Summary. Standardized protocols for processing radical prostatectomy specimens are critical for superior patient management. It provides accurate information to the clinician in a reliable and consistent format to enhance patient care and prognosis. In recent years, processing protocols have been proposed by various authoritative groups, with similar suggestions for most parts of the practice guidelines; however, discrepancy in processing approaches still exists. Standardization improves the quality and consistency of pathology reports. In this review article, we incorporate the processing schemes for radical prostatectomy addressed in literature and propose a comprehensively standardized approach to evaluate radical prostatectomy specimens.

Key words: Prostatic neoplasm, Radical prostatectomy, Pathologic staging, Specimen handling, Processing protocol

Introduction

Prostate carcinoma is the most common noncutaneous malignancy of men in the United States. In 2008 it is estimated that 186320 new cases will be diagnosed, increased by 399% from the 37324 new cases in 1985 (Jemal et al., 2008). The dramatic increase of prostate carcinoma in the past two decades is mainly due to screening for prostate specific antigen (PSA) in asymptomatic men and to transrectal ultrasonography with biopsy for early detection. Due to expanding understanding of carcinogenesis in prostate cancer, treatment modalities have expanded to include androgen-deprivation therapy, chemotherapy, radiotherapy, and combinations of these modalities in selected patients. Radiotherapy was fraught with a high frequency of positive biopsies following therapy (Scherr et al., 2003). Hormonal therapy by androgen deprivation has long been regarded as a palliative treatment reserved for those with advanced or recurrent disease (Debruyne, 2002). Therefore, radical prostatectomy is still the mainstream of treatment for localized prostate cancer. The popularity of radical surgery may result from the nerve-sparing prostatectomy technique introduced in 1982 by Dr. Patrick Walsh, which has significantly lowered the incidence of impotence after surgery without increasing the risk of local recurrence (Walsh and Donker, 1982).

Radical prostatectomy specimens provide most detailed and comprehensive pathological information of prostate cancer, including tumor type and volume, histological grade, margin status and cancer stage, which are fundamental important for predicting prognosis and selecting adjuvant therapy. In addition, harvesting fresh tissue from prostatectomy specimens for molecular analyses may be of major importance in prostate cancer research and further patient treatment. However, consistency and reproducibility for evaluating prostatectomy specimens require a standardized manner for tissue fixation, sampling, embedding and processing. In this review article, we incorporate the processing protocols for radical prostatectomy addressed in recent reports (Humphrey and Walter, 1993; Henson et al., 1994; Sakr et al., 1995; Amin et al., 1996; Fechner, 1996; Bostwick and Montironi, 1997; Hoedemaeker et al., 1998; Mazzucchelli et al., 2001; Montironi et al., 2001, 2003a,b; Epstein et al., 2005; Sung et al., 2007) to
propose a comprehensively standardized approach to radical prostatectomy specimen evaluation.

Initial handling of radical prostatectomy specimens

When radical prostatectomy specimens are delivered to the lab, they should be inspected immediately. Topography, surgical procedure and fixation status should be documented in the record and specimen identification, including patient’s name and medical record number, should be verified. The surgeon should be contacted if any of these properties is unclear. Specimens are weighted and measured in three dimensions, including apical to basal (vertical), left to right (transverse) and anterior to posterior (sagittal). The prostate should be weighed independent of the seminal vesicles to obtain the precise weight of the prostate itself. The dimensions of seminal vesicles and pelvic lymph-node-bearing tissue are also measured. Weight is usually a more reproducible parameter than linear dimensions because the resected prostate is an irregular structure, symmetric only in one axis.

Macroscopical evaluation of specimens

The specimens must be well-oriented. The pathologist should contact the urologist for clarification if any difficulty arises in orientation. The entire specimen is carefully inspected and palpated to localize zones of nodularity or induration. If there is asymmetry or a palpable abnormality, the location, size and texture of lesions should be recorded. Previous reports of needle biopsy or transrectal ultrasound should serve an aid to identifying the location and size of tumor. The prostatectomy specimens should be handled with care to avoid the disruption of the surrounding thin soft tissue, which may result in the false positive status of the surgical margins.

