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EDRN PCA3 Validation Trial and Urinary Reference Set

Version 2.0

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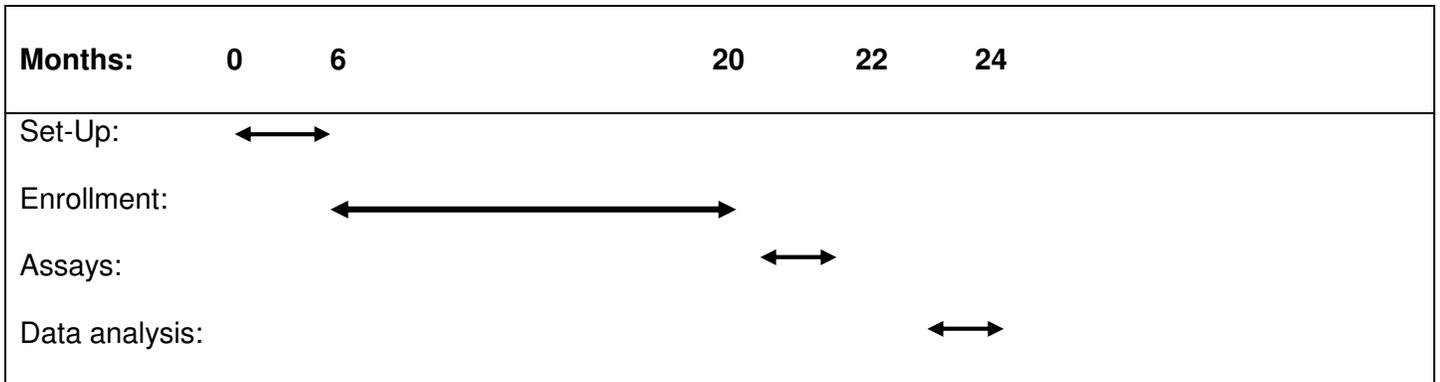
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STUDY SUMMARY

Prostate cancer (PCa) is the third leading cause of cancer-related deaths among United States men, accounting for 33% of such diagnosed cancers.(1) An estimated 186,320 new cases of PCa were diagnosed in 2006, with an associated mortality rate of 28,660.(15,16) Unfortunately the current screening tools, PSA and related tests, have limited ability to detect PCa. In fact, the Prostate Cancer Prevention Trial detected PCa in 6.6%, 10.1%, 17%, 23.9% and 26.9% of subjects with “normal” PSA values of <0.5, 0.6-1.0, 1.1-2.0, 2.1-3.0 and 3.1-4.0 ng/ml respectively. Since the majority of men between the ages of 45 and 75 had PSA values of <4.0 ng/ml, it has been suggested that 15% of high-grade cancer cases, were missed due by PSA-only evaluation.(30,33)

This study has two primary aims -- to provide necessary data to validate a promising new urinary biomarker for prostate cancer, PCA3 and to create a novel urinary reference set for future validations.

STUDY SCHEMA



Set up: Finalize protocol, finalize data elements, create data collection forms (as needed), create study database, modify specimen tracking (as needed), develop Manual of Operations, train sites on protocol and data entry, establish MTAs and obtain IRB approval (months 0-6).

Enrollment: Enrollment will begin once the set-up is complete. The enrollment period will last for 14 months (months 6-20).

Assays: Johns Hopkins Biomarker Reference Lab will perform analysis on all eligible samples and Gen-probe will analyze 10% for quality control. (months 20-22) or periodically throughout the duration of the study.

Data Analysis: The EDRN DMCC will perform statistical analysis (months 22-24)

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1 OBJECTIVES

Independent of serum PSA level, PCA3 score will refine the risk of having cancer detected on an extended template prostate biopsy.

Primary Specific Aims

Aim 1: To evaluate the positive predictive value (PPV) of PCA3 for initial biopsy population and negative predictive value (NPV) of PCA3 for repeat biopsy population in a multi-center prostate biopsy cohort of men without prior history of prostate cancer.

Aim 2: To develop a novel urinary reference set consisting of whole urine /urine sediment as well as plasma/serum necessary for future pre-validation studies of urinary biomarkers.

Secondary Aims

Aim 1: To evaluate the sensitivity, specificity, PPV, NPV and absolute risk prediction by PCA3 alone and multiplexed with other biomarkers and clinical variables in the detection of prostate cancer overall and in the detection of high grade cancer

Aim 2: To evaluate the correlation between PCA3 and prostate biopsy tumor grade

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2 BACKGROUND AND SIGNIFICANCE

2.1 Epidemiology

This study has two primary aims -- to provide necessary data to validate a promising new urinary biomarker for prostate cancer, PCA3 and to create a novel urinary reference set for future validations.

Prostate cancer (PCa) is the third leading cause of cancer-related deaths among United States men, accounting for 33% of such diagnosed cancers.(1) An estimated 186,320 new cases of PCa were diagnosed in 2006, with an associated mortality rate of 28,660.(15,16) Unfortunately the currently used screening tools, PSA and related tests, have limited ability to detect PCa. In fact, the Prostate Cancer Prevention Trial detected PCa in 6.6%, 10.1%, 17%, 23.9% and 26.9% of subjects with "normal" PSA values of <0.5, 0.6-1.0, 1.1-2.0, 2.1-3.0 and 3.1-4.0 ng/ml respectively. Since the majority of men between the ages of 45 and 75 had PSA values of <4.0 ng/ml, it has been suggested that 15% of high-grade cancer cases, were missed due by PSA-only evaluation.(30,33)

Even though other potentially useful biomarkers for the early detection of prostate cancer (PCa), have been described, few make it past the initial discovery phases and fewer yet are ever translated into a clinical assay. One of the most promising biomarkers for PCa is PCA3, aka DD3, a PCa-specific biomarker(4) initially reported by Bussemaker et al. in 1997.(2) In a seminal study, overexpression of DD3 was observed in 94% of PCa tissues studied but not detected in any normal or benign prostatic hyperplasia (BPH) tissues.(3,9,17,41) PCA3 is expressed in the urine and prostatic fluids of individuals with PCa. Assays used to detect PCA3 rely on urine produced following an attentive digital rectal exam (DRE) which improves the secretion of prostatic cells into the urethra.(34)

A simple and precise molecular urine PCA3 assay has been described by Groskopf et al with a ROC of 0.746. The sensitivity and specificity for predicting PCa on biopsy were 69% and 79%, respectively. In the same sample, specificity of PSA was only 28%.(8) Other studies have shown that urine and prostatic fluid PCA3 assays produce comparable results.(37) In the same study, the informative rate for the PCA3 assay improved from 74.4% to 96.7% with an attentive DRE.(32) Three multi-center studies have evaluated the diagnostic ability of urine PCA3 compared to a biopsy;(7,21,40) diagnostic accuracies ranged from 66-81%(7,21,39) and were superior to that of PSA ($p < 0.05$). (7,21) A median PCA3 of 73 was associated with positive biopsy.(38) In addition, PCA3 scores correlated with risk of PCa detected on biopsy such that a PCA3 value > 100 was associated with a 50% chance of a positive PCa diagnosis.(21) The PCA3 assay was found to be significantly higher among more aggressive, higher grade and larger tumors relative to more indolent or lower grade tumors.(24) Though these study findings are promising, they typically come from small trials of men presenting for an initial prostate biopsy and lacked the rigorous standards for tissue storage, handling, mailing and preparation necessary for a biomarker validation study. Moreover, they often failed to evaluate PPV and NPV which accounts for the prevalence of the cancer in the clinical population for which the test will eventually be administered.

In spite of the interest and investment into other promising urinary biomarkers, biosamples appropriate for validation beyond the initial discovery is currently a major limitation for funded investigators. Availability of a carefully annotated tissue bank with urinary and serum specimen resources will provide a novel resource for future biomarker validation studies. These include but are not limited to the gene fusion product, TMPRSS2:ERG(10,18), GSTP1, SPINK1 and EPCA-2 (Chinnaiyan, Getzenberg). A theoretical advantage of urinary biomarkers over tissue biomarkers is that urinary biomarkers are not dependent on needle ascertainment, which may or may not adequately sample the diseased tissue particularly among low volume cancers. By developing a comprehensive urinary and blood tissue resource in this application, it is likely that we would greatly foreshorten the validation path

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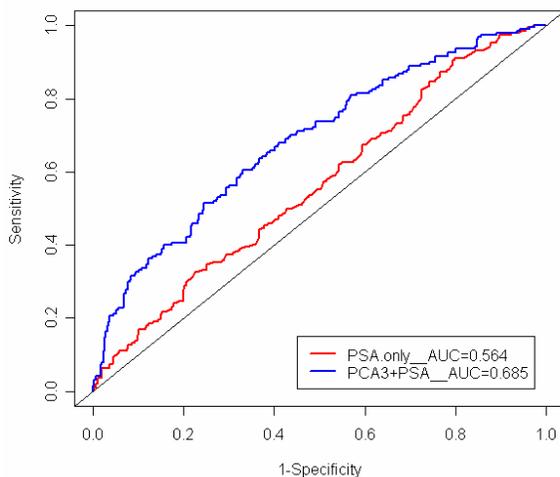
of future urinary biomarkers such as TMPRSS2:ERG. Moreover, this approach will allow for the comparison of existing and potential biomarkers, thus greatly adding to the value of this trial.

2.2 Preliminary Data

Two sources of data provide early evidence of the utility of PCA3 in detecting PCa. Deras et al. analyzed data on 190 PCa cases and 346 controls in a logistic regression.(6) Both PCA3 ($p<0.0001$) and history of at least one biopsy ($p<0.0001$) but not the interaction of these two variables predicted PCa detection. The PCA3 ROC did not differ in ability to detect PCA amongst first, repeat and all biopsies. Likewise, predictability curves were similar in shape amongst the three biopsy groups. The only noted difference was at the beginning of the predictability curve where PCa prevalence was higher for first biopsy.

A second sample from the University of Michigan (Wang 2009) echoed that of Deras et al.(5) and provided additional support for the use of PCA3 as well as multiplex biomarkers in the detection of PCa. Biopsies were indicated based on PSA scores for 88.2% of the sample (mean PSA was $9.6\pm 12.6\text{ng/ml}$). Fifty-eight (42.6%) men had biopsies positive for PCa. Using a PCA3 cutoff of 35, this test was significantly able to detect PCa ($p=0.005$), had a sensitivity of 52%, specificity of 77%, positive predictive value (PPV) of 63% and a negative predictive value (NPV) of 68%. Not surprisingly, increases in PCA3 values were associated with greater PCa detection. While 40% of PCa cases were found using a PCA3 range of 5-19, 70% were detected using a cutoff value of 100. As with the Gen-Probe data, based on PCA3, PCa prevalence was higher in men without previous biopsy although the ROC curves were similar in shape for those with and without history of biopsy. This would suggest that PCA3 may be useful for men with prior negative prostate biopsies, a clinical situation where PSA has little diagnostic value. Therefore, verification of the diagnostic value for PCA3 in a repeat prostate biopsy setting is clinically relevant and warranted. Moreover, the EDRN serum reference set consists only of men with initial prostate biopsies so we currently lack any tissue banks (blood or urine) to evaluate biomarkers for a repeat biopsy scenario.

Figure 1



While critics of PCA3 would suggest that PCA3 is not the end-all of prostate cancer biomarkers, it does incrementally add to our clinical ability to stratify risk for prostate cancer. In Figure 1, the EDRN DMCC compared the AUC for PSA alone and PCA3 in addition to PSA using raw data from a Gen-Probe multicenter study ($n=536$) and demonstrate that the addition of PCA3 to PSA alone increased the AUC of prostate cancer detection by 12%.

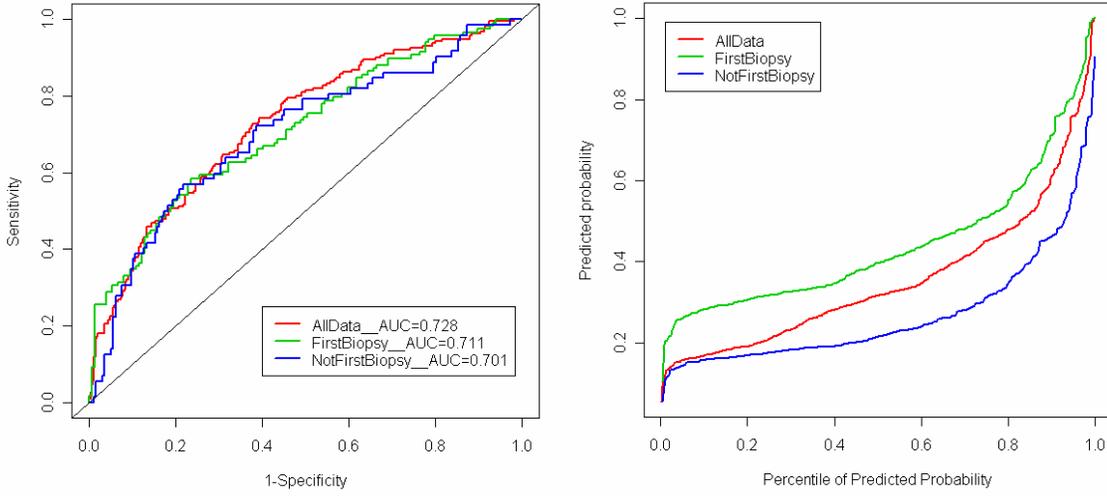
Further analysis of the same Gen-Probe preliminary data ($n=536$) by EDRN DMCC found that using a $\text{PPV}=0.75$ as desirable threshold for recommending biopsy and $\text{NPV}=0.85$ for recommending no biopsy, the cutoff point is 60 for $\text{PPV}=0.75$ for initial biopsy population and is 20 for $\text{NPV}=0.85$ for repeat biopsy population. Due to the substantial differences in PCa prevalence between these two population (44% for

first biopsy population and 27% for repeat biopsy population), almost all PCA3 predicted high risk men are in the initial biopsy group while almost all PCA3 predicted low risk men are in the repeat biopsy group (right panel of Figure 2). This observation forms the basis of the study design. Figure 2 indicated that for the two target populations PCA3 has very similar ROC curves (left panel) and the predicted

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risk curves are largely parallel (right panel). The risk distribution plotted against PCA3 on the right panel of Figure 2 also demonstrates the value of PCA3 as risk stratifying markers.

