the pre-amp water bath at 62°C ± 1°C for 10 ± 5 minutes.
5. Transfer the rack into a water bath at 42°C ± 1°C for 5 ± 2 minutes.
6. With the rack in the water bath, carefully remove the sealing card and, using the repeat pipettor, add 25 µL of the reconstituted Enzyme Reagent to each of the reaction mixtures. All reactions should now be orange in color.
7. Immediately cover the tubes with a fresh sealing card, remove from the water bath, and quickly mix the reactions by gently shaking the rack by hand.

    Note: Minimize the time the rack is outside the water bath to prevent the tubes from cooling.
8. Incubate the rack at 42°C ± 1°C for 60 ± 5 minutes.

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 using the SB100 Dry Heat Bath/Vortexer, refer to the S*B100 application sheet.*

1. Hybridization
    a. Remove the rack from the pre-amplification water bath and transfer to the post-amplification area. Add 100 µL of the reconstituted analyte-specific Probe Reagent, using the repeat pipettor. All reaction mixtures should now be yellow in color.
    b. Cover tubes with a sealing card and vortex for 10 seconds, or until the color is uniform, on the multi-tube vortex mixer.
    c. Incubate the rack in a 62°C ± 1°C water bath for 20 ± 5 minutes.
    d. Remove the rack from the water bath and incubate at room temperature for 5 ± 1 minutes.
2. Selection
    a. Using the repeat pipettor, add 250 µL of Selection Reagent to each tube. All reactions should now be pink in color.
    b. Cover tubes with a sealing card, vortex for 10 seconds, or until the color is uniform, and incubate the rack in a water bath at 62°C ± 1°C for 10 ± 1 minutes.
    c. Remove the rack from the water bath. Incubate the rack at room temperature for 15 ± 3 minutes.

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 *Quick Reference Guide* or *PROGENSA PCA3 Assay Software System Administrator's Manual and Operator's Manual*.

1. Prepare the LEADER HC+ Luminometer by placing one empty TTU in cassette position number 1 and perform the WASH protocol once.
2. Ensure there are sufficient volumes of Auto Detect 1 and 2 to complete the reactions.
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 on **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 step incubation.
5. Prepare a buffered bleach deactivation solution by mixing equal volumes of 5.25% (0.7 M) sodium hypochlorite and Buffer for Deactivation Fluid into a large-capped plastic container. Label and write the expiration date on the plastic container. This buffered bleach deactivation solution 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 with the buffered bleach solution. 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, 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. Therefore, it is imperative that the water baths be maintained within their specified temperature ranges.
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 *Test Procedure*.

F. Vortexing

Proper vortexing is important to the successful performance of the PROGENSA PCA3 Assay. To vortex reactions, set the multi-tube vortex mixer speed to the lowest setting, secure the rack, and turn on power. Slowly increase speed until the liquid goes halfway up the tube. Vortex for 10 seconds, the indicated amount of time, or

until the color is uniform. Turn speed to lowest setting before turning off the multi-tube vortex mixer and removing the rack. The reaction mixtures should never touch the sealing cards.

G. Water Baths

1. The level of the water in the water baths must be maintained 3.8 to 5.0 cm (1.5 to 2.0 in.) deep as measured from the supporting metal tray (on the bottom of the water bath) to the surface of the water. This will ensure proper heat transfer.
2. To avoid cross-contamination, water baths should be dedicated to a specific assay step.

H. 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 (bleach solution). Allow bleach solution to contact surfaces for at least 1 minute and then follow with a water rinse. **Do not allow the bleach solution to dry.** Chlorine solutions may pit equipment and metal. Thoroughly rinse bleached 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 any Wash Solution remaining in the dispense station priming trough.