Fresh tissue harvest for research

A few modern molecular or genetic approaches for prostate cancer investigation require extremely fresh tissue to achieve optimal results. Several techniques have been devised to harvest fresh tissue from radical prostatectomy specimens prior to fixation. The goal is to avoid compromising the evaluation of pathological parameters without inadequate or unrepresentative sampling for molecular studies (Bova et al., 1993; Wheeler and Lebovitz, 1994; Sakr et al., 1995; Hoedemaeker et al., 1998). Details of fresh prostatic tissue harvesting are beyond the scope of this article, but we summarize these sampling techniques briefly to accommodate future developments in prostatectomy tissue analysis.

Needle biopsy method

Hoedemaeker et al. tried to locate the prostate cancer by combining information from palpation, from previous needle biopsy reports and from transurethral ultrasonography images. Fresh tissue is then harvested from tumor areas by 14-gauge biopsy needle or skin biopsy punch (Hoedemaeker et al., 1998). Damage to the surgical margin is avoided by directing the biopsies parallel to the prostatic surface or by performing them via the urethra.

Scraping method

Guided by gross inspection, the fresh tissue is sampled by scraping the tumor area with a surgical blade (Sakr et al., 1995). When gross identification of cancer is difficult to establish, a frozen section of the suspect area is performed.

Mid-slicing with punch biopsy method

A single slice cutting through the mid-prostate posteriorly and then used a punch biopsy instrument to harvest the fresh tissue from at least 10 separate sites (Wheeler and Lebovitz, 1994). In order to facilitate identification of sampling regions during histological examination, the sampling holes were filled with green ink.

Peeling method

Bova et al. incised a peel of resection from the surface near grossly identifiable cancer for evaluation of margin status and assessment of the extent of extraprostatic extension (Bova et al., 1993). Underlying prostate cancer tissue may then be harvested freshly for a research purpose without compromising the diagnosis of margin status.

Fixation

A number of pathologists section the prostate before fixation (True, 1994) and some experts claim that sectioning the prostate fresh does not compromise accurate pathologic staging (Sakr et al., 1995). However, cutting unfixed prostate may result in bulging of parenchyma, especially in the foci of nodular hyperplasia. This makes the sections have non-uniform thickness, posing difficulties for embedding, processing and consistent histological sectioning. In addition, proper fixation preserves the surgical margins and enhances subtle characteristics of tumor foci such as firmness and discoloration. Therefore, we recommend fixing the radical prostatectomy specimens before sectioning, unless harvesting fresh tissue for additional molecular studies as mentioned above.

There are several methods used for fixation of radical prostatectomy specimens. In our lab, we immerse the whole prostate in 10% neutral buffered formalin for 24 hours at room temperature. To obtain complete fixation and avoid tissue distortion, the volume of fixative should be at least 5-10 times the prostate volume and the prostate should not touch the sides of container.
except on the bottom.

To enhance quick and uniform penetration of the fixative, microwave irradiation has been incorporated to reduce fixation time (Ruijter et al., 1997) in some institutes. In addition, some investigators have proposed injecting 10% neutral buffered formalin directly into the prostate (Hollenbeck et al., 2000). They injected approximately 100 ml of formalin solution in a systemic pattern from all sides of the prostate with fine hypodermic needles. The rationale behind the injection procedure is that formalin slowly diffuses from the surface toward the center of the prostate when it is immersed in fixative. The cross-linked protein at the surface may prevent further diffusion of fixative toward the center of the specimen (Montironi et al., 2003a). We, however, find this procedure time-consuming, difficult to standardize and occasionally distorting to the tissue when compared to simple immersion fixation.

**Inking**

Because the outer surface of the prostate is fibromuscular connective tissue, its “capsule” may be grossly and microscopically imperceptible, especially in the vicinity of transformed epithelium. Therefore, a colored ink is necessary to discriminate whether the section edge is a true positive margin or just a sectioning artifact. The entire external surface of the prostate should be carefully inked either by immersion into India ink solution or by painting the surface with different colors of ink to ensure proper orientation of subsequent sections (Fig. 1A).

India ink is preferred by some pathologists because it was more easily observed under the microscope, but saturated colored inks, when applied liberally, allow both margin identification and orientation. After immersion or painting with ink, the wet specimen is placed into a mordant, such as acetic acid or Bouin’s solution, and air-dried to fix inks.