Figure 2



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3 ELIGIBILITY CRITERIA

3.1 Overview

The Early Detection Research Network (EDRN) is a National Cancer Institute (NCI) funded multi-center collaborative research group. Resources include a biomarker development laboratory at the University of Michigan, biomarker reference laboratories and clinical epidemiological validation centers at the University of Michigan, Beth Israel Deaconess-Harvard University, University of Texas at San Antonio and Johns Hopkins University (see Appendix 6 for list of participating sites). Objectives of the EDRN include development and independent validation of promising biomarkers and technologies for early cancer detection and collaboration amongst academic and industrial leaders in molecular biology, molecular genetics, clinical oncology, computer science, public health and clinical application for early cancer detection. This study will fully utilize the EDRN infrastructure to validate a promising new urinary biomarker for prostate cancer, PCA3 and to create a novel urinary reference set for future validations.

Total number of 850 men will be recruited into the study

3.2 Inclusion Criteria

- Males greater than or equal to age 18
- Patient scheduled for prostate biopsy (at least 10 cores and extended template) for any of the following reasons:
 - PSA >2.0 ng/ml
 - Elevated PSA velocity (>0.4 ng/ml/yr)
 - Lower PSA value with other risk factors for PCa (e.g. family history)
 - Prior ASAP or HGPIN
 - Abnormal DRE
 - % free PSA <15%
- NOTE: finasteride/dutasteride and other medications for BPH and ED are allowable but will be recorded

3.3 Exclusion Criteria

- History of PCa
- Participating in an intervention trial for prostate disease
- Prior surgical (open or endoscopic) or minimally invasive treatment for BPH (e.g., TUNA/TUMT)
- Prior saturation biopsy defined as at least 30 cores
- Prior prostate biopsy within 6 months (180 days) of consent date
- Prior PCA3 test performed for clinical purpose

3.4 Exposure Variables

- PCA3
- Clinical factors (eg, age, race, PSA, number prior biopsies, ASAP, HGPIN, prostate size, prior prostate disease, family history)
- Other biomarkers (eg from multiplex profile, ERG2, etc.)

3.5 Outcome Variables

- Positive and negative predictive values, ROC curve.

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- Detection of cancer
- Detection of aggressive cancer (see analyses section for definition)

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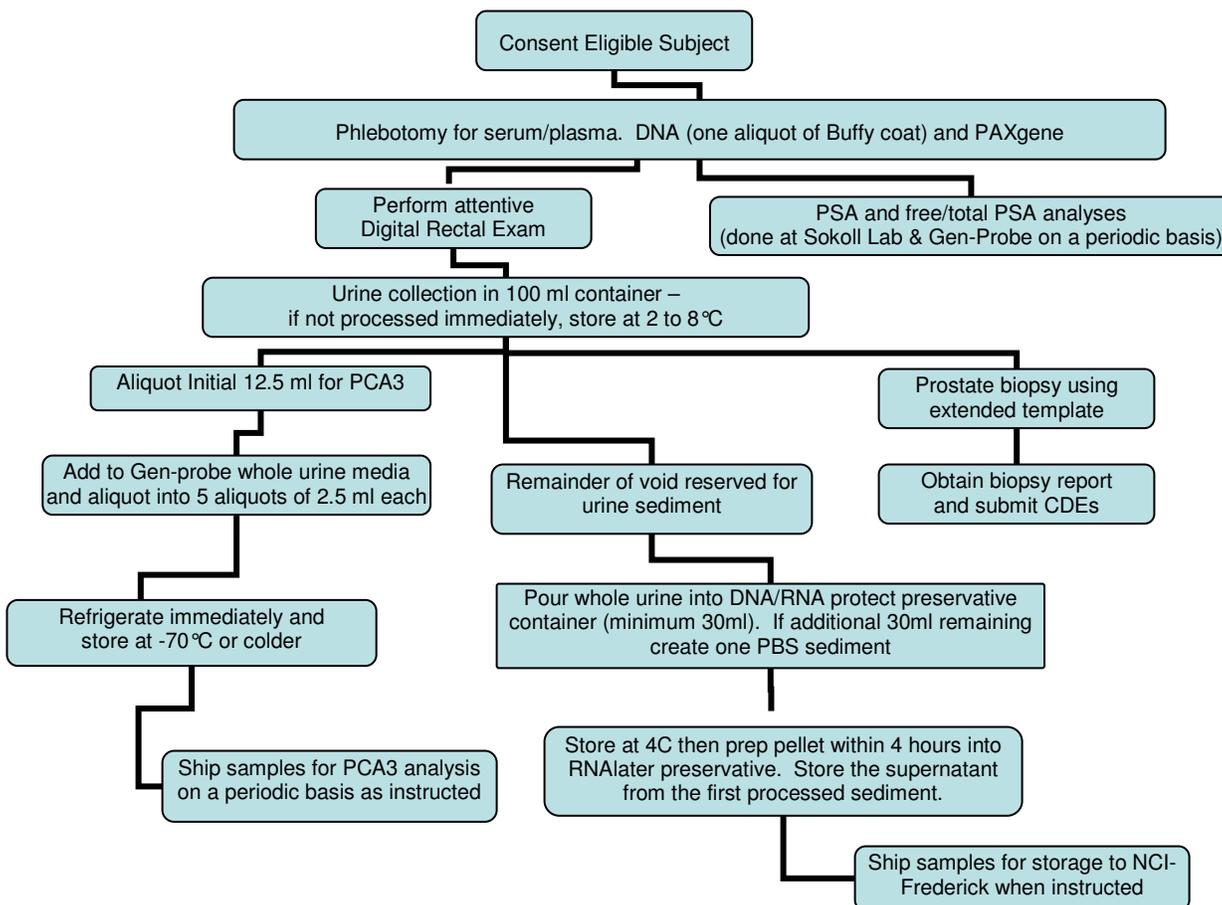
4 SPECIMENS

If the two (2) specimen types (blood and urine) are collected on the same day, then they must be processed in the following order: Eligibility/Consent, Pre-DRE blood, DRE, Post-DRE urine, Prostate biopsy.

If procured on different days, the order for blood and urine collection does not matter. But **there is a limited 90 day window between consent and prostate biopsy - both blood and urine must be procured in that window and prior to the prostate biopsy.** If another DRE is performed on the same day as blood collection then that blood sample also needs to be Pre-DRE. Information about specimen collection (Pre-DRE and Post-DRE) is captured in VSIMS.

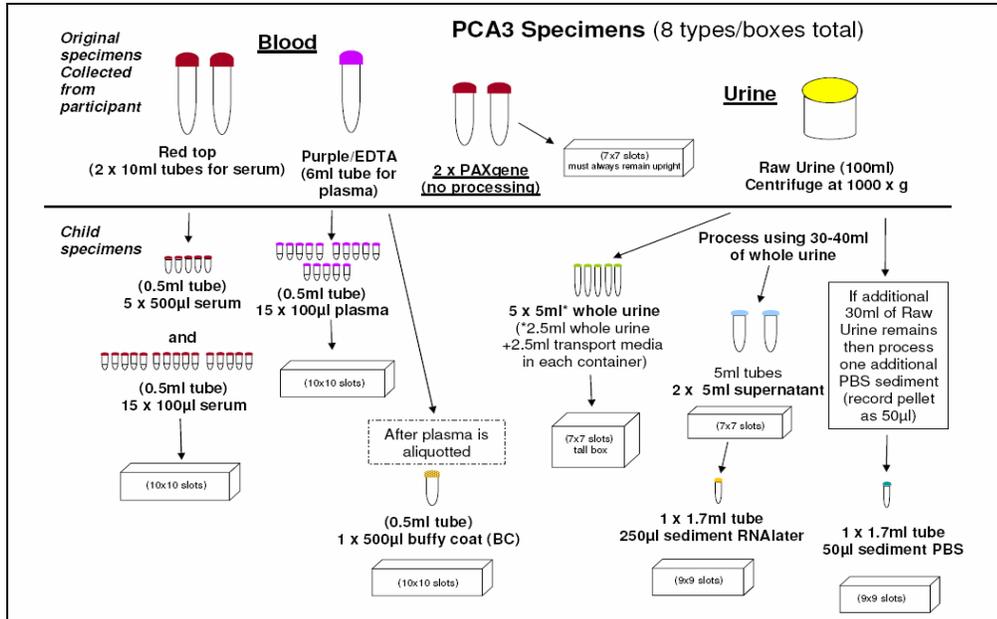
1. Pre-DRE blood. Which can be collected up to 3 months (90 days) before index biopsy.
2. Post-DRE urine. Which can be collected up to 3 month (90 days) before index biopsy

Figure 3: Flow chart for specimen procurement



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Figure 4: Flow chart for specimen processing



NOTE: on a periodic basis the DMCC will randomly select one whole urine and one 500µl serum to be sent to Sokoll lab and 10% to Gen-Probe for QC.

4.1 Blood

Up to 3 months (90 days) before the index biopsy, blood will be collected per the site-specific routine for phlebotomy. Serum total PSA (allows us to calculate PSA density) and free-PSA test will periodically be measured at the reference lab only for the purpose of the research using the same company (e.g. Beckman Coulter). **See Appendices 2 - 3 for details of collection and processing of serum, plasma, DNA (one Buffy Coat) and PAXgene tubes which has been adapted from current EDRN Prostate Reference Set study.**

Do not collect blood (or urine) from a patient who has evidence of urinary tract infection (treated or untreated) within the past 2 days.

Do not collect blood (or urine) from a patient who has had a cystoscopy or catheter within the past 2 days.

All blood collection supplies will be supplied by Michigan. PAXgene tubes are sent directly from SourceMDx to the sites.

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4.2 Urine

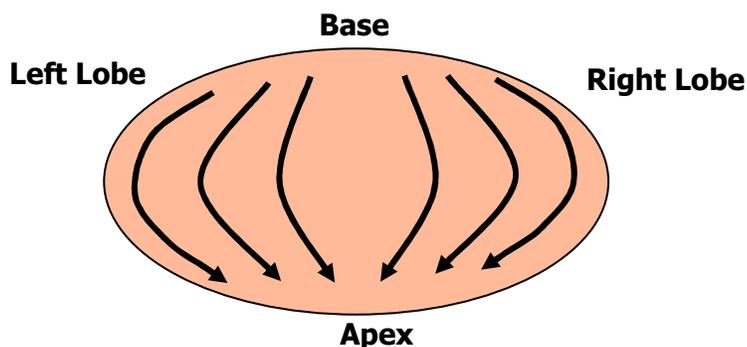
4.2.1 Urine Specimen Collection and Digital Rectal Examination (DRE) Procedures:

Do not collect urine (or blood) from a patient who has evidence of urinary tract infection (treated or untreated) within the past 2 days.

Do not collect urine (or blood) from a patient who has had a cystoscopy or catheter within the past 2 days.

- Step 1. Up to 3 months (90 days) before the index prostate biopsy, a post-DRE urine specimen will be collected. The following materials for urine sample collection are needed:
- 1- 100 mL uncoated plastic urine collection container (supplied by Michigan)
 - Transfer pipettes (provided by Gen-Probe)
 - Gen-Probe Urine Specimen Transport Tubes (provided by Gen-Probe)
 - 1 Set of Urine Specimen ID labels (provided by DMCC)
- Step 2. **Attentive DRE Procedure:**
The urine sample will be collected following an attentive DRE. The clinician will perform an attentive DRE as indicated below and document any suspicious nodules or other abnormalities (Figure 5). Apply firm pressure on the prostate from the base to the apex and from the lateral to the median line of each lobe as shown below. Apply enough pressure to slightly depress the prostate surface. Perform exactly three strokes per lobe.

Figure 5: Attentive Digital Rectal Exam of the Prostate



- Step 3. **Whole Urine Procurement Procedure:**
Following the DRE, the subject will collect up to 100 mL of urine in a 100 mL urine collection cup labeled with provided Specimen IDs. Men should be instructed to fill the urine cup starting with the first drop of urine until the cup is full or their bladder is empty. If the subject is unable to provide this quantity, collect at least 12.5 mL. Record the time and volume of urine collection. A sterile technique is not required for the procurement of the urine specimens.
- The first 12.5 mL of urine will be prepped in Gen-Probe Urine Specimen Transport Tubes for PCA3 (see step 4 below). If less than 12.5 mL is procured, then the entire specimen will be prepped in Gen-Probe Specimen Transport Tubes.

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- The remainder of the voided urine will be prepped for urine sediment isolation (see Section 4.2.1.1)

Step 4. In order to test the urine sample with the PCA3 Assay, the Initial Voided sample must be processed with the Gen-Probe Urine Specimen Transport Tubes. If not processed immediately, the urine sample should be placed promptly on ice or at 4°C and refrigerated for no longer than four hours.

Step 5. Instructions for processing urine:

1. Invert Initial Voided urine sample (in urine collection cup) 5 times to resuspend cells. Do not shake as it will cause foam and bubbles.
2. Using the transfer pipette, transfer 2.5 mL of urine to an appropriately labeled Gen-Probe Urine Specimen Transport Tube. The correct volume of urine has been added to the transport tube when the fluid level is between the black fill lines.
3. Screw on the transfer tube cap tightly then invert the transport tube 5 times to mix.
4. Four (4) additional aliquots of processed urine specimens will be made by following the same procedures 1 through 3 above, volume permitting. There should be a total of 5 processed urine specimens. Label the specimens with the provided Specimen Labels. All processed urine specimens should be frozen at -70°C or colder following processing/labeling.
5. Enter information into VSIMS Specimen Tracking System

4.2.1.1 Urine Sediment

After the whole urine has been processed for the PCA3 assay as described above, the remainder will be used for urine sediment processing (if there is < 30 ml of urine after whole urine procurement (Step 5), then urinary sediment processing will not be possible).

Urine samples should be placed on ice or at 4°C immediately and refrigerated for no longer than 4 hours if not processed sooner.

- Step 1. Invert the urine collection cup 5 times to resuspend the cells.
- Step 2. Depending on the remaining volume of urine **after PCA3 aliquoted**, follow the following plan for creating urinary sediment:
- Step 3. If there is 60-90 ml – divide sample into two equivalent samples, one for RNA later prep and the second one for a PBS pellet
- Step 4. If there is 30< 60 ml then create just one sample 30-40 ml for RNA later sample (PBS pellet will not be created).
- Step 5. If there is <30 ml, then no urine sediment will be processed

4.2.1.2 RNA later sediment

- Step 1. Transfer 30-40 mL urine to a 50 mL conical and process as described below.
- Step 2. Centrifuge: 10 min, 1000 x g, at 4°C. Do not use brakes.
- Step 3. Remove supernatant, taking care not to disturb the pellet
- Step 4. Decant the supernatant into separate tubes as individual 10 ml aliquots. Label with provided Specimen IDs.