   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% (0.7M to 1.0M) sodium hypochlorite solution to the 4 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 at least 15 minutes and then dispose of the contents (waste).

   g. Rinse the waste bottle with water to remove any waste remaining inside.

   h. Cap the empty waste bottle and place it in the vacuum trap enclosure. Attach the quick disconnect fittings 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 (bleach solution). Follow the bleach solution step with a water rinse and then dry the surfaces completely with paper towels.

5. Racks

   Submerge the racks in 2.5% to 3.5% (0.35 M to 0.5 M) sodium hypochlorite (bleach solution), ensuring that they are covered by the bleach 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.

I. 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 with buffered bleach as described in the *Test Procedure*. Do not reuse the TTUs.
3. Perform regular decontamination of equipment and work surfaces as described above 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 PCA3 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 c/mL difference between the replicates exceeds 600%. Testing of the specimen for that analyte must be repeated.

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 (3). 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 *Quick Reference Guide* or *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 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 mRNA copies to PSA mRNA copies, multiplied by 1000. 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 cutoff, the result should be interpreted as NEGATIVE. If the PCA3 Score is above or equal to the cutoff, the result should be interpreted as POSITIVE. The laboratory director will establish the cutoff (see *Performance Characteristics* for more information).

Under some conditions, a PCA3 Score range (>[Calculated Score] or <[Calculated Score]) is provided. If <[Calculated Score] is below the cutoff, the result should be interpreted as NEGATIVE. If >[Calculated Score] is above the cutoff, the result should be interpreted as POSITIVE. If a numerical value is required, specimen dilution and retesting may generate a PCA3 Score instead of a PCA3 Score range (see *Retesting - Dilution of Out-of-Range High Specimens*).

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 lower case for PCA3 results and upper case 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 *Quick Reference Guide* or *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 4a or Table 4b to determine if further action is necessary.

Note: The presence of a status or analysis code does not automatically mean that retesting is required.

3. Interpreting the Results in the Exceptions Summary

   The Exceptions Summary may not list any specimens. 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 4a 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 4a: 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 and manually match results.
Invalid (a, b, e, h, or i)	In range (no code)	--	Yes	Retest PCA3 and manually match results.
In range (no code)	Out-of-range high (F)	<[Calculated Score]**	Optional	1. Manually match to get <[Calculated Score] OR 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 Score]	Optional	1. Manually match to get >[Calculated Score] OR 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 Score]	No	Manually match to get <[Calculated Score].
Out-of-range low (g)	Out-of-range high (F)	<[Calculated Score]	No	Manually match to get <[Calculated Score].

*Applies only to invalid specimens within a valid run.
**For out-of-range values, the Calculated 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 4b for instructions.

Table 4b: 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.

*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**.

   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). Refer to the PROGENSA PCA3 Specimen Diluent Kit package insert. 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).

Limitations

A. The PROGENSA PCA3 Assay should not be used for patients who are taking medications known to affect serum PSA levels such as finasteride (Proscar, Propecia), dutasteride (Avodart), and anti-androgen therapy (Lupron). The effect of these medications on PCA3 gene expression has not yet been evaluated.

B. 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 PCA3 testing should be collected when the clinician believes prostate tissue has recovered.

C. 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.

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. For detailed information, refer to the package insert provided in the PROGENSA PCA3 Urine Specimen Transport Kit.

F. Results from the PROGENSA PCA3 Assay should be interpreted in conjunction with other laboratory and clinical data available to the clinician. (Test results may be affected by improper specimen collection, technical error, or specimen mix-up.)

Performance Characteristics

A. Clinical Results

1. Diagnostic Sensitivity and Specificity

Performance characteristics for the PROGENSA PCA3 Assay were established using specimens from subjects enrolled at four geographically diverse North American clinical sites. The subject population consisted of 529 men scheduled for prostate biopsy. The subject demographics are shown below:

a. Average Age ± SD = 64 ± 8 years (median 63, range 32 to 89)

b. Average serum PSA level = 7.9 ± 21.9 µg/L (5.6, 0.3 to 484)

c. Average prostate volume (determined by trans-rectal ultrasound) = 44 ± 25 cc (39, 5 to 225)

d. 34% (180/529) biopsy-positive for prostate cancer

Figure 3 shows the correlation of PCA3 Score with the probability of positive biopsy. As the PCA3 Score increased, the occurrence of cancer-positive biopsy in subjects increased.