**Sectioning**

**Apex (distal) and bladder neck (proximal) margin**

**Perpendicular section**

The apex and bladder base of the prostate are removed by transverse sections 4-5 mm from the distal and proximal margins, perpendicular to the rectal surface. Both margin specimens are then cut parasagittally at 4 mm intervals perpendicular to the inked surface. An alternate method would be to section in a radial fashion similar to the cutting of a cone biopsy of the uterine cervix. Whatever the method of cutting, all margin sections are microscopically examined.

**Shave (en face) section**

Some pathologists prefer shave margins to sample a larger surface region and to disclose the two-dimensional extent of margin exposure. They take thin margin specimens of 1 to 2 mm thickness on the surface of apex and bladder neck (Hall et al., 1992). If any cancer cell is identified in the shave specimens, the margin is regarded as positive. Although this method avoids missing a tiny positive margin between the margin sections of perpendicular sampling, the thicker shave section may artificially result in a false-positive histological margin.

The false positive rate of the margin status is dependent on the method of en face sampling and on the criteria of individual pathologist for evaluating margins; therefore, the more consistent perpendicular section approach is recommended by us for evaluating the margin status.

**Seminal vesicles**

Complete sampling

The bilateral seminal vesicles are amputated at the junction between the base of the seminal vesicles and the prostate surface. The seminal vesicles are cut longitudinally with a “sandwich” technique. The sandwich technique involves stabilizing the seminal vesicle against the cutting board with gauze or a safety device, and slicing with the knife parallel to the board surface. Both halves are submitted with attached soft tissue in one or two blocks. The right and left seminal vesicles should be placed into different labeled cassettes.

Partial sampling

Some pathologist didn’t submit all of the tip and body of each seminal vesicle and they cut transverse sections through the base of the seminal vesicle where it enters the prostate (Srigley, 2006). This sampling strategy is based on the hypothesis that the base part of the seminal vesicle will be the first part involved by tumors invading the seminal vesicle (Epstein et al., 2005).

**Prostate**

After sampling the seminal vesicles and margins, the remaining prostate specimen is serially sectioned by knife transversely at 3 to 5 mm intervals perpendicular to the rectal surface. Some investigators use a prostate slicing device (Schmid et al., 1992) or a commercial meat slicer (Ayala et al., 1989) to obtain more consistent and precise thickness of slices but these devices are not widely available and require regular maintenance.

**Gross identification of prostate cancer**

Unlike other malignant cancers, in which tumors form a distinct, firm mass, prostate cancers often have similar consistency and color to the surrounding benign tissue, which causing difficulties in discriminating between cancer and benign regions. Especially after the introduction of PSA screening combined with needle biopsy to diagnose early cancer in asymptomatic men,
the increasing incidence of small tumors in prostate, which infrequently forming the distinct mass and often obscured by nodular hyperplasia, makes accurately grossly identification of cancer extremely difficult, or even impossible. In one study of clinically organ-confined cancer, Hall et al found macroscopically identifiable tumors in 90% (94 of 104 cases) stage A and B patients (Hall et al., 1992). However, other investigators have reported a much lower detection rate for grossly identifiable cancer. In a study of 211 consecutive radical prostatectomy specimens, Renshaw found carcinoma identified by gross inspection in only 63% of cases, and the false-positive rate for gross examination was up to 19% (Renshaw, 1998). Bostwick

![Fig. 1. Processing of radical prostatectomy specimen.](image-url)

**A.** Inking. The entire surface of prostate was painted by two colors of ink to ensure proper orientation and margin identification. The right lobe was painted by yellow color and the left lobe by black color. **B.** Sectioning and sampling of radical prostatectomy specimen. The apex and bladder neck margin are removed by transversal sections and cut parasagittally perpendicular to the inked surface. The seminal vesicles are amputated at the base connected to the prostate proper and longitudinally cut by a sandwich technique. The remaining prostate proper is serially sectioned transversely at 3 to 5 mm intervals perpendicular to the rectal surface. **C.** A routine section of prostatectomy specimen. Sections were obtained by slicing each transverse sections (C1) into two halves (C2) or four quadrants (C3) to fit for the conventional cassettes (C4). **D.** A whole-mount section of prostatectomy specimen. The entire transverse sections of the prostate are mounted on oversize glass slides without subdivision. The orientation is well preserved and entire tumor is studied in three dimensions to facilitate tumor identification and quantification, and evaluation of their relationship between surrounding structures.
et al. also reported a similar low identification of prostate carcinoma by gross inspection (Bostwick et al., 1997).