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- Step 5. Add 5 mL ice cold PBS (PBS must be free of calcium and Magnesium Chloride) to the remaining pellet
- Step 6. Re-suspend pellet thoroughly by pipetting up and down with a sterile pipet.
- Step 7. Transfer suspension to a 15 mL conical tube.
- Step 8. Centrifuge: 10 min, 1000 x g at 4°C. Do not use brakes.
- Step 9. Remove and discard this supernatant, taking care not to disturb the pellet
- Step 10. Add 1 mL ice cold PBS.
- Step 11. Re-suspend pellet thoroughly by pipetting up and down with a sterile pipet.
- Step 12. Transfer suspension to 1.7 mL labeled eppendorf tube.
- Step 13. Microfuge: 10 min, 700 x g at 4°C.
- Step 14. Decant.
- Step 15. Resuspend (vortex) pellet in 250 microliters of RNAlater. Store at 4°C for 24 hours. After 24 hours, transfer the sediment tube to -70°C or colder in appropriate freezer box until shipping. NOTE: If specimen is collected on a Friday, the sample may be stored for up to 72 hours.
- Step 16. Label specimen with provided Specimen IDs
- Step 17. Enter information into VSIMS Specimen Tracking System

4.2.1.3 PBS sediment –

- Step 1. Transfer at least 30 mL urine to a 50 mL conical and process as described below.
- Step 2. Centrifuge: 10 min, 1000 x g, at 4°C. Do not use brakes.
- Step 3. Decant supernatant, taking care not to disturb the pellet
- Step 4. Add 5 mL ice cold PBS (PBS must be free of calcium and Magnesium Chloride) to the remaining pellet
- Step 5. Re-suspend pellet thoroughly by pipetting up and down with a sterile pipet.
- Step 6. Transfer suspension to a 15 mL conical tube.
- Step 7. Centrifuge: 10 min, 1000 x g at 4°C. Do not use brakes.
- Step 8. Remove and discard this supernatant, taking care not to disturb the pellet
- Step 9. Add 1 mL ice cold PBS.
- Step 10. Transfer suspension to 1.7 mL labeled eppendorf tube.
- Step 11. Microfuge: 10 min, 700 x g at 4°C.
- Step 12. Decant and store at -70°C in appropriate freezer box until shipping.
- Step 13. Label specimen with provided Specimen IDs
- Step 14. Enter information into VSIMS Specimen Tracking System

4.2.2 Urine Specimen Transport and Storage Conditions

Urine samples (in the urine collection cup) must be processed immediately or stored on ice or at 4°C and processed within 4 hours of collection. The processed urine specimens should be frozen at -70°C or colder until shipment.

Frozen processed urine specimens (in the transport tube) may be shipped overnight, on dry ice or with cold packs, to NCI-Frederick. Once received, these specimens must be stored at -70°C or colder.

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Following PCA3 Assay testing, the processed urine specimens may be stored -70°C or colder. Processed urine specimens must be thawed to room temperature before testing and may be subjected to no more than one freeze/thaw cycles.

4.3 Biopsy

All biopsies slides will be primarily reviewed at the clinical site of origin, per usual protocol. 10% of cases will be randomly selected by DMCC for quality assurance. The purpose of this review is to evaluate the consistency of the primary outcome variable. If any of the QA samples show a discrepancy in the diagnosis of cancer (absent/present), then the DQMB will review and decide further action. Neither participants nor their urologists will be informed of the findings from this review since there is not a gold standard to reconcile discordant pathology review results. The outcome of the review is for research quality assurance and investigational purposes only and will not affect the management of the participant's standard of care. Secondly, we will measure other pathologic variables such as Gleason score, # cores involved by cancer, % core involved by cancer for consistency.

An extended pattern as defined by the National Comprehensive Cancer Network (NCCN) will be utilized. Briefly, this consists of a sextant template, with at least six additional cores from the lateral peripheral zone and also biopsies directed to lesions found on palpation or imaging or transition zone, as may be clinically indicated(22). All biopsies will be performed with ultrasound guidance and prostate size will be measured with height, width, length and total volume. DRE results (most recent) will be documented at or within 6 months (180 days) prior to the biopsy date and hypoechoic lesions will be documented at the time of the study biopsy.

4.3.1 Timing of Prostate Biopsy

Up to 3 month (90 days) after Consent, a prostate biopsy will be performed according to the procedure described in Section 4.3. above.

4.4 Specimen Tracking

The Specimen Tracking component of VSIMS will be used to track the creation, shipping and receiving of specimens for the study. The VSIMS Specimen Tracking System utilizes 2-D bar coding technology. Each specimen tube is labeled with a barcode label with a unique specimen identification number. The shipping site scans the participant ID and the specimen ID assigned to the specimen and the system automatically enters the date, time and shipping site. The shipping site then prints the packing list to send with the specimen shipment. The receiving site then pulls up the shipment by packing list number and scans all specimens into the system. If the condition of a specimen that is received is not "normal" it will be indicated in the system. The Sites/Sokoll Lab will receive a Batch List identifying what samples are to be analyzed for PCA3 and what sample is selected for QC (10% at Gen-Probe)

The barcode labels are produced at the DMCC using a thermal printer and 2-D barcode technology. This allows a small label to be produced that can contain a lot of information. The labels have been tested to adhere in -80°C freezers.

4.4.1 Identification and shipping of specimens

Samples will be shipped to NCI-Frederick on a regular basis. NCI-Frederick will be periodically notified to ship samples to Johns Hopkins (Sokoll Lab) and Gen-Probe. NCI-Frederick will immediately receive the PAXgene tubes and re-ship to SourceMDx at the end of the study. Samples are to be shipped to:

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Christine Demasco-Cody
Fisher BioServices
4600 Wedgewood Blvd, Suite H
Frederick, MD 21703
301-694-5911
christine.demasco@thermofisher.com

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5 DATA MANAGEMENT, COLLECTION AND QUALITY CONTROL

5.1 Data Management

This study will be coordinated the EDRN Data Management and Coordinating Center (DMCC) and will utilize the EDRN's Validation Study Information Management System (VSIMS) for data management and specimen tracking as VSIMS is now used for all prospective validation studies in EDRN. All study data will be entered into VSIMS that has been customized for the PCA3 Validation Study. VSIMS is a secure, web-based system that includes the main components needed for a multi-site validation study. The major components of the system include:

- 1) data submission via online data entry
- 2) confirm eligibility function
- 3) specimen tracking
- 4) study reports
- 5) issue tracking and communication
- 6) data transfer
- 7) study contact information and protocol

There are multiple levels of security that make VSIMS a secure system, such as 128-bit encryption for all data transfers and for all users of VSIMS be authenticated prior to entry. Each VSIMS user is required to complete an access application for each protocol in order to be provided a user account and protocol-specific access. Additional security measures include audit and event logs, connection time-outs and deactivation of inactive accounts.

Ideally, data will be directly entered into VSIMS and data collection forms will not be used. This should be possible for data abstracted from medical records, radiology reports, laboratory reports, etc. It may be necessary logistically, to collect some data on forms and then enter them into VSIMS. Data collected on data collection forms will be stored in a secure location. All participants will be assigned a unique participant ID created by the DMCC. Forms will be labeled with the participant's unique participant ID; no other identifier will be put on data collection forms. The participant ID and the specimen ID will be the only identifiers used in the database program and specimen repository. Each specimen aliquot will be labeled with a unique specimen ID that is linked in the database to the participant ID so that specimens, assay results and participant data can be linked. The specimen ID labels will be generated at the DMCC with 2-D barcodes so that IDs can be scanned into VSIMS in order to minimize data entry error.

5.1.1 Clinical Site Responsibilities

Each site must have a designated person responsible for assuring that data and specimens are collected/processed and entered into VSIMS according to the protocol and study's Manual of Operations. Each site must also have a computer with internet connection. Sites are also responsible for notifying the coordinating center when in need of additional supplies. Sites are also responsible for submitting invoices to the University of Michigan for confirmed participants before receiving payment.

5.1.2 DMCC Responsibilities

The EDRN DMCC will serve as the coordinating and data management center for the study. They will be responsible for the following:

- Assist in finalizing the protocol

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- Oversee IRB approvals from sites
- Develop Manual of Operations in conjunction with study PI
- Add study-specific components to VSIMS including: data elements and forms, eligibility confirmation, specimen tracking and study reports
- Establish and maintain database for the study
- Supply participant and specimen ID labels
- Coordinate regular conference calls and site training
- Train sites and conduct periodic Site Monitoring Visits
- Oversee enrollment, data collection and entry, timeliness, accuracy and completeness
- Communicate with sites and labs
- Establish Data Quality and Monitoring Board
- Work with PI and investigators to monitor the study, modify study design when necessary
- Create shipping lists for NCI-Frederick to send samples to various locations (Sokoll Lab, Gen-Probe and SourceMDx)
- Select 10% of the tissue samples for QC
- Analyze data with PI and study investigators and prepare manuscripts for publications

5.1.3 PI Responsibilities

The PI and his team at the University of Michigan will be responsible for the following:

- Supervise conduct of the study in conjunction with DMCC: supervise and coordinate enrollment, sample collection and shipment, hold monthly conference calls with sites, participate in data analysis, report progress to site and NCI, lead the report of study findings

5.2 Data Collection

Men at risk but without any prior history of prostate cancer who are scheduled for a prostate biopsy at a participating clinical site will be identified, screened and consented within 3 months (90 days) prior to their actual biopsy date (see Appendix 1). Initial data is obtained by asking the participant questions or by having the participant complete a self-administered a questionnaire. Additional data is obtained after medical source documents are finalized.

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The following Common Data Elements are being measured – see Appendix 4 for details

Identifiers

- Subject ID
- Date of Consent

Demographic

- Age
- Race/Ethnicity
- Weight/Height

Smoking History

Clinical

- Participation in an intervention trial (note that participation in prostate specific intervention trials is exclusionary)
- Prostate Health History
- Medications
- Prostate Biopsy History and Exam
- Labs (PSA)
- TRUS Procedure Details
- Pathology and TNM Staging
- Prostate Cancer Details
- Non-cancer prostate biopsy pathology details

Specimen Collection and Processing

- Aliquot number
- Time from blood/urine collection until freezer
- Specimen type
- Storage temperature before shipping to NCI facility

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6 STUDY ASSAY

6.1 Assay

PCA3 testing of processed urine specimens will take place at John Hopkins University (Dr Sokoll) and Gen-Probe (Dr. Groskopf) (see Appendix 5). Testing will be performed and analyzed according to the method described by Groskopf(8). The Hopkins EDRN Biomarker Reference Laboratory will assay 100% of the samples and the Gen-Probe laboratory will assay 10% as quality control.

Processed urine specimens will be tested in duplicate at both laboratories with the PCA3 Assay on the Gen-Probe Systems. The calibration curve will be tested in triplicate and the controls will be tested in duplicate. The mRNA results for PCA3 and PSA for each subject must be generated from the same processed urine specimen tube.

Following PCA3 Assay testing, the processed urine specimens should be stored frozen at -70°C or colder.

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7 ACCESS TO SAMPLES

After the completion of the PCA3 validation study all remaining specimens will be available for future research, if the patient consented to have their samples stored. Investigators (both internal to EDRN and external to EDRN) may apply for access to these samples. A specimen approval committee will be established and an application form will be posted on the EDRN public website.

7.1 Specimen Committee

The Specimen Committee is comprised of:

- PI of study John Wei
- Chair GU Collaborative group of EDRN
- Biostatistician: Ziding Feng
- NCI Program Director

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8 DATA ANALYSIS AND STATISTICAL POWER CONSIDERATIONS

8.1 Primary outcome analysis

For the initial biopsy group, we will calculate PPV($PCA3 > 60$) and its 95% confidence interval and test null hypothesis that PPV at this threshold is not significantly larger than 55% (close to prevalence 44%). For the repeat biopsy group, we will calculate NPV($PCA3 < 20$) and its 95% confidence interval and test null hypothesis that NPV at this threshold is not significantly larger than 75% (close to prevalence 73%). Both one-sided tests will use significant $0.05/2=0.025$ to adjust for two tests. The tests and confidence intervals will use exact tests and confidence intervals for binominal proportions.

From Gen-Probe preliminary data, the observed PPV($PCA3 > 60$) is 77% for initial biopsy group and the observed NPV($PCA3 < 20$) is 86% for repeat biopsy group. At PPV=75% or NPV=85% for respective population, the study needs 305 repeat biopsy men (assuming 44% of them with $PCA3 < 20$) and 273 initial biopsy men (assuming 18% of them with $PCA3 > 60$) to achieve 90% power. The observed ratio of the number of initial and repeat biopsy men is 1:1 in Gen-Probe data and is hypothesized to be similar to academic medical centers due to high referral rate of men with prior biopsy history.

There are a number of advantages not to over-sample one group as this study specimen repository will be used for other biomarker and marker panel evaluation for which the required sample size ratio between two groups will vary. Therefore, the minimum total sample size is $n=610$ men who are biopsy candidates.

Since the actual composition of two groups and the prevalence of men with $PCA3 > 60$ or < 20 may differ from Gen-Probe preliminary study, we should inflate the calculated sample size by 20%.

Therefore, the study sample size will be $n=850$.

At the 2009 Houston GU collaborative group meeting, the group voted not to exclude finasteride users because diagnosis value of PCA3 on the subgroup is valuable given the potential increasing use in near future. However, we do not want to compromise the study power for the non-users group of finasteride. Since the finasteride users represent 5-10% of men in this age group and this rate is expected to go up, we will increase the sample size by 100. Therefore, the final study sample size is $n=850$.

In the analysis, we will first compare the distributions of PCA3 in negative biopsy control group for finasteride users and non-users. The primary data analysis is based on the data from the non-users. A secondary analysis will use a logistic regression with finasteride usage as an indicator variable, PCA3 and PCA3 by finasteride use interaction in the model. If the interaction effect is not significant, we will combine two groups and repeat the primary analyses. If the interaction effect is significant, we will perform parallel analyses for finasteride user group with the recognition that the sample size is small and the performance estimates (ROC curve, PPV, NPV) are treated as exploratory.

8.2 Interim analysis

An interim analysis is planned when recruitment reaches 300. If at the interim analysis, the up bound of 95% confidence interval does not reach 75% for PPV for initial biopsy group and 85% for NPV for repeat biopsy group, the PCA3 aim of the study will terminate. If this target is reached for one of the two groups, the PCA3 aim of the study will continue for this group but not for the other. The final analysis for the validation study will adjust for this interim analysis using UMVUE proposed by Pepe et al (Pepe MS, Feng Z, Longton G, Koopmeiners J Conditional estimation of sensitivity and specificity from a phase 2 biomarker study allowing early termination for futility. *Statistics in Medicine* 2009:

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28:762-779)) to maintain correct type I error. The DMCC has conducted a simulation study to evaluate the study power influenced by this interim analysis. The power loss is very small. In particular with the 20% inflation to final sample size $n=850$, the study will have more than 90% power. Regardless of the outcome of the interim analysis, recruitment of 850 participants will proceed to assemble the reference set.