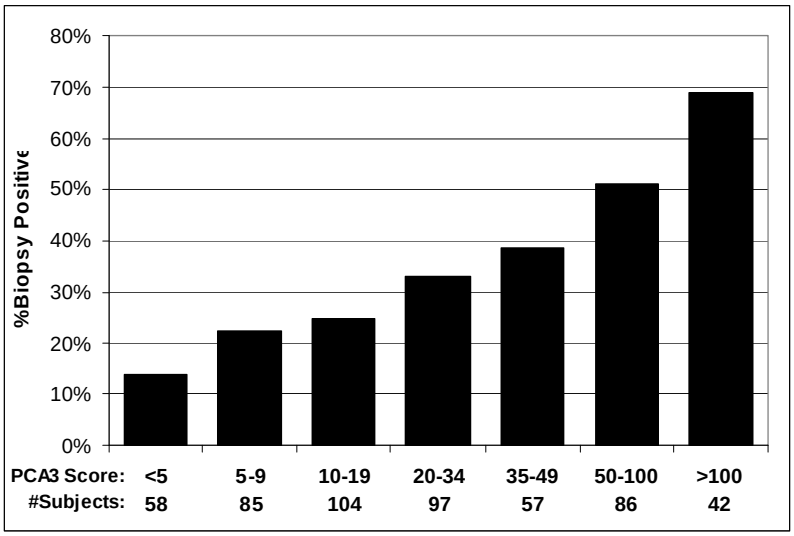

Figure 3. Correlation of PCA3 Score with Probability of Positive Biopsy

Receiver operating characteristic (ROC) analysis was performed, using prostate biopsy as the reference method, according to CLSI GP10-A (1995) (4). For the PROGENSA PCA3 Assay, the area under the curve (AUC) was 0.685 (95% confidence interval = 0.637 to 0.733). Table 5 shows diagnostic sensitivity and specificity at different PCA3 Score cutoff values. Each laboratory should establish the cutoff for diagnostic sensitivity or specificity (see *Interpretation of Results*).

Table 5: PROGENSA PCA3 Assay Diagnostic Sensitivity and Specificity at Different PCA3 Score Cutoffs

PCA3 Score Cutoff	5	10	15	25	35	50	95
Sensitivity	96%	85%	77%	63%	53%	41%	17%
Specificity	14%	33%	47%	61%	74%	84%	95%

2. Specimen Stability Studies

a. Stability in whole urine: First catch urine was collected from 10 subjects and stored at 2°C to 8°C or at 30°C prior to processing by addition to urine transport medium (UTM). At 2°C to 8°C, significant PCA3 and PSA mRNA degradation was observed in some specimens after 4 hours. Thus, whole urine must be processed within 4 hours. At 30°C, significant degradation was observed in less than 1 hour. Therefore, whole urine must always be refrigerated or kept on ice before processing.

b. Stability in processed urine: Twelve specimens were incubated at 4°C or 30°C for up to 38 days. At 4°C, PCA3 and PSA mRNA were stable for 21 days; at 30°C, 5 days. Specimens stored at -20°C and -70°C have demonstrated PCA3 and PSA mRNA stability for up to 90 days.

c. Freeze-thaw stability: Specimens were cycled between 37°C and -70°C 6 times. No decrease in PCA3 or PSA mRNA copy levels was observed.