The transverse sections of prostate should be carefully examined for grossly identifiable cancer. Prostate carcinoma appears as a solid gray to yellow-white mass, with wide variation in gross appearance. Grossly apparent tumors are usually at least 5 mm in greatest dimension, and have a firm consistency due to tightly packed neoplastic glands or desmoplastic stroma. The color may be yellow-white due to tissue necrosis and membrane lipid accumulation. In addition, they are often more solid and homogenous than the surrounding multinodular benign tissue. Some cancers appear as a yellow granular mass which contrasts sharply with the normal sponge or microcystic prostatic parenchyma. In cases with less apparent lesions, structural asymmetry is an important clue for tumor identification. Many subtle lesions are recognized best by their contrast with the contralateral lobe.

After gross inspection of all cross slices, they are serially laid out on the board and photographed to document the block legend. This photograph also records the location and appearance of prostatic lesions, which may also serve a reference image for trainees to become familiar with the gross appearance of prostate cancer (Fig. 1B).

**Sampling and embedding**

There is no consensus among pathologists about how to sample and embed radical prostatectomy specimens. A recent study by Hollenbeck et al. compared complete whole-mount sections and partial sampling. These investigators found no significant difference in pathological outcome between these two sampling methods (Hollenbeck et al., 2000). In a large multi-institutional series, however, Desai et al reported that whole-mount sections of radical prostatectomy specimens increase detection of adverse pathological features, such as extraprostatic extension and seminal vesicle invasion (Desai et al., 2002). Each method of sampling has its own advantages and disadvantages, and should be adopted according to the need and amenity of individual institutes.

**Partial sampling**

Complete histological examination of all prostatic tissue is usually reserved for research in academic institutes, not for routine treatment of prostate cancer in community hospitals. In a survey by the American Society of Clinical Pathologists, only 12% of pathologists embedded the entire prostate for sectioning (True, 1994). Given the limitations of cancer identification by gross examination of radical prostatectomy specimens (Renshaw, 1998), a systemic strategy is critical for partial sampling to succeed at accurate diagnosis and staging. A variety of partial sampling strategies have been described in the literature; therefore, the protocol of partial sampling should be documented in the report.

The protocol by Sehdev et al. (2001)

In a study of 78 patients of clinical stage T1c prostate carcinoma with adverse biopsy features, Sehdev et al proposed the following partial sampling method (Sehdev et al., 2001). In addition to apical and proximal margins and the bases of the seminal vesicles, all the posterior slices and one midanterior section from right and left sides are submitted. If either of the anterior sections shows a sizable tumor, the entire ipsilateral anterior lobe is completely embedded.

The protocol by Hall et al. (1992)

Hall et al. evaluated 104 radical prostatectomy specimens from clinical stage A and B patients. They claimed the following partial sampling method would offer accurate information for pathological analysis (Hall et al., 1992). In each case, proximal and distal margins, the apical slice adjacent to the distal margin, the base of the seminal vesicles and the margins of vasa deferentia are embedded. In addition, any grossly identifiable tumor in clinical stage B patients is submitted in systematically labeled cassettes. If no cancer is grossly identified in clinical stage B patients, alternate slices of the posterior aspect are submitted. For clinical stage A patients, any grossly identifiable lesion and alternate slices of grossly normal prostate are submitted. When adopting the partial sampling strategy for practice, the remaining prostatic sections should be well preserved in the original orientation. Thus when additional sections are needed, they can be taken from a positively identified location in the gland.

**Complete sampling**

Complete sampling requires the entire prostate to be submitted for histological examination. The rationale for complete sampling of prostatectomy is the unique characteristic of indistinct gross appearance of prostate cancer, sometimes indistinguishable from benign tissue and resulting in inadequate sampling if submitting only in parts. Nevertheless, even for completely embedded specimens, less than 0.2% of all the prostatic tissue is available for examination as slides (Humphrey and Walter, 1993). Theoretically, 15,600 slides would be required per case for truly complete microscopical examination (Humphrey and Walter, 1993). There are two methods for complete sampling of radical prostatectomy specimens-- complete sampling with whole-mount sections and complete sampling with routine sections.