8.3 Evaluation of future biomarker panel

The additional urine and serum collected from this study will be available for validating other biomarker panels for prostate cancer. We assume these candidate markers have passed discovery phase and if it is a biomarker combination, that the algorithm has been finalized for validation. It is difficult to do specific power analysis without knowing performance characteristics but the sample size of 850 is larger than that of the ongoing %proPSA validation study for the same clinical application and we expect we will have more than 90% power for biomarkers with similar performance characteristics as %proPSA or PCA3.

Another utility of this reference set is for combining serum and urine-based biomarkers. In this setting, we assume that the reference set will be used for biomarker discovery and initial pre-validation. We will split the reference to training and test sets. The training set will be used for developing an algorithm to combine serum biomarkers with urine biomarkers. The test set will evaluate the variation of the performance of the algorithm on an independent sample. The definitive validation study will likely to be done using other samples unless the test set ($n=425$) is judged to be adequate for a specific clinical application.

An example of a biomarker that can be combined with PSA and PCA3 is TMPRSS2:ERG (23,29,31,35,36,42), which results from intronic DNA deletions on chromosome 21q22.2-3 between ERG and TMPRSS2.(28,36) These gene fusion products, particularly TMPRSS2:ERG, are detectable in urine of individuals with PCa(19) and commercial assays for this application are under development. Hessels et al. evaluated TMPRSS2:ERG alone and in combination with PCA3 for the early detection of PCa.(11) Post-DRE urine was collected for 78 men with biopsy-diagnosed PCa and 30 without PCa but with abnormal DRE and/or PSA >3 ng/ml. Biomarkers were measured using RT-PCR (PCA3 & TMPRSS2:ERG) followed by Southern blot hybridization (TMPRSS2:ERG). Sensitivities in predicting PCa were 37%, 62% and 73% for TMPRSS2:ERG, PCA3 and the biomarker combination. Thus, addition of the fusion product, TMPRSS2:ERG, to PCA3 appears to improve PCa detection.(12)

8.4 Secondary analyses

PCA3 as a panel member to improve prediction of PCa risk: The preliminary data from Gen-Probe indicates that PCA3 and biopsy history are two most significant predictors ($p < 0.0001$) in a logistic regression model with PCA3, previous negative biopsy, PSA, race, age, DRE and family history of PCa. These variables except PCA3 are used in the PCPT PCa risk calculator. Therefore, with this validation study data, we will confirm whether PCA3 still significantly improves the prediction over variables used in PCPT. Note that the PCPT risk calculator was built from the PCPT cohort that does not represent the biopsy candidate population (many with low PSA).

Since PSA and abnormal DRE often trigger biopsy, we would expect the contribution of PSA and DRE to be much smaller in biopsy candidate population. Since EDNRN just completed a %[-2]proPSA validation study and in this PCA3 validation study we also plan to measure %fPSA and %[-2]proPSA, another secondary analysis will examine whether PCA3 makes independent contribution to diagnosis of prostate cancer in addition to fPSA and proPSA. We will again use a logistic regression model using with all clinical variables above plus fPSA, proPSA and PCA3, then proceed a backward selection to eliminate any biomarkers (PSA, fPSA, proPSA and PCA3) if it does not make a significant independent

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contribution in the model, i.e. p -value < 0.05 for its regression parameter. The clinical variables will always be included in the model as they are easy to collect.

After the model is selected, we will use the linear score of the logistic regression as a “biomarker”, plot its ROC curve and predictiveness curve (PC) (Huang Y, Pepe M, Feng Z. Evaluating the predictiveness of a continuous marker. *Biometrics* 2007;63:1181-1188. PMID: 17489968). PC curve is a plot of the estimated absolute risk as vertical axis against the percentile of the biomarker as x-axis. PPV(v) or NPV(v) could be easily obtained by using the area below the curve from $[v, 1.0]$ for PPV(v) or the area above the curve from $[0, v]$ for NPV(v). We will use v that corresponds to PPV(v)=0.75 for initial biopsy population and NPV(v)=0.85 for repeat biopsy population. The confidence intervals for PPV and NPV at these cutoffs will be calculated by 10,000 bootstrap samples to take into account the variability of selecting cutoff point v . PCA3 would be judged complementary to other markers if at least one of PPV(v) and NPV(v) is better than that by the model from clinical variables plus other biomarkers. To test the statistical significance, the difference of PPV and NPV for two models will be computed from 10,000 bootstrap samples to get a 95% C.I. The improvement would be judged as statistically significant if the low bound of this confidence interval is larger than zero.

PC is mainly used to describe the potential use of the prediction model in making clinical decisions. One can see from PC, for any chosen cutoff point the corresponding absolute risk of positive biopsy and the percent of men who have risk above this cutoff. Both predicted and observed PCs will be plotted to show the goodness of the fit of the PC.

We will repeat the above analyses for predicting high grade prostate cancer ($GS \geq 7$). The preliminary data did not indicate a strong association between biopsy tumor grade and PCA3 although an association was observed between PCA3 and pathologic grade. We will evaluate this again in this study to see if PCA3 alone, or in combination with other clinical variables and biomarkers, will improve the prediction of high grade (Gleason Score 7 or above) and high volume PCa. All pathology reports will be obtained and measures of tumor volume.

Definitions of Low, High and Intermediate risk based on needle biopsy:

Patients with AJCC clinical T stage T1c, T2a and PSA level of 10 ng/mL or less and biopsy Gleason score of 6 or less will be defined to have low risk.

Patients with AJCC stage T2c disease or a PSA level of more than 20 ng/mL or a biopsy Gleason score of 8 or more will be defined to have high risk.

Patients with PSA levels higher than 10 and 20 ng/mL or lower, a biopsy Gleason score of 7, or AJCC clinical stage T2b will be defined to have an intermediate risk.

In order to see if PCA3 and biomarker panel has differential diagnostic values across three groups, we will plot ROC curves for PCA3 and the panels of biomarkers from the analyses described above for each of three risk groups of prostate cancer patients with negative biopsy group as controls. If the performance on high risk prostate cancer group is better than that for low risk prostate cancer group, then the test has more clinical utility in selecting prostate cancer patients of high risk of progression or recurrence.

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9 ETHICAL AND REGULATORY CONSIDERATIONS

9.1 Data Quality Monitoring Plan

A Data Quality and Monitoring Board (DQMB) will be established and meet approximately every 6 months (180 days). This board will include the principal investigator, the PI of the DMCC of the EDRN, the Chair of the EDRN GU committee and an external member (Dr. Jorge Marrero at University of Michigan). Issues regarding quality of the data, accrual progress and other matters related to the implementation of the trial will be discussed.

Adverse events are only reported to the sites local IRB. If questions regarding adverse events arise during the course of the study then the DQMB may formally review.

9.2 Demographics

The patient population under study includes adult males (≥ 18 years old) with a clinical indication for prostate biopsy (e.g. elevated PSA, Abnormal DRE). The proportion of minority patients including African Americans is determined by referral patterns at the participating centers. Collection sites will be enlisted with the goal of approximately 12% enrolled subjects being African Americans. Women and children are not at risk for prostate conditions and are therefore excluded from participation.

9.3 Confidentiality

Patient confidentiality will be maintained to the extent that no publication will be made to include specific patient identifiers. Patient and sample identification must be maintained so that outcome data can be obtained. However, all specimens are coded with a unique research number and date. These codes will only be broken in order to obtain follow-up outcome data on these patients. All information in the pathology database can be retrieved by participant ID number for data correlation with results obtained by investigators.

Patients will be informed that clinical and pathological data including prostate tissue, urine, plasma and serum will be used for the discovery of genes and their products (i.e. proteins) that may help in diagnosing, preventing, or treating prostate cancer. Patients will be informed that these studies will use their pathology and clinical data to help with this discovery process. These data will be maintained in a confidential manner in a centralized database with several levels of password protection. If tissue samples and clinical data are sent to another lab or institution, all identifiers that could trace these samples back to the patient will be removed. Outside investigators will receive the samples with a unique research code for which only they hold the key.

DNA samples, if obtained, will be linked to a database that contains the clinical and pathology data. However, all patient identifiers will be removed before actual analyses are performed. Specifically, the samples will be assigned a unique research ID number that enables the DMCC to link the molecular data to the clinical and pathology databases in a confidential manner. Samples will only be collected from subjects who have signed an informed consent with regards to the use of their clinical data, prostate tissue, DNA, urine and serum/plasma for research purposes. This authorization (or lack of) will be maintained in the database.

To address the issue of tumor burden, we will examine the number of needle cores involved with cancer as an approximation of tumor burden. Minimal tumor burden will be defined as involvement of a single needle core; moderate tumor burden will be defined as 2 cores up to 25% of cores involved; and significant tumor burden will be defined as more than 25% of cores involved.

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9.4 Risk /Benefit

There is minimal risk in drawing blood from a vein including discomfort; possible bruising and swelling around the needle puncture site and rarely an infection. There is also a remote chance of fainting. No known risks are associated with collection of urine samples.

Should the validation study prove positive at the conclusion of this study, i.e. reject the null hypothesis, the PCA3 test results will remain blinded and the results will NOT be shared with the subjects.

In this multi-site observational cohort study, there is NO study-related intervention, and adverse outcomes (eg urinary tract infections, hematuria, rectal bleeding, and death) are expected to occur due to the biopsy procedure, prostate cancer, and/or the subject's own comorbid health conditions. As such, there will not be a requirement to report adverse events to the study or in VSIMS. This should not preclude any clinical site from reporting an event to their local IRB if required at their institution. Any unforeseen issues regarding plausible attribution of adverse events that arise during the course of the study will be discussed by the DQMB, who will formally determine attribution to study protocol and any subsequent need to report to all sites.

There will no cost nor monetary compensation to subjects for their participation in this study.

9.5 Withdrawal

A subject may withdraw consent for the use of their clinical data, tissue, DNA and/ or blood samples from the study at any time. The request should be documented in a letter to the principal investigator at the site, who will be responsible for communicating this information to the DMCC.

9.6 Protection of Human Subjects

The study will be thoroughly explained and informed consent will be obtained at time of consent. If the patients agree to participate, a questionnaire will be administered to collect information on their medical and social history. Study participants will have blood drawn and urine collected for study purposes. The enrollment visit will add about 15 minutes to the normal standard of care. This study does not involve any type of invasive test or therapeutic intervention.

All data collection forms will be kept in a locked location at the study coordinator's site. Access to VSIMS-PCA3, the study data management system, is password protected. Specimens will be uniquely labeled with a Specimen ID, and linked to a unique participant ID. Patient privacy is our utmost concern in this study.

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FINAL

APPENDIX 1 – CONSENT FORM TEMPLATE

IR Number: _____

IRB Consent Approval Date: _____

IRB Project Approval Expiration Date: _____

Institution Name

Consent To Be Part Of A Research Study Called PCA3 Validation Trial and Urinary Reference Set

What is the purpose of this consent form?

This form is called a consent form. The purpose of this form is to let you know about a research study being done here at the cancer center. It tells you about the purpose, risks and benefits and describes what is involved in the study. It also tells you what other choices you have.

Do I have to take part in this study?

No, you do not have to take part in this study. It is up to you. To help you decide if you want to take part in this study, you should:

- Read this form.
- Ask your doctor questions about the study.
- Write down your questions.
- Have your doctor (attending physician) explain anything that you do not understand.
- Discuss it with your family or close friends.
- Take time to think about whether you want to take part in this study.

Research studies only include people who want to take part in the study. Please take time to make your decision. We encourage you to discuss your decision with your doctors, family and friends.

1. General Information about the Study and Researchers

Study title: EDRN PCA3 Validation Trial and Urinary Reference Set

Study sponsor: National Institutes of Health (NIH) (Early Detection Research Network)

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Names, degrees and affiliations of the researchers conducting the study:

Principal Investigator Site John Wei, M.D., M.S. University of Michigan 1500 East Medical Center Dr Room 1013, Women's Trailer Ann Arbor, MI 48109-0362 (734)-615-4628	Local Clinical Investigator PI Name Facility Name Address City, State Zip Phone	Study Coordinating Center Jackie Dahlgren Fred Hutchinson Cancer Research Center 1100 Fairview Avenue N.,M3- A306 P.O. Box 19024 Seattle, WA 98109 206-667-3438
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2. Purpose of the Study

Study purpose:

Prostate cancer is the third leading cause of cancer-related deaths among men in the United States, accounting for 33% of such diagnosed cancers. An estimated 186,320 new cases of prostate cancer were diagnosed in 2006, with an associated mortality rate of 28,660. Unfortunately the current screening tools, such as PSA and related tests, have limited ability to detect prostate cancer. Even though other potentially useful biomarkers for the early detection of prostate cancer have been described, few make it past the initial discovery phases and fewer yet are ever translated into a clinical assay. One of the most promising biomarkers for prostate cancer is PCA3. The Purpose of this study is to show that PCA3 and other biomarkers will improve our ability to quantify the risk of having prostate cancer.

3. Information about Study Subjects

Participating in the study is completely voluntary. You do not have to participate. You may withdraw from the study at any time. You have the right to choose whether or not you want to take part in this research study. Once you have started the study, you may decide to stop taking part in the study at any time. You will not lose any benefits that you already have by leaving the study early.

Who is eligible to take part in the study?

Patients without prostate cancer who are scheduled to undergo a prostate biopsy at one of the participating clinical sites will be eligible to participate in this study. Subjects must be older than or equal to 18 years of age, no matter their ethnic origin.

Note: it is very important for you to give the researchers accurate and complete information about your medical history and condition.

How many people (subjects) are expected to take part in the study?

We estimate that about 850 subjects will participate in this study. An estimated 244 patients will eventually be diagnosed with prostate cancer.

How long will I be asked to participate in the study – and how long will it take to complete the study?

Research data for patients is obtained one time at baseline. Research data will be kept indefinitely.

4. What Happens to Subjects in This Study

What exactly will be done to me if I agree to be a research subject?

If you agree to participate, we will draw 3 tablespoons of your blood, perform a digital exam of your prostate and then obtain a sample of urine for this research study. We will then perform a series of tests on the blood and urine for markers which have been associated with prostate cancer. The tests

FINAL

we will perform on the blood and urine will allow us to determine if these tests can function as markers for early detection of this tumor. The samples of blood and urine obtained from you will form a valuable resource for the discovery and possible validation of new blood and urine tests that may allow us to detect prostate cancer.