B. Analytical Results

1. Analytical Sensitivity

An analytical sensitivity panel comprised of diluted *in vitro* mRNA transcript was used to evaluate assay sensitivity. One operator tested the panel in twelve runs of five replicates, using a single reagent lot. The limit of detection and limit of quantitation were calculated according to CLSI EP17-A (2004) (5). The limit of detection of the PCA3 analyte was 80 c/mL, and for the PSA analyte it was 1,438 c/mL. The limit of quantitation of both analytes was Calibrator 2.

2. Analytical Specificity

a. Unspliced transcript: The assay was designed to detect only the prostate cancer-specific exon 3-exon 4 spliced PCA3 mRNA (2). The assay did not detect 1 million c/mL of unspliced PCA3 mRNA significantly above background.

b. Prostate specificity of PCA3 mRNA in urine: Specimens from post-radical prostatectomy subjects (n = 97) were tested with the PROGENSA PCA3 Assay, and PCA3 mRNA levels were compared to those from pre-biopsy subjects (n = 464). The median PCA3 mRNA c/mL was below the assay limit of detection for post-prostatectomy subject specimens, while the median PCA3 mRNA c/mL for pre-biopsy subject specimens was 7,243 c/mL; these data confirm that PCA3 mRNA in urine is from the prostate.

c. Tissue specificity: Total RNA was extracted from tissues of two unique male donors per tissue type, added to specimen diluent (10 ng per reaction), and tested with the PROGENSA PCA3 Assay. Prostate tissue was the only type detected above the PCA3 mRNA limit of detection of the tissue types listed in Table 6.

Table 6: Male Tissue Types Tested for PCA3 mRNA

Tissue Type	
Bladder (Normal)	Kidney
Bladder (Tumor)	Penis
Bone marrow	Prostate
Ductus deferens	Seminal vesicle
Epididymus	Testis

d. Interfering substances: The substances listed in Table 7 were added to aliquots of pooled processed male urine. The specimens were tested with the PROGENSA PCA3 Assay according to CLSI EP7-A2 (2005) (6). At the concentrations listed, no assay interference was observed.

Table 7: Substances Tested for PROGENSA PCA3 Assay Interference

Therapeutic Agents		Therapeutic Agents, continued	
Substance	**Test Concentration**	**Substance**	**Test Concentration**
Acetaminophen/Codeine	5.34 µmol/L	Uroxatral	30 mg/L
Atorvastatin	25 mg/L	Doxazosin	1.33 µmol/L
Lisinopril	0.74 µmol/L	Terazosin	7.8 µmol/L
Amlodipine	245 µmol/L	Finasteride	15 mg/L
Atenolol	37.6 µmol/L	Tamsulosin	1.2 µg/L
Sulfasalazine	754 µmol/L	Metformin	310 µmol/L
Esomeprazole	120 mg/L	Sildenafil	12.9 pmol/L
Allopurinol	294 µmol/L	Saw palmetto	1600 mg/L
Diphenhydramine	19.6 µmol/L	Selenium	0.275 mg/L
Acetaminophen	1324 µmol/L		
Acetylsalicylic acid	3.62 mmol/L	**Urine Constituents**	
Ibuprofen	2425 µmol/L	**Substance**	**Test Concentration**
Furosemide	181 µmol/L	Uric acid	1.4 mmol/L
Ciprofloxacin	30.2 µmol/L	Hemoglobin	2 g/L
Levaquin	48.6 µmol/L	White blood cells	4.56×10^7 cells/L
Doxycycline	67.5 µmol/L	Red blood cells	3.06×10^7 cells/L
Fluoxetine hydrochloride	11.2 µmol/L	Albumin	50 g/L
Flutamide	1500 mg/L	Bilirubin (unconjugated)	342 g/L
Dutasteride	1.5 mg/L	IgG	60 g/L

3. Accuracy

Accuracy of the PROGENSA PCA3 Assay was assessed according to CLSI EP15-A2 (2005) (7). PCA3 and PSA mRNA transcripts were quantified by UV-vis spectrophotometry, added to processed normal female urine (no detectable PCA3 or PSA mRNA), and concentrations measured in the PROGENSA PCA3 Assay. Percent (%) recovery was calculated as a ratio of measured c/mL to added c/mL, multiplied by 100.

