

PCPT Specimen Collection and Handling:

Prostate tissue

Biopsies

The study required that each biopsy performed should be at least a sextant biopsy. 80% of the biopsies were sextant. The biopsy sites included were, at minimum, left apex, left mid, left base, right apex, right mid, and right base. Laterally directed biopsies in these areas (e.g., left lateral mid) or digitally-directed biopsies to palpable lesions also were submitted in some cases. Once received and verified for proper identification and accuracy, specimens were placed into tissue-processing cassettes permanently marked with PCPT ID number, collection date and biopsy location. All participant-identifying information was stripped from the samples according to HIPPA standards. Specimens were processed for paraffin microtomy using standard vacuum paraffin infiltration. Stained slides and blanks were prepared as described below. One set of stained slides was returned to the originating center for routine diagnostic interpretation by their pathologists.

For each of the paraffin-embedded specimens the following approach was taken. The block was faced until tissue was first encountered. A ribbon of three sections (5 μm) was collected on each of slides #1A and #1B and stained with hematoxylin and eosin (H&E). Ten subsequent slides, each with one paraffin section (5 μm) were collected and stored. Two additional slides (#2A and 2B), each with three sections were collected and stained with H&E. Another ten unstained slides each with one paraffin section (5 μm) were collected for storage. Finally, two more slides with three 5 μm sections (#3A and 3B) were cut and stained with H&E. Slides 1-3A were shipped to the sites for diagnosis by site pathologists. Slides 1-3B were reviewed by pathologists at the Anatomic Core Laboratory (the PDL). The cut surface of the paraffin block was sealed with molten paraffin. A summary of this protocol is seen in Figure 1. The result of this processing protocol is that, for each biopsy core, the archive at our facility contains approximately 23 cut slides (3 H&E, 20 unstained) and one paraffin block containing residual tissue. In some cases where cancer was suspected, archive material (2-3 unstained slides) was used for immunohistochemistry or additional H&E stains in order to render a diagnosis.

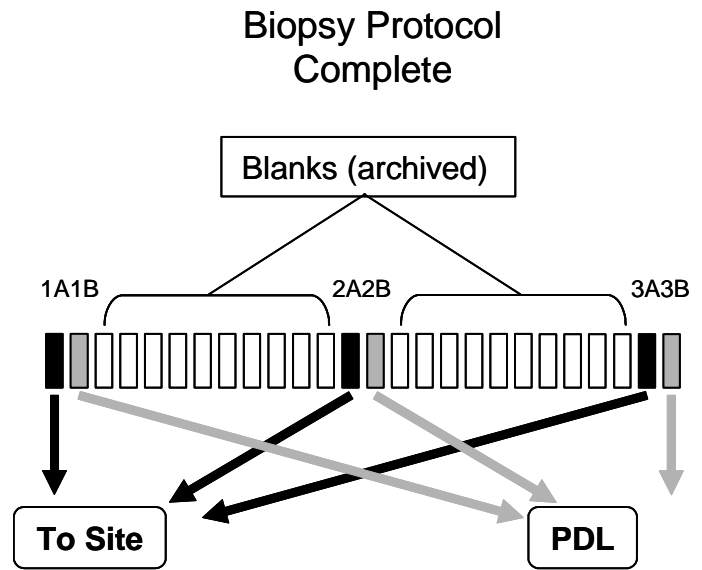


Figure 1

Staff pathologists at the Anatomic Core Laboratory (PDL) reviewed the H&E stained slides that were prepared for each case. Each slide was evaluated for the presence of carcinoma, PIN, acute inflammation, chronic inflammation, atrophy in all its forms (see below), reserve cell hyperplasia, atypical adenomatous hyperplasia, and various subtypes of hyperplastic nodules (adenomatous, fibroadenomatous, fibromyoadenomatous, fibromatous and leiomyomatous). In addition, each biopsy was scored as good, adequate or inadequate with respect to biopsy quality. Inadequate biopsies were those that failed to contain recognizable prostate and were solely composed of extra-prostatic connective tissue. "Good" biopsies are those that contained large amounts of tissue from which additional sections could easily be cut from the remnant block. All other biopsies were considered "adequate." The unstained slides were archived in our laboratory unless needed for diagnostic purposes by the P.I. or Clinical Center's pathologist. Any focus that was considered as

carcinoma, suspicious for carcinoma, atypical or PIN was reviewed by the P.I. for final diagnosis before sites were notified. With respect to quality assurance, using the method described above, each H&E stained slide was read by at least two diagnostic pathologists (the P.I. and the pathologist at the originating Center) and examined for the presence of carcinoma or PIN. Discrepancies were pursued using special stains and/or additional outside consultations until a consensus was reached.

Radical Prostatectomies: When a participant in the PCPT underwent prostatectomy, the site had the option of sending the specimen to the PDL for whole-mount processing. Formalin-fixed specimens received were logged-in, measured and weighed, and inked with permanent green ink on the right and blue ink on the left. The prostates and seminal vesicles were then serially sectioned every 4 mm from apex to base in the oblique transverse plane. The slices were then processed by vacuum paraffin infiltration, embedded into large format paraffin blocks, sectioned and mounted on special double-size glass slides for routine hematoxylin and eosin staining. A set of slides was returned to the originating sites for interpretation by site pathologists. A second set of slides was reviewed by the P.I. of the PDL.. For prostatectomies that were not processed at the PDL, duplicate sets of slides were sent from the site to the PDL for review and diagnosis by the P.I.

Bloods:

Annual Blood Collection: The PCPT required collection of approximately 15 ml of blood from participants at the first visit and at each subsequent annual visit. Approximately 3% of subjects had an additional 15 ml of blood collected for quality control analysis. Bloods were collected into tubes, without anticoagulant, containing a gel to separate serum from clot after centrifugation and kept at room temperature for 30-60 min before centrifugation. Serum was poured off and the sample frozen as quickly as possible after separation. Samples were generally expressed to Esoterix (Calabasas, CA) within 24 hrs of collection but some were stored for up to 7 days at -20°C before shipment. Upon receipt, the sample was thawed to remove an aliquot for PSA analysis. The remaining serum was frozen in 0.5 ml aliquots (at least 4 for each specimen).

Additional Blood Collection with Anticoagulant (Plasma, White Blood Cell (WBC), and Red Blood Cell (RBC) Preparations: During the study, it was decided to collect an additional blood sample with anticoagulant specifically for isolation of DNA for genotyping studies. This process began March 2000 and continued until the early termination of the study. Additional informed consent was obtained for this new collection. Three acid washed 7 ml EDTA tubes were used to collect approximately 20 ml of blood and were kept from light. Samples were overnight shipped chilled to NCI-Frederick (Frederick, MD) for processing. Plasma was separated into 5 x 1.5 ml aliquots, WBC's into 2 aliquots and the RBC's into a 4.5 ml aliquot. All samples are currently stored at -70°C at NCI Frederick.