We will ask you several questions about your medical history and review your medical records. After the blood test and interview, no additional study data will be collected although your doctor and site investigator may continue to follow you.

If you agree to have your blood and urine samples stored for future research, the samples will be stored indefinitely.

You will not be provided with the results of any tests done on your samples.

Will I receive any other information about my participation in the study?

By signing this consent form, you agree that the results of the marker studies will not be made available to you. If we learn any important new information that might affect your health, welfare, safety, or willingness to stay in the research study, the research staff will tell you. You may be asked to sign another consent form if you wish to stay in the research study at that time.

5. Risks and Potential Benefits of the Study

What risks will I face by participating in the study?

The known or expected risks for people participating in the study include:

The main aspect of this study is drawing blood. Drawing blood may cause temporary discomfort and bruise where the needle enters the vein. A potential risk is a feeling of light-headedness; if this occurs, the patient will be asked to lie down for a few minutes. Blood will be removed only if the patient agrees and if they are getting blood removed as part of their care. Blood will be removed by experienced personnel in the blood drawing station to minimize discomfort.

Another risk is that you may be asked questions that are sensitive in nature. You do not have to answer questions that you are not comfortable answering.

What will be done to reduce or monitor these risks?

The blood draw will be performed by trained individuals in the blood drawing stations at the (INSTITUTION NAME).

What happens if I am hurt or become sick as a result of the study?

The researchers have taken steps to minimize the known or expected risks. However, you may still experience problems or side effects, even when the researchers are careful to avoid them. If you believe that you have been harmed, notify the researchers listed in Section 10 of this form. The (INSTITUTION NAME) will provide first aid or emergency care. The cost of this first aid or emergency care may be billed to your insurance company, but if it is not covered by your insurance, the (INSTITUTION NAME) will pay for it. Additional medical care will be provided if the (INSTITUTION NAME) determines that the injury is caused by the research. If you sign this form, you do not give up your right to seek additional compensation if you are harmed as a result of being in this study.

Please note: It is important that you tell the researchers about any injuries, side effects, or other problems that you experience during this study. You may also need to tell your regular doctors.

Can I expect any benefit for myself or others from participating in the study?

We cannot promise that you will personally receive any benefits from being in this study. Although there is no direct benefit to you, the results of this study may lead to the development of method(s) for

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the early detection of prostate cancer. We will investigate multiple factors that may be involved in the cancer development that could then be applied for the early detection of this tumor.

If I participate in this study, can I also participate in other studies?

Yes, but please do inform us if you decide to participate in another study.

6. Other choices (alternatives to PARTICIPATING IN THE STUDY)

If I decide not to be in this study, what may happen to me or what other choices are there?

Your customary care will be not affected whatsoever. This is not a treatment study.

Please ask the researchers or your doctor about other choices you may have.

7. Ending the Study

If I want to withdraw from the study, what should I do?

You can withdraw from this study at any time without loss of any non-study related benefits to which you would have been entitled before participating in the study. If you want to withdraw, you may do so by notifying the study representative listed in Section 10 of this form.

If I decide to leave the study early, what is likely to happen to me? Are there likely to be any dangers in doing so?

You always have the right to end your participation in research for any reason, any time. There are no dangers from leaving this study early.

8. Financial Information

Will subjects or their insurers be billed for any costs of the study? If so, which and what happens if insurance does not cover the costs?

No. The principal investigator will cover all cost for the blood draws and data collection associated with the research study.

Are subjects paid or given anything for being in the study?

No. There will be no financial gain from participating in this study.

Who has an ownership interest in the study intervention or in the study's sponsors?

The National Institutes of Health has ownership of the samples and clinical data obtained.

9. Confidentiality of Subject Records

(INSTITUTION NAME) policies and certain federal and state laws require that personal health information be kept confidential but allow disclosures in specific situations. You will be informed of these confidentiality policies and laws that apply to this study and you will also be asked to sign an authorization to permit the researchers in this study to obtain access to, use and disclose your personal health information in this study as described in this consent form.

Why would my health information be disclosed?

There are many reasons why your health information may be used or disclosed in the course of this study. For example, the researchers may need the information to verify that you are eligible to participate in the study, or to monitor the results, including side effects. Other university and government officials, safety monitors and study sponsors may need the information to ensure that the study is conducted properly. Also, information may need to be disclosed to insurance companies or others responsible for your medical bills in order to secure payment.

What information will be disclosed?

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If you agree to participate in this study and sign your name on the last page, you will also be given an authorization form to sign. If you agree to sign that authorization form, you will be giving the (INSTITUTION NAME), including its Health System (hospitals, health centers, clinics and health care providers) and other providers involved in your care permission to disclose your medical information (doctors' notes, lab results, x-rays, hospital charts, etc.) to the researchers.

However, your name and other information that would directly identify you will not be used in any publications or presentations resulting from this research study, unless you give us separate written permission.

The researchers may need to use the information collected in this study to create a tissue bank and databank of information about your condition or its treatment. Any information or specimens shared with other investigators and/or institutions will be coded in such a way that your identity will not be disclosed.

How will the researchers protect my privacy?

All the data will be recorded in data sheets and then entered into a password-protected database. We shall put the information collected about you during the study into a research record. This research record will not show your name, but will have codes entered in it, that will allow the information to be linked to you. However, we shall keep your research record confidential, to the extent provided by federal, state and local law. We shall not allow anyone to see your record, other than people who have a right to see it (i.e., principal investigator, co-investigators, etc). You will not be identified in any reports on this study.

Other than the research staff, who might see information about me collected during the study, or other related medical records?

(INSTITUTION NAME) faculty, staff and contractors responsible for oversight of the research: Funding agencies and government officials who oversee research (such as the National Cancer Institute, Federal Office for Human Research Protections, the Institutional review boards here and at Fred Hutchinson Cancer Research Center.

The Early Detection Research Network's Data Management Coordinating Center at the Fred Hutchinson Cancer Research Center in Seattle, WA.

When does my permission expire? What happens to information about me after the study is over?

The authorization form that you sign along with this consent will indicate when your permission will expire. In some cases, you will be asked to grant permission for us to access your medical records for the duration of the study. Your permission for us to retain the data for study purposes does not expire. Even after the study is complete or after you decide to withdraw from the study, information about you may be used or disclosed as follows:

- To preserve the integrity of the other information collected during the study.
- As part of a data set used for research, educational and other lawful activities that does not include your name, social security number, or other identifying information.
- To (INSTITUTION NAME) faculty, staff and agents responsible for oversight of the research.
- As required by applicable federal or state law. For example, if you withdraw from the study at any time, a record of your withdrawal and the reasons you gave for withdrawing will be kept as part of the study record. In addition, government officials who are responsible for oversight and review of clinical trials may require certain disclosures.

In addition, the information may be used to create an anonymous database or repository of information about your blood tests, size of the tumor, tissue testing results, genetic analysis in the future.

FINAL

Several genetic databases are available to help researchers understand different diseases. These databases contain DNA sample information and other information helpful to study diseases. DNA comes from cells in your body and contains all your genetic information. As part of this study we would like to put your genetic information into these databases. Your DNA undergo genome-wide analysis (GWAS) and genotype and phenotype data may be shared for research purposes through the NIH GWAS data repository. Your information may benefit future research. All of your personal information would be removed. Your name, address, etc will not be in the database. Only genetic information and information about your condition will be sent to the database. There is a small risk that your genetic information could be matched against other genetic databases to get your name. Once we release your data to the central database we are no longer in control of the information.

It is important to understand that once your personal health information has been disclosed to the researchers, the protections of federal privacy regulations issued under the Health Insurance Portability and Accountability Act of 1996 ("HIPAA") may no longer apply. However, the researchers are required to maintain the confidentiality of that information. In addition, as long as the information is held in any part of the (INSTITUTION NAME), it is protected by the (INSTITUTION NAME) privacy policies. For more information about these policies, please ask your doctor for a copy of the (INSTITUTION NAME) "Notice of Privacy Practices", or visit our website at (INSTITUTION URL).

FINAL

10. Contact Information

For more information about the study or the study procedures or treatments, or to withdraw from the study, contact:

(LOCAL PI NAME, ADDRESS AND PHONE NUMBER)

(OTHER LOCAL NAME, ADDRESS AND PHONE NUMBER)

To report any illness or injury you experience during the study, contact the researchers listed above and your regular doctor.

For more information about your rights and responsibilities as a research subject, or to express a concern about the study, contact:

(INSTITUTIONAL IRB NAME, ADDRESS AND PHONE NUMBER)

11. Record of Information Provided

Your signature on the next page means that you have received copies of all of the following documents:

- This Informed Consent Document. Note: In addition to the copy you receive, copies of this document will be stored in a separate research file and entered into your regular medical record.
- Other (specify):

12. SIGNATURES

Research Subject:

I understand the information printed on this form and in the attached materials. I have been given copies of all of these. I have discussed this study, its risks and potential benefits and alternatives to participation in the study with _____. My questions so far have been answered. I understand that if I have any additional questions or concerns about the study or my rights as a research subject, I may contact one of the people listed above. I also understand that I will receive a copy of this document at the time I sign it and later upon request.

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In order to direct us on how we may use your blood and urine samples collected for this study and the reference set, please mark one box and initial your choice for each of the 4 questions below.

1. I consent to the use of my samples for this study
 Yes No _Initials:_____
2. I consent to the use of my samples for future prostate cancer research
 Yes No _Initials:_____
3. I consent to the use of my samples for any type of future research
 Yes No _Initials:_____
4. I consent to the authorization of future contact for research purposes
 Yes No _Initials:_____
5. I consent to sending genetic information to one or more databases for future research
 Yes No _Initials:_____

If we want to use your tissue from your biopsy in the future for a purpose not described in this consent, we must first send a request to the Institutional Review Board (IRB) for review and approval for all proposed new research.

Signature of Subject: _____ Date: _____

Name (Print legal name): _____

Patient ID: _____ Date of Birth: _____

If applicable: Name, Address, Telephone and Signature of Person Legally Authorized to Give Consent:

Relationship to Subject:

Parent Spouse Child Sibling Legal Guardian Other:

Principal Investigator (or Designee):

I have given this research subject information on the study that I believe to a reasonable degree of medical certainty is accurate and sufficient for the subject to understand fully the nature, risks and benefits of the study and the rights and responsibilities of a research subject. There has been no coercion or undue influence.

Name: _____ Role on Study: _____

Signature: _____ Date of Signature: _____

FINAL

APPENDIX 2 – BLOOD SPECIMEN COLLECTION

Phlebotomy Materials and Procedure (recommendation from DMCC at FHCR)

Materials:

Gloves, sharps Container, 21- gauge Butterfly needle, attached tubing and Luer adapter, Tourniquet, Antiseptic wipes, bandages, centrifuge as per routine phlebotomy procedures at clinical sites

Collection Procedure:

Do not collect blood (or urine) from a patient who has evidence of urinary tract infection (treated or untreated) within the past 2 days.

Do not collect blood (or urine) from a patient who has had a cystoscopy or catheter within the past 2 days.

See http://www.bd.com/vacutainer/pdfs/blood_collection_tubes_product_insert_VDP40035.pdf

NOTE: Tubes with additives must be thoroughly mixed. Erroneous test results may be obtained when the blood is not thoroughly mixed with the additive.

- Step 1. Assemble the supplies to be used in obtaining the specimen. Do not label the vacutainer tubes until specimen is obtained.
- Step 2. Put on disposable gloves.
- Step 3. The patient should be comfortably seated in a venipuncture chair. The arm should be positioned on a slanting armrest in a straight line from the shoulder to the wrist. The arm should not be bent at the elbow.
- Step 4. Apply a tourniquet 2 inches above the antecubital fossa or above area to be drawn with enough pressure to provide adequate vein visibility. Have the patient form a fist. Select the site for venipuncture.
- Step 5. Clean the forearm of the patient with antiseptic wipe in a circular motion beginning at the insertion site. Allow the antiseptic to dry.
- Step 6. Anchor the vein by placing the thumb 2 inches below the site and pulling the skin taut to prevent the vein from moving. The holding finger is placed below the site, not above, to prevent accidentally sticking the finger with the needle.
- Step 7. Using the dominant hand, insert either the butterfly needle Push the evacuated tube onto Luer adapter
- Step 8. Release the tourniquet once blood flow is established.
- Step 9. Carefully remove the tubes when full without dislodging the needle. The tube will automatically stop filling when the vacuum is gone leaving the tube approximately three-fourths full.
- Step 10. Lightly place a sterile gauze pad over the venipuncture site. Gently remove the needle.
- Step 11. Apply pressure to the site with sterile gauze. Apply bandage. Instruct the patient to leave the bandage on for at least 15 minutes.
- Step 12. Dispose of the needle in a sharps container.
- Step 13. Remove gloves and wash hands.

FINAL

APPENDIX 3 –SPECIMEN SUPPLIES AND BLOOD PROCESSING

Serum, Plasma, Buffy Coat and PAXgene Collection, Processing and Storage

Objective:

To collect biologic samples on men prior to prostate biopsy, in association with clinical information and common data elements appropriate for evaluation of risk and prognosis of prostate cancer. All blood collection supplies are being provided by the University of Michigan (PAXgene tubes are supplied by Source MDx however they are distributed to the sites through University of Michigan).

Materials:

- Serum: Two 10ml red top glass tube, no additive, no clot activator with silicone coated interior, (BD366430).
<http://catalog.bd.com/bdCat/viewProduct.doCustomer?productNumber=366430>
- Plasma-EDTA: One 6ml EDTA plastic tube (367899)
<http://catalog.bd.com/bdCat/viewProduct.doCustomer?productNumber=367899>
- PAXgene: Two tubes
- Aliquot containers for all blood specimens: 0.5ml Polypropylene Micro Tubes, screw top, conical skirted (Sarstedt 72.730)
<http://www.sarstedt.com/php/main.php>
- 2-D barcode labels and worksheets for each subject contributing specimens (provided by the DMCC)

1) Serum Specimens (processing consistent with

http://www.bd.com/vacutainer/pdfs/blood_collection_tubes_product_insert_VDP40035.pdf)

Serum is obtained from whole blood collected in red top vacutainer tubes with no additives or clot activators. The blood specimen should be allowed to clot for minimum of 30 minutes and maximum of 60 minutes at room temperature, stored at 4⁰C for up to 4 hours and then centrifuged 20 minutes at 1300g-force, 4 degrees Celsius. Serum (supernatant post-centrifugation) will then be placed in Five 500µl and Fifteen 100µl aliquots and stored in 0.5 ml aliquots.