Table 8: Copy Recovery of the PROGENSA PCA3 Assay

Analyte	Known Concentration, c/mL	Measured Concentration, c/mL	% Recovery
PCA3	750	808	108%
	7,500	7,618	102%
	18,750	18,722	100%
	75,000	70,287	94%
PSA	20,000	23,684	118%
	250,000	278,373	111%
	500,000	599,941	120%
	1,750,000	1,960,775	112%

4. Linearity and Range

The linear range of the PROGENSA PCA3 Assay was determined according to CLSI EP6-A (2003) (8) based on linear regression analysis (least squares). Two sets of dilution series were prepared from specimens containing high concentrations of PCA3 and PSA mRNA. One set was diluted into processed female urine and one set was diluted into specimen diluent. Dilutions spanned the entire assay range between the lowest and highest positive calibrators for each analyte. For both PCA3 and PSA analytes, assay-measured results showed a direct proportional relationship between the dilutions tested and the analyte c/mL reported. There was no significant diluent matrix effect. See Figure 4.

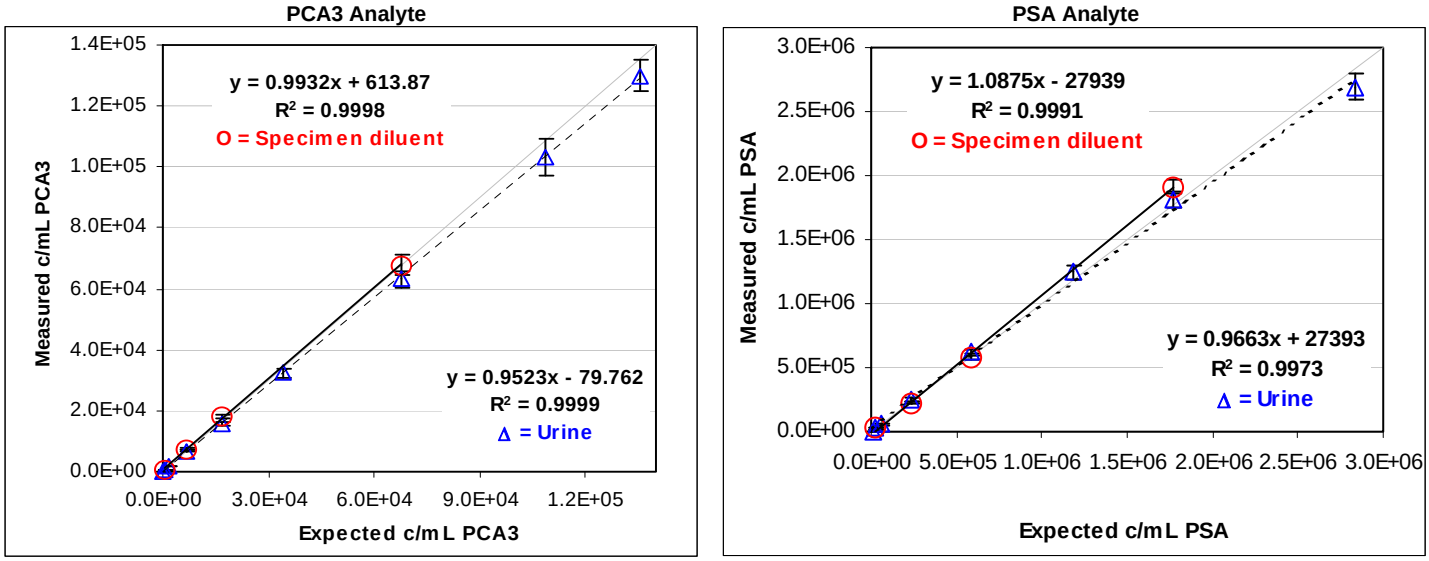

Figure 4. PROGENSA PCA3 Assay Linearity for PCA3 and PSA Analytes

5. Precision

Assay precision was assessed according to CLSI EP5-A2 (2004) (9). Repeatability is precision under conditions of minimum variability, and reproducibility is precision under conditions of maximum variability.