**Complete sampling with whole-mount sections**

With whole-mount sections one submits the entire prostate as intact transverse sections mounted on
oversize glass slides without subdivision. The whole-mount sections are identified consecutively with capital letters, always starting from the most apical section. In this manner, the whole radical prostatectomy specimen is available for histological examination, orientation is clearly preserved and entire lesions are studied in three dimensions disclosing their relationships to surrounding structures (Fig. 1D). In our laboratory, we have adopted this approach as routine processing for radical prostatectomy specimens because it offers better integrity of specimens for orientation and tumor volume determination in research studies. However, whole-mount processing requires special handling of larger tissue samples and additional facilities are needed to store the unconventional size blocks and slides. Conventional (rotating) microtomes usually cannot cut whole-mount prostate blocks. We have found that a horizontal sliding microtome (Vibratome, St. Louis, MO) works best for sectioning the large blocks of whole-mount prostate samples. Moreover, immunohistochemistry can only be performed manually on oversize slides and consumes a larger amount of reagents than conventional tissue sections.

Complete sampling with routine sections

One may also submit the entire prostate in conventional cassettes after cutting the transverse slices sufficiently small to fit. Sections are obtained by slicing each transverse section into two halves or four quadrants (Fig. 1C). This approach, yielding a mean of 26 slides per case (Schmid et al., 1992), makes the entire radical prostatectomy specimen available on conventional slides for histological examination. Complete sampling with routine sections eliminates the demand for special storage, dedicated tissue processing and manual immunohistochemical staining. However, it also forfeits the integrity of prostate specimens processed by whole-mount sections. In a study comparing whole-mount and routine sections for complete sampling of 52 patients with clinical stage B prostate cancer, Cohen et al found that discrepancies of pathological features between the two different approaches were found in 25% of cases (Cohen et al., 1994). Of the 20 cases with extraprostatic extension on whole-mount sections, 3 (15%) were missed on routine sections (Cohen et al., 1994).

No residual cancer in radical prostatectomy

If no cancer is identified on the submitted sections, pathologists should embed other slices for histological examination. When tumor is not readily identified on the slides even after submission of the entire prostate, one should cut deeper sections of the selected regions based on the information of previous needle biopsy or even re-cut after block-flipping of all available blocks. If cancer remains undetectable even after re-cutting, one should review the previous sample of needle biopsy and the possibility of incorrect specimen labeling should be considered. Genetic analysis for sample identity can be performed under such a circumstance.

Processing of pelvic lymph node specimens

A precise assessment of metastatic status in pelvic lymph nodes is vital for staging, for prognosis and for selection therapy. However, there is controversy about how to sample tissue from pelvic lymphadenectomy specimens one should examine routinely. Some pathologists suggest submitting all of the pelvic lymphadenectomy tissue only in patients with biopsy Gleason score greater than 7. For cases with biopsy Gleason score 7 or lower, they would only embed grossly identified lymph nodes. If palpable lymph nodes are sectioned entirely, there should be sufficient sensitivity to metastatic carcinoma for correct tumor staging (Epstein et al., 2005). Weingartner proposed approximate 20 pelvic lymph nodes may serve a guideline for a sufficient pelvic lymph node dissection (Weingartner et al., 1996). In one study involving 310 pelvic lymphadenectomy specimens, however, Epstein et al found metastatic tumor in presumed adipose tissue rather than in grossly recognized lymph node in 6.5% of cases (Epstein et al., 1986). Based on this finding, we recommend submitting pelvic lymphadenectomy specimens entirely to avoid misdiagnosis in about 1 case in 20 from examining palpable lymph nodes only.

Conclusion

Radical prostatectomy provides the most precise and comprehensive information for predicting prognosis and deciding about adjuvant therapy for prostate cancer. Consistency and reproducibility of evaluations from radical prostatectomy requires a standardized protocol for tissue fixation, sampling, embedding and processing. Although the recent publication of processing methods has led to a convergence of various procedures, differences still exist in protocols in handling, sampling and processing specimens. Pathologists should be well acquainted of the updated and standardized methods for handling, sectioning and sampling the radical prostatectomy specimens to obtain consistent results of the routine histopathological examination and further enhance patient management.

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