The goal is for the serum to be centrifuged and transferred to the Micro Tubes within 4 hours of collection, (however up to 18 hours is acceptable; time at 4⁰C will be recorded as a specimen-specific CDE). Samples are then frozen at –70⁰C or colder until shipping to NCI-Frederick. Hemolyzed serum samples are to be excluded.

2) Plasma and cellular fraction (Buffy Coat) specimens (processing consistent with

http://www.bd.com/vacutainer/pdfs/blood_collection_tubes_product_insert_VDP40035.pdf)

Plasma and cellular fractions are being collected using an EDTA containing tube. Plasma is obtained from whole blood collected in plastic vacutainer tubes containing EDTA from Becton-Dickinson. Tubes are inverted for mixing as per phlebotomy routine and BD instructions. The blood specimen should be placed immediately on ice or 4⁰C and centrifuged 10 minutes at 1500g-force. Plasma should be stored at 4⁰C until aliquotted. Plasma will then be placed in Fifteen 100µl aliquots and stored in 0.5 ml aliquots.

FINAL

The goal is for the plasma to be centrifuged and transferred to the Micro Tubes within 4 hours of collection, however up to 18 hours is acceptable. Plasma samples are then frozen at -70°C or colder until shipping to NCI-Frederick.

After removal of EDTA-plasma do not vortex or mix. Carefully take out buffy coat (between plasma and RBC). Place 100 μl of buffy coat into one 0.5ml tube and then freeze at -70°C or colder and store locally (remaining buffy coat may be kept locally).

3) PAXgene tubes

These tubes will be collected last. Tubes are to be stored between -20 and -80°C at the site and shipped to Source MDx, via NCI-Frederick, within 90 days of collection. PAXgene tubes will be distributed to sites by the University of Michigan. After processing at SourceMDx samples will be sent to NCI Frederick for permanent storage.

4) Sample Aliquots

- a) Sites are being asked to collect a minimum of Five 500 μl and Fifteen 100 μl of serum aliquots to NCI-Frederick. The remaining serum can be kept locally. If a site is unable to provide twenty aliquots, please send what is obtained. Serum is stored in 0.5 ml aliquots.
- b) Sites are being asked to collect a minimum of fifteen 100 μl EDTA plasma aliquots to NCI-Frederick. Any remaining plasma from the EDTA tube can be kept locally. If a site is unable to provide fifteen aliquots, please send what is obtained. Plasma is stored in 0.5 ml aliquots.
- c) Sites are being asked to collect one 100 μl EDTA Buffy Coat to NCI-Frederick. The remaining EDTA Buffy Cells can be kept locally. Buffy coat is stored in 0.5ml tubes.
- d) Sites are being asked to collect two PAXgene tubes and ship both tubes to NCI-Frederick who will then ship the tubes to Source MDx for processing.

Materials for collecting urine sample and labeling the urine specimens: Some of the supplies for processing urine are supplied by Gen-Probe and distributed by University of Michigan.

- 100 mL uncoated plastic urine collection container
- Instruction sheet for urine collection cup
- Transfer pipette for processing whole urine
- Five Gen-Probe/PROGENSA Urine Specimen Transport Tubes for processing whole urine
- Five non-pierceable caps for urine specimen transport tubes
- 1.5 mL Polypropylene screw top cryovial tubes for processing and storing urine sediment
- Two 5ml tubes for storing supernatant
- ID Labels: One set of 2-D barcode labels for labeling all original and child urine specimens provided by the DMCC.
- Urine Specimen Collection Worksheet: contains copies of 2-D barcode labels and information that is necessary to record about urine specimen collection and processing. This worksheet will stay with the specimens until they are put in the freezer, at which point it will be put into the participants study chart. Worksheets provided by the DMCC.

FINAL

APPENDIX 4 – DATA COLLECTION FORMS

Green text indicates Eligible for enrollment

Red text indicates Ineligible for enrollment

** is Primary Key.

* is required field.

PCA3 Consent Documentation (Form ID: 776)
Baseline
PCA3 Validation Study and Urinary Reference Set
EDRN

- **421** EDRN Participant ID (Go To: **423**) _____
- *423** EDRN Protocol ID (Go To: **422**) _____
- *422** EDRN Site ID (Go To: **929**) _____
- *929** EDRN Staff ID (Go To: **1288**) _____
- *1288** Date participant signed consent form (MM/DD/YYYY): _____ (Go To: **2493**)
Month Day Year
- *2493** Has the participant authorized the use of their specimens for this study? (Go To: **End of Form**)
- No** **Yes** (Go To: **2491**)
- *2491** Has the participant authorized the use of their specimens for future prostate cancer research? (Go To: **2492**)
- No Yes
- *2492** Has the participant authorized the use of their specimens for ANY TYPE of future research? (Go To: **2631**)
- No Yes
- *2631** Has the participant authorized future CONTACT for research purposes? (Go To: **2824**)
- No Yes
 Not collected
- *2824** Has the participant authorized sending his or her genetic information to one or more databases for future research? (Go To: **1097**)
- No Yes
 Not collected
- 1097** Comments: (Go To: **End of Form**)

FINAL

Version: 2.0

FINAL

Note:

Click on the DE ID link to see the details of selected DE(s) of interest.

** is a Primary Key and a Required Field.

* is a Required Field.

PCA3 Participant Questionnaire (Form ID: 779)
Baseline
PCA3 Validation Study and Urinary Reference Set
EDRN

- **421** [EDRN Participant ID](#) (Go To: **423**)
- * 423** [EDRN Protocol ID](#) (Go To: **422**)
- * 422** [EDRN Site ID](#) (Go To: **929**)
- * 929** [EDRN Staff ID of the person who collected the data:](#) (Go To: **1219**)
- * 1219** [Date of baseline visit:](#) (MM/DD/YYYY) (Go To: **1293**)
- * 1293** [Are you Hispanic or Latino?](#) (Go To: **1315**)
_0 No _1 Yes
_9 Unknown/refused
- * 1315** [What is your race?](#) (Check all that apply.) (Go To: **1567**)
_1 White _2 Black or African-American
_3 American Indian or Alaska Native _4 Asian
_7 Native Hawaiian or other Pacific Islander _97 Other, specify: (Go To: **1294**)
_99 Unknown/refused
- * 1294** [Race \(Other, specify\)](#) (Go To: **1567**)
- * 1567** [Which race do you consider to be your PRIMARY racial background?](#) (Go To: **1300**)
_1 White _2 Black or African-American
_3 American Indian or Alaska Native _4 Asian
_7 Native Hawaiian or other Pacific Islander _97 Other, specify: (Go To: **1579**)
_99 Unknown/refused
- * 1579** [PRIMARY racial background \(Other, specify\)](#) (Go To: **1300**)

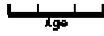
FINAL

* [1300](#) Did you ever smoke cigarettes regularly, at least one a day for a year or more? (Go To: **886**)

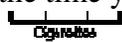
- ₀ No ₁ Yes (Go To: **1299**)
₉ Unknown/refused

* [1299](#) Do you currently smoke cigarettes regularly, at least one a day? (Go To: **1297**)

- ₀ No (Go To: **1298**) ₁ Yes
₉ Unknown/refused

* [1298](#) How old were you when you permanently quit smoking cigarettes? (Go To: **1297**) 

* [1297](#) How old were you when you began smoking cigarettes regularly? (Go To: **1325**) 

* [1325](#) During the time you have smoked, on average, how many cigarettes did you smoke per day? (Go To: **1301**) 

* [1301](#) How many years total have you smoked? (Go To: **886**) 

* [886](#) Have you ever been told by a doctor that you have any of the following genitourinary conditions? (Check all that apply.) (Go To: **1571**)

- | | |
|---|---|
| <input type="checkbox"/> ₁ [Males only] BPH (Benign prostatic hypertrophy) | <input type="checkbox"/> ₂ Hematuria (blood in the urine) |
| <input type="checkbox"/> ₃ [Males only] Prostatitis (an inflamed prostate) | <input type="checkbox"/> ₄ Urinary tract infection |
| <input type="checkbox"/> ₆ Urethritis (inflammation of the urethra) | <input type="checkbox"/> ₇ Other type of genitourinary tract infection |
| <input type="checkbox"/> ₈ Urinary stones | <input type="checkbox"/> ₉ Interstitial cystitis |
| <input type="checkbox"/> ₄₄ None | <input type="checkbox"/> ₉₉ Unknown/refused |

* [1571](#) Have you ever had any of the following procedures or problems? (Check all that apply.) (Go To: **End of Form**)

- | | |
|--|--|
| <input type="checkbox"/> ₁ [Males only] Transurethral resection of the prostate (TURP) | <input type="checkbox"/> ₂ [Males only] Transurethral incision of the prostate (TUIP) |
| <input type="checkbox"/> ₃ [Males only] Laser treatment for the prostate (interstitial laser or Niagra PVP) | <input type="checkbox"/> ₄ [Males only] Microwave or heat treatment for the prostate (TUNA or TUMT) |
| <input type="checkbox"/> ₁₀ Simple Prostatectomy | <input type="checkbox"/> ₁₁ Radical Prostatectomy |
| | <input type="checkbox"/> ₄₄ None (Go To: 1572) |

* [1572](#) Have you ever taken any of the following medications or supplements for a prostate or genitourinary condition? (Check all that apply.) (Go To: **1574**)

- ₁ 5-alpha reductase inhibitors (eg ₂ Alpha-blockers (eg Doxazoin, Terazosin,

FINAL

Finasteride, Avodart or Proscar)

₃ Anti-cholinergics

₅ Saw Palmetto

Other medications for prostate related conditions, specify: (Go To: **1573**)

Tamsulosin, others)

₄ Androgens (eg Testosterone, AndroGel, others)

₄₄ None (Go To: **1307**)

[1573](#) Other prostate or genitourinary medications, specify: (Go To: **1574**)

[1574](#) When were the medications or supplements for your prostate or genitourinary condition last taken? (Go To: **1575**)

₁ Within the past month

₂ More than 1 month ago

[1575](#) Total number of months taken (Go To: **1307**)

* [1307](#) Have you ever had cancer (other than basal/squamous cell skin cancer) confirmed by a doctor? (Go To: **End of Form**)

₀ No (Go To: **2650**)

₁ Yes (Go To: **1341**)

₉ Unknown/refused

* [1341](#) Cancer type/location: (select all that apply, and enter answers to 2732, 1333, and 1302 for EACH type of cancer selected.) This begins a loop to enter information about each type of cancer selected. **Do not use the Back button after you click Next on this page.** (Go To: **End of Form**)

₁ Bladder (Go To: **2732**)

₂ Bone (Go To: **2732**)

₃ Brain (Go To: **2732**)

₄ Breast (Go To: **2732**)

₆ Colon (Go To: **2732**)

₇ Esophagus (Go To: **2732**)

₈ Head & neck (mouth, nose, and throat) (Go To: **2732**)

₉ Kidney (Go To: **2732**)

₁₀ Liver (Go To: **2732**)

₁₁ Leukemia (Go To: **2732**)

₁₂ Lung (Go To: **2732**)

₁₃ Lymphoma, including Hodgkins (Go To: **2732**)

₁₅ Pancreas (Go To: **2732**)

₁₆ **Prostate**

₁₇ Rectum (Go To: **2732**)

₁₈ Skin (melanoma, no basal or squamous) (Go To: **2732**)

₁₉ Stomach (Go To: **2732**)

₂₀ Thyroid (Go To: **2732**)

₂₄ Testis (Go To: **2732**)

₂₇ Hypopharynx (Go To: **2732**)

₂₈ Larynx (Go To: **2732**)

₂₉ Nasopharynx (Go To: **2732**)

₃₀ Oropharynx (Go To: **2732**)

₃₁ Oral Cavity (Go To: **2732**)

FINAL

₉₅ Other (Go To: **2732**)

₉₉ Unknown/refused

* [2732](#) Cancer type name: (Go To: **1333**)

* [1333](#) Date of diagnosis (MM/YYYY) (Go To: **1302**)
Month Year

* [1302](#) Age at diagnosis (Go To: **2650**)
Age

* [2650](#) Are you participating in any prostate disease treatment studies? (Go To: **End of Form**)

₀ No (Go To: **1568**)

₁ Yes

₉ Unknown/refused

* [1568](#) Have any of your living or deceased first-degree blood relatives been diagnosed with prostate cancer? (Go To: **2194**)

₀ No

₁ Yes (Go To: **1569**)

₉ Unknown/refused

[1569](#) How many of your living or deceased first-degree blood relatives have been diagnosed with prostate cancer? (Go To: **1570**)

[1570](#) How many of your first-degree blood relatives have died of prostate cancer? (Go To: **2194**)

* [2194](#) Have any of your living or deceased second-degree blood relatives been diagnosed with prostate cancer? (Go To: **1097**)

₀ No

₁ Yes (Go To: **2195**)

₉ Unknown/refused

[2195](#) How many of your living or deceased second-degree blood relatives have been diagnosed with prostate cancer? (Go To: **2654**)

[2654](#) How many of your second-degree blood relatives have died of prostate cancer? (Go To: **1097**)

[1097](#) Comments: (Go To: **End of Form**)

Shaded areas indicated questions to be completed by research staff.

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** is a Primary Key and a Required Field.

* is a Required Field.