For repeatability, a 3-member test panel comprised of diluted *in vitro* mRNA transcript was prepared. One operator at one site tested the panel in 20 runs of 5 replicates over 20 days, using a single calibrator and control lot, reagent lot, and equipment set. Table 9 shows the repeatability precision of the PROGENSA PCA3 Assay at different test concentration levels.

Table 9: PROGENSA PCA3 Assay Repeatability

Analyte	Panel Member	Average c/mL	Repeatability SD	Repeatability CV
PCA3	1	1,228	145	12%
	2	12,020	809	7%
	3	61,108	2,489	4%
PSA	1	48,091	3,715	8%
	2	484,457	41,026	8%
	3	2,001,430	131,554	7%

For reproducibility, an 8-member test panel comprised of pooled specimens (1 to 3) and diluted *in vitro* mRNA transcript (4 to 8) was prepared. Three operators tested the panel in 18 runs over 3 days, using a single calibrator and control lot, 3 reagent lots, and 3 equipment sets. Tables 10 and 11 summarize total, intra-run, and inter-run, operator, equipment, and lot precision of the PROGENSA PCA3 Assay for analyte c/mL and for PCA3 Score.

Intra-run, inter-operator, and inter-run variability were, in descending order, the largest contributors to overall assay variance. Reagent lot and equipment showed little contribution to overall assay variance. These results demonstrate that the assay performs reproducibly, and the primary source of variation is random error (intra-run).

Table 10: PROGENSA PCA3 Assay Reproducibility: Copy/mL Analysis

Analyte	Panel Member	n	Measured c/mL	Total CV	Intra-Run CV	Inter-Run CV	CV, Inter-Operator	CV, Inter-Equipment	CV, Inter-Lot
PCA3	1	36	248	27%	24%	7%	15%	11%	0%
	2	36	7,021	11%	6%	9%	9%	0%	0%
	3	36	31,469	8%	6%	5%	9%	0%	4%
	4	36	1,469	15%	13%	7%	6%	0%	1%
	5	36	14,844	7%	5%	2%	6%	0%	4%
	6	36	72,372	7%	4%	6%	0%	1%	0%
	7	36	430	26%	26%	0%	11%	0%	1%
	8	36	62,274	13%	8%	8%	3%	0%	5%
PSA	1	34	52,739	9%	6%	6%	7%	4%	2%
	2	34	218,789	10%	6%	7%	7%	4%	0%
	3	32	1,073,920	11%	4%	6%	9%	8%	0%
	4	34	37,185	9%	5%	7%	3%	0%	1%
	5	32	386,504	10%	4%	8%	6%	3%	4%
	6	34	1,518,748	12%	5%	8%	4%	3%	7%
	7	32	11,007	14%	8%	9%	0%	6%	0%
	8	34	1,694,404	11%	7%	7%	0%	1%	6%

Table 11: PROGENSA PCA3 Assay Reproducibility: PCA3 Score Analysis

Panel Member*	n	Mean Score	Total CV	Intra-Run CV	Inter-Run CV	CV, Inter-Operator	CV, Inter-Equipment	CV, Inter-Lot
1	34	5	27%	26%	5%	23%	8%	0%
2	34	32	14%	9%	10%	12%	0%	2%
3	32	30	12%	7%	5%	17%	7%	6%
7	32	39	28%	24%	2%	8%	11%	7%
8	34	37	21%	14%	12%	0%	0%	9%

*Panel members 4 to 6 contained only PCA3 or PSA mRNA transcript and therefore were not included in this analysis.

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