PCA3 Clinical Data (Form ID: 780)
Baseline
PCA3 Validation Study and Urinary Reference Set
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**[421](#) EDRN Participant ID (Go To: [423](#))

* [423](#) EDRN Protocol ID (Go To: [422](#))

* [422](#) EDRN Site ID (Go To: [929](#))

* [929](#) EDRN Staff ID of person who collected the data: (Go To: [1219](#))

* [1219](#) Date of chart abstraction: (Go To: [2651](#))

* [2651](#) Any prior PCA-3 administered for clinical purpose? (Go To: **End of Form**)

₀ No (Go To: [2274](#))

₁ Yes

₉ Unknown/refused

* [2774](#) Did the participant exhibit any evidence of a urinary tract infection within 2 days prior to either specimen collection? (Go To: [2775](#))

₀ No

₂ Yes, evidence of treated UTI

₃ Yes, evidence of untreated UTI

₉₉ Unknown

* [2775](#) Did the participant have a cystoscopy within 2 days prior to either specimen collection? (Go To: [2776](#))

₀ No

₁ Yes

₉₉ Unknown

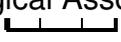
* [2776](#) Did the participant have a catheter inserted within 2 days of either specimen collection? (Go To: [1318](#))

₀ No

₁ Yes

₉₉ Unknown

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- * [1318](#) Any prostate biopsies prior to index biopsy? (Use to verify input on participant's form; this entry takes precedence.) (Go To: **578**)
- ₀ No ₁ Yes (Go To: **1698**)
₉ Unknown/refused
- *1698 Number of previous biopsies: (Go To: 2625)?
- * [2625](#) Date of most recent biopsy prior to index: (MM/DD/YYYY) (Go To: **2652**)
 (>6mo from consent. ≤6mo from consent)
- * [2652](#) Were any of the prior biopsies saturation biopsies? (Go To: **End of Form**)
- ₀ No (Go To: **578**) ₁ Yes
₉ Unknown/refused
- * [578](#) Age at specimen collection (Go To: **1292**)  (≥18. <18)
- * [1292](#) Height in inches: (Go To: **1295**) 
- * [1295](#) Weight in pounds: (Go To: **1576**) 
- * [1576](#) American Urological Association (AUA) symptom score: (Go To: **1691**) 
- * [1691](#) Quality of life due to urinary symptoms: (Go To: **979**)
- ₀ Delighted ₁ Pleased
₂ Mostly satisfied ₃ Mixed
₄ Mostly dissatisfied ₅ Unhappy
₆ Terrible ₉₉ Unknown/refused
- * [979](#) Date of most current digital rectal exam (DRE): (Go To: **976**)

- * [976](#) Results of most current digital rectal exam: (Go To: **973**)
- ₁ Normal ₄ Enlarged/Asymmetry
₃ Enlarged, benign Abnormal ₉₉ Unknown/refused
₅ (firm/induration/nodularity), suspicious for cancer (Go To: **1580**)

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[1580](#)

Amount of induration/nodularity on digital rectal exam (DRE), if not normal:
(Go To: **973**)

₁ < 50% right

₃ < 50% left

₅ Bilateral

₂ ≥ 50% right

₄ ≥ 50% left

₆ Abnormal DRE but extent not specified

* [973](#)

Date of index biopsy

Month Day Year

* [1577](#)

Indication for current prostate biopsy (Check all that apply.) (Go To: **2653**)

₃ Abnormal DRE

₂₂ Elevated PSA velocity (>0.4 ng/ml/yr)

₂₄ Prior ASAP or HGPIN

₉₅ Other (Ineligible if this is the ONLY selection)

₂₁ PSA >2.0 ng/ml

₂₃ Lower PSA value with other risk factors for PCa (e.g. family history)

₂₅ % free PSA < 15%

* [2653](#)

Extended template biopsy? (Go To: **1581**)

₀ No

₁ Yes

[1581](#)

Hypoechoic or suspicious foci seen? (Go To: **1692**)

₀ No

₁ Yes

₉₉ Unknown

[1692](#)

Prostate size (by TRUS): (Go To: **1586**)

* [1586](#)

Presence of prostate cancer: (Go To: **980**)

₁ Malignancy absent

₂ Malignancy present (Go To: **975**)

* [975](#)

Prostate T-Stage, Clinical (Go To: **1594**)

₄ T1a

₆ T1c

₉ T2b

₁₂ T3a

₅ T1b

₈ T2a

₁₀ T2c

₁₃ T3b

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₁₄ T4

₁₅ TX

*[1594](#) Prostate N-Stage, Clinical: (Go To: **1694**)

₁ NX

₂ N0

₅ N1

*[1694](#) Prostate M-Stage, Clinical : (Go To: **980**)

₁ MX

₂ M0

₃ M1

₄ M1a

₅ M1b

₆ M1c

*[980](#) Total number of cores taken: (Go To: **1097**) (≥10)

[1097](#) Comments: (Go To: **End of Form**)

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** is Primary Key.

* is required field.

PCA3 Index Biopsy (Form ID: 782)
Baseline
PCA3 Validation Study and Urinary Reference Set
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**[421](#) EDRN Participant ID (Go To: [423](#))

*[423](#) EDRN Protocol ID (Go To: [422](#))

*[422](#) EDRN Site ID (Go To: [929](#))

*[929](#) EDRN Staff ID of person who collected the data: (Go To: [844](#))

*[844](#) Date pathology report completed: (Go To: [839](#)) Month Day Year

[839](#) ID number of local pathology report: (Go To: [1595](#))

*[1595](#) Inflammation present? (Go To: [1598](#))

₀ No

₁ Yes

[1598](#) Atrophy? (Go To: [1586](#))

₀ No

₁ Yes

₉₉ Unknown

*[1586](#) Presence of prostate cancer: (Go To: [2158](#))

₁ Malignancy absent

₂ Malignancy present

*[2158](#) Number of specimens to record: (This begins a loop to enter information about multiple biopsy specimens as needed.) (Go To: [2159](#))

*[2159](#) VSIMS number for specimen container: (auto-entered by VSIMS and cannot be updated.) (Go To: [2709](#))

[2709](#) Site's identifier for the specimen container: (Go To: [2160](#))

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*[2160](#) Tissue position (laterality): (Go To: **2211**)

- ₁ Right ₂ Left
₉₉ Unknown/refused

[2211](#) Tissue position (zone): (Go To: **2213**)

- ₁ Transition Zone (TZ) ₂ Seminal vesicle
₃ Anterior zone ₄ Peripheral zone

[2213](#) Tissue position (regionality): (Go To: **2212**)

- ₁ Apex (A) ₂ Middle (M)
₃ Base (B)

[2212](#) Tissue position (position within region): (Go To: **2712**)

- ₁ Lateral (lat) ₂ Medial (med)

*[2712](#) Specimen status: (If normal, go on to next container and skip the following.) (Go To: **1097**)

- ₁ Normal ₃ Positive for malignancy (**Go to 1589**)
₄ Abnormal but negative for malignancy (Go To: 984) ₉₉ Unknown

*[1589](#) Primary Gleason grade: (Go To: **1590**)

*[1590](#) Secondary Gleason grade: (Go To: **1696**)

[1696](#) Tertiary Gleason Pattern: (Go To: **1697**)

*[1697](#) Overall Gleason Score: (auto-entered by system) (Go To: **2713**)

*[2737](#) Histological type (prostate): (Go To: **2713**)

- ₄ Adenocarcinoma (Go To: **2738**) ₅ Sarcoma cell carcinoma
₆ Squamous cell carcinoma ₇ Transitional cell carcinoma
₉₉ Unknown

[2738](#) Histological subtype: (check all that apply) (Go To: **2713**)

- ₄ Ductal ₁₃ Mucinous
₁₅ Neuroendocrine ₁₉ Small cell carcinoma
₉₉ Unknown

*[2713](#) Method used to record quantity of cancer in specimen: (Go To: **984**)

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₁ Percent cancer (Go To: **2210**)

₂ Length of cancer & length of specimen (Go To: **2208**)

₉₉ Unknown

[2210](#) Percent of cancer in tissue: (Go To: 2838)

*[2838](#) Notation for percent cancer in tissue: (Go to: 984)

₁ < less than

₁₀ No notation

₉₇ Other, specify: (Go To: **2839**)

2839 Notation for percent cancer in tissue (Other, specify) (Go to: 984):

[2208](#) Length of cancer in tissue specimen (mm): (Go To: **2209**)

[2209](#) Length of tissue specimen (mm): (Go To: **984**)

*[984](#) High grade prostatic intraepithelial neoplasia (PIN)? (Go To: **1596**)

₀ No

₁ Yes

*[1596](#) Atypical small acinar proliferation (ASAP)? (Go To: **1597**)

₀ No

₁ Yes

*[1597](#) Atypia/Suspicious? (Go to: **1591**)

₀ No

₁ Yes

₉₉ Unknown

[1591](#) Perineural invasion: (end of specimen container loop) (Go To: **1097**)

₁ Present

₂ Absent

₉₉ Unknown

[1097](#) Comments: (Go To: **End of Form**)

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PCA-3 PSA History (Form ID: 890)
Baseline
PCA3 Validation Study and Urinary Reference Set
EDRN

****421** EDRN Participant ID (Go To: **423**)

* **423** EDRN Protocol ID (Go To: **422**)

* **422** EDRN Site ID of the person who collected the data: (Go To: **929**)

* **929** EDRN Staff ID (Go To: **1582**)

* **2156** Number of PSAs performed: (This begins a loop to enter information about multiple PSAs, years.) (Go To: **1582**)

* **1582** Date of PSA: (Go To: **947**)

* **947** PSA (ng/ml) (Go To: **2502**)

2502 PSA test used: (Go To: **1097**)

- | | |
|---|--|
| <input type="checkbox"/> ₁ Bayer Immuno-1 PSA Test | <input type="checkbox"/> ₃ Hybritech PSA Test |
| <input type="checkbox"/> ₄ Roche Diagnostics Free PSA Test | <input type="checkbox"/> ₅ Abbot Diagnostics (Architect, A |
| <input type="checkbox"/> ₆ Beckman Coulter, Inc (Access, UniCel DxI) | <input type="checkbox"/> ₇ Beckman Coulter, Inc. (WHO stan |
| <input type="checkbox"/> ₈ Ortho Clinical Diagnostics (Vitros ECi) | <input type="checkbox"/> ₉ Roche Diagnostics (Elecsys 2010, E170) |
| <input type="checkbox"/> ₁₀ Siemens ADVIA Centaur | <input type="checkbox"/> ₁₁ Siemens Dimension HM |
| <input type="checkbox"/> ₁₂ Siemens Immulite | <input type="checkbox"/> ₁₃ Siemens Immulite, third generation |
| <input type="checkbox"/> ₁₄ Tosoh Bioscience | <input type="checkbox"/> ₉₇ Other, specify: (Go To: 2503) |
| <input type="checkbox"/> ₉₉ Unknown | |

2503 PSA test used (Other, specify): (Go To: **1097**)

1097 Comments: (Go To: **End of Form**)

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** is Primary Key.
* is required field.

PCA-3 Ineligibility (Form ID: 783)
Baseline
PCA3 Validation Study and Urinary Reference Set
EDRN

*[421](#) EDRN Participant ID (Go To: **423**)

*[423](#) EDRN Protocol ID (Go To: **422**)

*[422](#) EDRN Site ID (Go To: **929**)

*[929](#) EDRN Staff ID (Go To: **2186**)

*[2186](#) Reason(s) for ineligibility: (Go To: **1097**)

- | | |
|---|---|
| <input type="checkbox"/> ₂ Participant did not meet all inclusion/exclusion criteria | <input type="checkbox"/> ₄ Biopsy not performed |
| <input type="checkbox"/> ₁₀ Participant decided not to participate | <input type="checkbox"/> ₁₅ Pathology report unavailable |
| <input type="checkbox"/> ₁₆ Specimen(s) not collected | <input type="checkbox"/> ₉₇ Other, specify (Go To: 2184) |

*[2184](#) Reason for ineligibility (Other, specify): (Go To: **1097**)

[1097](#) Comments: (Go To: **End of Form**)

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- ** is Primary Key.
- * is required field.

PCA-3 Protocol Deviation (Form ID: 784)
Baseline
PCA3 Validation Study and Urinary Reference Set
EDRN

- [**421](#) EDRN Participant ID (Go To: **2642**)
- [**2642](#) Date of protocol deviation (MM/DD/YYYY): (Go To: **423**)
 Month Day Year
- [*423](#) EDRN Protocol ID (Go To: **422**)
- [*422](#) EDRN Site ID (Go To: **929**)
- [*929](#) EDRN Staff ID (Go To: **1105**)
- [*1105](#) Date site learned of protocol deviation: (Go To: **1106**)
- [*1106](#) Type(s) of protocol deviation(s): (Check all that apply.) (Go To: **1097**)
- | | |
|---|---|
| <input type="checkbox"/> ₁ Participant did not sign a consent form | <input type="checkbox"/> ₂ Participant did not meet all inclusion/exclusion criteria |
| <input type="checkbox"/> ₆ Specimen(s) collected without documentation of approval | <input type="checkbox"/> ₁₂ Protocol procedures not followed |
| <input type="checkbox"/> ₉₇ Other, specify: (Go To: 1107) | |
- [*1107](#) Type of protocol deviation (other, specify): (Go To: **1097**)
- [1097](#) Comments: (Go To: **End of Form**)

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Note:

Click on the DE ID link to see the details of selected DE(s) of interest.

** is a Primary Key and a Required Field.

* is a Required Field.

PCA3 PCA3 to PASS (Form ID: 1071)
Baseline
PCA3 Validation Study and Urinary Reference Set
EDRN

******[421](#) PCA3 Participant ID: (Go To: **423**) 

* [929](#) EDRN Staff ID (Go To: **2869**) 

* [423](#) EDRN Protocol ID (Go To: **422**) 

* [422](#) EDRN Site ID (Go To: **2869**) 

* [2869](#) PASS Participant ID: (Go To: **1097**)

[1097](#) Comments: (Go To: **End of Form**)

Shaded areas indicated questions to be completed by research staff.

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APPENDIX 5 – PCA3 ASSAY

PROCEDURE NAME:

PCA3 Assay

INTENDED USE:

The PCA3 Assay is an in vitro nucleic acid amplification test that quantitatively detects the prostate cancer gene PCA3 mRNA and PSA mRNA in male urine specimens to generate a PCA3 Score. (3)

PRINCIPLE:

The PCA3 assay is based on generation of amplicon by nucleic acid amplification that is detected by chemiluminescent DNA probes. The assay utilizes target capture, transcription-mediated amplification (TMA), and hybridization protection assay (HPA) technologies to streamline specimen processing, amplify target mRNA, and detect amplicon, respectively. The PCA3 Assay quantitatively detects PCA3 and PSA mRNA in male urine specimens. (3)

SUMMARY AND EXPLANATION:

PCA3 (also known as “PCA3DD3” or “DD3PCA3”) is a non-coding prostate-specific gene that is highly over-expressed in prostate cancer cells (1, 2). It is mostly undetectable in other tissues, including bladder and testis, and has very low expression in normal prostate cells. 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 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 in 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.

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EQUIPMENT:

Method Specific Equipment:

DTS® 402 Systems: SB100 Dry Heat Bath/Vortexers
Data Acquisition Software, v. 6.0.3 (XP) or 4.0.0.1 (NT)
DAS Worklist Editor, v. 4.0.2.2
Micropipettor, 200 to 1000 µL
PCA3 Assay Protocol for LEADER® HC+ Luminometer
TableCurve 2D software, v. 5.01
"PCA3 40 Specimen Assay,"v. 20070403a, Microsoft Excel workbook
Repeat pipettor tips (2.5 mL, 5.0 mL, 25 mL)
Tips, Pipetman P1000 style, APTIMA Combo 2®
Microsoft Excel

See General Equipment List for additional information.

REAGENTS, CONTROLS, CALIBRATIONS:

The following reagents are stable when stored at 2° to 8°C:

PCA3/PSA Amplification Reagent
PCA3/PSA Enzyme Reagent
PCA3/PSA Probe Reagents
PCA3/PSA Calibrators 1-5
PCA3/PSA Positive Control

The following reagents are stable when stored at Room Temperature (15°C to 30°C)

PCA3/PSA Enzyme Reconstitution Solution
PCA3/PSA Probe Reconstitution Solution
PCA3/PSA Selection Reagent
PCA3/PSA Target Capture Reagents **DO NOT** store at temperatures below 15°C
Wash Solution
Oil Reagent
Auto Detect Reagents 1 and 2: **WARNING: IRRITANT, CORROSIVE**
Buffer for Deactivation Fluid
Sealing Cards
APTIMA Adapter Kits
Urine Specimen Collection Kits

After reconstitution the following are stable for 30 days when stored at 2°C to 8°C.

PCA3/PSA Enzyme Reagents
PCA3 and PSA Amplification Reagents
PCA3 and PSA Probe Reagents (see note)

Note: The Probe Reagent and reconstituted Probe Reagent are photosensitive. Store the reagents protected from light.

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DO NOT FREEZE THE REAGENTS

Controls and Calibrators are stable when stored at 2° to 8°C

See Reagent Manual for additional information

SPECIMEN REQUIREMENTS:

See “Specimen Handling and Processing” in the General Laboratory Procedure Manual for additional information.

Acceptable Specimens

Unprocessed Urine:

- Collect in sterile urine container.
- **Must be maintained at 2°C to 8°C or kept on ice (DO NOT FREEZE),**
- Transport and process within 4 hours of collection.

Processed Urine:

- Urine mixed with UTM (Urine Transport Media).
- Total liquid volume must fall between two black indicator lines on transport tube. (If using transport tubes) **REJECT** if volume does not fall between these two lines
- Or urine will be added in equal volume to UTM and aliquoted for use in testing. *See General Laboratory Procedure Manual for details.*
- **Must be transported at or below 30°C and may be frozen.**
- Must be received within 5 days of collection.
- Store at 2°C-8°C for up to 14 days or keep frozen at -70°C or below for up to 90 days. Samples may freeze-thaw up to 4 times.

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QUALITY ASSURANCE:

Calibrators and Controls (Transcripts)

- **Must be run with all assays and on the same rack as test specimens.**
- Transcripts 1, 2, 4, 6, and 8 (**Calibrators**) are to be run in **three replicates each**. Transcripts 3 and 7 (**Controls**) are to be run in **two replicates each**.
- Test **specimens** are to be run in **two replicates each**.
- Store Calibrators and Controls at **2° to 8°C**
- Mix the Calibrators and Controls **by inversion** to ensure homogeneity. **DO NOT VORTEX.**

Note: Within the PCA3/PSA Transcripts package, vials are labeled 1 to 8 and should be used as listed in the table below. The assigned copy level of the transcripts will be provided in the packaging.

The following table describes placement of the Transcripts (Calibrators and Controls) and specimens.

	Sample Description	Transcript	Target PCA3 Concentration (c/ml)	Target PSA Concentration (c/ml)	** PCA3 and PSA Positive Calibrators and Controls are value assigned, so the actual c/mL values for
1-3	Calibrator 1	1	0	0	
4-6	Calibrator 2	2	250	7,500	
7-9	Calibrator 3	4	2,500	75,000	
10-12	Calibrator 4	6	25,000	750,000	
13-15	Calibrator 5	8	125,000	3,000,000	
16-17	Low Control	3	1,250	37,500	
18-19	High Control	7	62,500	1,500,000	
20-n	Test Specimen	--	unknown	unknown	

Calibrators 2 to 5 and Controls A and B will be slightly different than the target concentrations listed in the above 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.

The following criteria **must be met in order for a run to be considered valid:**

Average RLU for Calibrator 2 (Transcript 2) replicates > RLU Cutoff

Where RLU Cutoff = Average RLU of Calibrator 1 (Transcript 1)
+ 1.645 standard deviations of Calibrator 1 (Transcript 1) RLU replicates
+ 1.645 standard deviations of Calibrator 2 (Transcript 2) RLU replicates

- Average interpolated Calibrator 5 (Transcript 8) recovery = 70% to 130%
- Average interpolated Low Control (Transcript 3) recovery = 40% to 160%
- Average interpolated High Control (Transcript 7) recovery = 65% to 135%
- If a run is invalid as a result of failing to meet the above criteria, the invalid run will have no ratio results calculated.
- The run must be repeated for the **invalid analyte**.
- If the run is valid for the other analyte, those results may stand and be used in data analysis with the repeat, valid run, of the first analyte.
- Ratio results may need to be manually calculated.
- Within a run, individual urine specimen results may be deemed **INVALID** and will be indicated in the Raw Run Report. Although individual replicates for a specimen may be valid, a specimen

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will be invalidated if the interpolated c/mL difference between the replicates exceeds 600%. Testing for that urine specimen must then be repeated.

PROCEDURE:

All work should be done in a unidirectional flow to avoid contamination.

In the Pre-Amplification Area wear Light Blue gloves for all steps!

- Put on gloves (Light Blue) to begin work.
- Clean equipment and work surfaces with **50% bleach**. (add equal parts bleach to equal parts water). Allow bleach to contact surfaces for at least 1 minute and then follow with DI water rinse/wipe. (See “Decontamination” in the Laboratory Procedure Manual for more details).
- Cover the bench surface with a plastic backed absorbent laboratory bench cover.
- Turn on SB100. On the **pre-amplification** SB100 instrument, press the “|” symbol on the power switch. The Main menu displays after 5 secs. (*see General Laboratory Procedure Manual for details*)
- Press the ◀ key to select the Select Run Mode Menu.
- Press the ▲ key to select the Run Protocol Menu. SB100 will begin to ramp to assay temp.
- **CHANGE GLOVES (light blue)**

REAGENT RECONSTITUTION:

- Pair appropriate reconstitution solution with each lyophilized reagent.
- MIX solutions and return to the plastic bottle and cap. Record initials and date of reconstitution. (*see Reagent Manual for details*)

SPECIMEN PREPARATION:

- Allow Urine to reach room temperature.
- Inspect specimen volume. Total initial liquid volume must fall between the two black indicator lines on the tube. (*see General Laboratory Procedure Manual for details*)
- Mix by inversion. **DO NOT VORTEX**
- Place appropriate number of Ten-Tube Units (TTUs) in TTU rack.
- Set up one rack for the PCA3 assay and another rack for the PSA assay (***be sure to label racks for identification***).

Note: If the number of test specimens is low enough, both assays may be run on a single rack.

- In the ten-tube unit (TTU) racks, place enough TTUs to accommodate the Transcripts (Calibrators and Controls) and specimens for each assay.
- **Label** the TTUs with the sample/specimen IDs. **DO NOT** leave empty tubes between calibrators, controls or patient samples. ***See the table above for placement.***
- Transcripts 1, 2, 4, 6, and 8 (**Calibrators**) are to be run in **three replicates each**.
- Transcripts 3 and 7 (**Controls**) are to be run in **two replicates each**.
- Test **specimens** are to be run in **two replicates each**.
- All transcripts and test specimens must be run on the same rack.

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- Verify Concentration Lot ID and Expiration date. (see *QC Manual for more details*).
- **CHANGE GLOVES (Light blue)**
- **Add 100 ul** of TCR to each reaction tube using the repeat pipettor.
- **Add 400 ul** of each control, calibrator or specimen to the bottom of the appropriate tube in the TTU.
- **Cover** with sealing card(s).
- Place rack into **Pre-Amp SB100**, cover with frame and lock in place.
- **Press ►** to continue. The SB100 will: Gently vortex the rack, incubate the rack at 62°C for 35 minutes, vortex the rack for 60 seconds, ramp down from 62° to 23°C, and Incubate the rack for the remainder of the 30 minutes at 23°C.

During this incubation period perform the following:

- Generate an assay run worklist with the Worklist Editor on the Pre-amp computer (see *General Laboratory Procedure Manual*).
- Perform Target Capture System start-up Maintenance. (see *General Laboratory Procedure Manual*)
- Place sufficient TTCs in rack.
- **CHANGE GLOVES (Light blue)**
- When incubation is complete. Place rack on Target Capture System (TCS) magnetic base. **Incubate** for 5-10 minutes.
- Carefully remove sealing card(s).
- Turn on vacuum pump and leave on while processing samples.(see *General Laboratory Procedure Manual for details*)
- **Aspirate** by firmly attaching aspiration manifold to the first set of tips. Aspirate **all** liquid. Eject tips into their original tip cassette. Repeat for all rows of TTUs.
- **Add 1 ml** of Wash by placing the dispense manifold over each TTU and using the dispense station pump, deliver wash solution, cover with sealing card(s), place in SB100, cover with frame and lock in place.
- **Press ►** (rack will be vortexed).
- Remove rack from SB100 and place back in TCS magnetic base for 5-10 minutes.
- **Press ►** to preheat SB100 to 62°C.
- **Aspirate** wash
- Check all tubes to make sure that there are magnetic beads present

Amplification

- **CHANGE GLOVES (Light Blue)**
- **Add 75 ul** of Amplification Reagent and **200 ul** of Oil. Check for **red** color.
- **Cover** with sealing card(s)
- Place rack into Pre-Amp SB100, cover with frame and lock in place.
- **Press ►** key to continue.(SB100 will vortex for 10 sec., incubate at 62°C for 10 min. and then ramp from 62°C to 42°C.
- When prompted (SB100 has reached 42°C), remove the frame and sealing cards to **Add 25 ul** of **Enzyme Reagent** to the **bottom** of each tube **while rack is in SB100, this must be**

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completed for all specimens within 90 seconds. (Do Not pipet down side of tube). Check for **orange** color.

- **Cover** with sealing card(s), cover with frame and then lock in place.
- **Press ►** key to continue, (this will start the 60 min. incubation at 42°C).
- **CHANGE GLOVES (Light Blue)**
- During the 60 minute incubation, turn off vacuum pump and remove aspirator manifold. Place manifold in **50% bleach** for 10 minutes (longer exposure can damage it). Rinse thoroughly under running water then final rinse in **DI water** and dry. Reconnect to TCS and turn on Pump for 5 minutes.
- **Empty** waste trap (after 300 samples or 1 week) and rinse with water. **Add 400 ml** undiluted bleach and place back into waste trap.
- Clean pre-amp work area and equipment with **50% bleach**. Follow with DI water rinse/wipe.

POST-AMPLIFICATION

In the Post-amplification Area wear Grey gloves for all steps!

- **CHANGE GLOVES (Grey)**
- Clean **Post-amp** work area and equipment with **50% bleach**. Follow with DI water rinse/wipe (see *General Laboratory Procedure Manual "Decontamination"* for details).
- **Turn on post-amp SB100 and start Protocol 2**
- **Add 100 ul** of Probe Reagent to each tube. Check for **yellow** color.
- **Cover** with sealing card(s) and frame.
- Place rack in the **Post-amp SB100**, cover with frame and lock in place.
- **Press ►** key to continue. (SB100 will vortex for 10 sec. and incubate at 62°C for 20 min.)
- When incubation is over, **remove the rack** from the SB100 and **incubate at room temperature** for 5 minutes.
- **Press ►** key to start the timer
- **NOTE: the ► key must be pressed within 5 minutes of the probe incubation. If not, the instrument will time out and the protocol will be aborted.**
- **Turn on Computer and LEADER HC+ and perform start-up** (see *General Laboratory Procedure Manual* for details)
- Prepare Deactivation Fluid by adding 1 part Gen-Probe deactivation buffer and 1 part, household bleach to container. (see *"Decontamination" in the General Laboratory Procedure Manual* for details).
- **CHANGE GLOVES (Grey)**
- After 5 minute incubation, remove sealing card(s).
- **Add 250 ul** of Selection Reagent. Check for **pink/red** color.
- **Cover** with sealing card(s).
- Place rack in the Post-amp SB100, cover with frame and lock in place.
- **Press ►** key to continue. (SB100 will vortex for 10 sec., incubate at 62°C for 10 minutes and then cool the rack to 23°C).
- When last incubation is complete, remove the rack from the SB100 and turn off SB100 if there are no further tests to be run.

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- **TTUs must be read in LEADER HC+ Luminometer within 1 hour and 45 minutes of last incubation.**

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DETECTION

- Leader HC + Luminometer should be on and ready to perform detection. **Initial start up procedure should be performed before continuing to next step.** (see the *General Laboratory Procedure Manual* for details).
- Load sample TTUs (load PCA3 TTUs first, read and then repeat steps for PSA), **close and lock lid.**
- In the APTIMA Assay Software, click **New Run or F3.**
- From the pull-down menu, choose the appropriate protocol.
- Select the appropriate worklist created earlier. (or manually enter the number of samples, lot and expiration date of kit if worklist is not being used)
- Click on **Next or F8**
- Click **“Start LEADER HC+.”**
- Click on **“No”** so that wash is not performed again and enter **“Done”** in comment line.
- Reading will begin.
- Run must be completed within 2 hours of the end of the selection step incubation.
- When run is finished, the assay software will generate two run reports, a Raw Run and a Ratio Report, if the runs are back-to-back.
- Transfer completed data to a jump drive or disc for transfer into data files at another computer not located in the Post-amp area. (See *General Laboratory Procedure Manual*)
- When the run is finished, remove the used TTUs from the Luminometer and place the TTUs into the container with the buffered bleach solution. (see *General Laboratory Procedure manual for Decontamination details*).
- Shut down LEADER HC+ Luminometer and Computer (see *General Laboratory Procedure Manual for details*)

DATA ANALYSIS

- At another computer, copy or move both the DAT and RAW data files into a Raw Data folder to archive them.(these have been stored in a jump drive or disc)
- Continue analysis by following the PCA3 Assay Data Analysis protocol found in the *General Laboratory Procedure Manual*.

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APPENDIX 6 – PARTICIPATING SITES

1. Dr. John Wei – University of Michigan
2. Dr. Martin Sanda – Harvard-BIDMC
3. Dr. Ian Thompson – University of Texas, San Antonio
4. Dr. Alan Partin – Johns Hopkins University
5. Dr. Yair Lotan – UT Southwestern Medical Center at Dallas
6. Dr. Mohamed Bidair – San Diego Clinical Trials
7. Dr. Daniel Lin – University of Washington
8. Dr. Samir Taneja – New York University
9. Dr. Adam Kibel – Washington University St. Louis
10. Dr. Rosalia Viterbo – Fox Chase Cancer Center
11. Dr. Erik Busby – University of Alabama