Best practice: gross examination and sampling of surgical specimens from the urinary bladder

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ABSTRACT
This review examines aspects of handling biopsies and surgical specimens from the urinary bladder with the aim of providing guidance to ensure that the pathologist is fully able to inform clinicians of all relevant factors that might have bearing on management or prognosis. It also offers recommendations on good practice in reporting in the setting of the specialist multidisciplinary meeting and emphasises quality control of the process, referring to recently published guidelines and consensuses while admitting that many of the recommendations may not be supported by a strong evidence base. The role of urine cytology and the value of frozen sections in urological practice are discussed. Participation in regular clinical audit and the national urological pathology External Quality Assurance (EQA) are recommended.

INTRODUCTION
An improved clinical outcome in urinary tract disease, particularly urothelial neoplasia, depends on close co-operation between clinicians, radiologists and pathologists. In recognition of this, best practice requires management of patients by multidisciplinary teams. Guidelines by National Institute for Health and Clinical Excellence (NICE) recommend multidisciplinary meetings (MDMs) for management of urological cancers.¹ Cases at high risk of disease progression should be discussed at the specialist MDM, while low risk cases may be managed by local clinical teams based on agreed protocols. Patients with muscle-invasive cancer must be referred to centres performing at least 50 procedures a year.

CLINICAL INFORMATION
The importance of good clinical information cannot be overstated. This information is important in deciding the approach to the specimen and also indicates the information required from the reporting pathologist. For instance, there is little point in an exhaustive examination of a specimen of a primary tumour if the patient has distant metastasis, and the resection is palliative. The pathologist is reliant on the clinician to provide the relevant clinical information, and this can be actively encouraged by agreeing on protocols for items of clinical information (box 1) or even devising a special request form. The latter would improve provision of relevant clinical information and also facilitate audit. This should be given consideration, especially as major urological surgery is increasingly being carried out in fewer centres as a result of national reconfiguration of cancer treatment.

EXAMINATION OF BIOPSIES AND TUR SPECIMENS
Collection of specimen
Either pots of suitable fixative may be provided to clinical areas or the tissue may be collected fresh (transported dry and unfixed on ice) for research and tissue banking. Specimens from different sites should be appropriately labelled. Adequate fixation is essential for good morphology required for grading of papillary neoplasia and for the recognition of flat in situ neoplasia. The type of fixative and length of fixation should be consistent in any particular laboratory to ensure good morphology and an optimum substrate for subsequent immunohistochemistry (IHC). Rapid processing with microwave-assisted fixation may be appropriate under some circumstances, however, not at the expense of good morphology.

Macroscopic description and processing
Endoscopic biopsies of urothelial tumours and mucosa are usually 2–3 mm in diameter. Macroscopic description should include the number and size of the biopsies. Accurately recording the number of the biopsy fragments ensures that the BMS and pathologist can be confident that all pieces received have been examined. TUR resections may be described as either the settled volume or total weight of the material submitted.

It is suggested that biopsies and smaller TUR specimens up to 10 g should be embedded fully. For larger specimens, sufficient tissue must be...
Box 1 Clinical information for bladder tumours

- Demographic information of patient and details of destination for the report.
- Clinical history—whether this is the first presentation; if not, details of previous resections.
- Details of treatment (radiotherapy and local or systemic chemotherapy), as these have an impact on tumour volume and morphology.
- Cystoscopic appearance: size, number and location of tumour(s), whether entirely papillary or any solid areas present, and also the state of background mucosa, for example, red patches suggesting carcinoma in situ.
- Clear indication of sites of lesions sampled (these may be represented diagrammatically).
- Special investigations performed for example blue-light cystoscopy or bladder washings for cytology; serum PSA levels to guide sampling of the prostate if cystoprostatectomy is planned.

processed to allow assessment of muscle invasion. In cases of tumours without histological invasion of either the lamina propria or the muscularis propria, all tissue should then be processed. Some may consider this a counsel of perfection, as some resections may generate a large number of blocks; however, these cases are relatively rare. A biopsy of the tumour base in a separate container is recommended by European Association of Urology (EAU) guidelines. Although this should include muscularis propria, it is not reliable for staging but may be regarded as a marker of complete local resection.

Levels
Small biopsies and resections of less than 1 g should be examined at a minimum of three levels. Levels are imperative on biopsy material, as orientation of the tissue at embedding is impossible. Biopsies are frequently not fully covered by urothelium, or much urothelium is lost due to epithelial discohesion in pathological conditions. Sections through the tissue are needed to find appropriately orientated urothelium (or any urothelium at all). Levels are also recommended in small TUR specimens (one or two blocks only). When levels are taken, it is important to keep some spare sections. There are two reasons for this. First, assessment of stage in urothelial neoplasms is the most important and often the most difficult call. Identification of tumour cells in lamina propria or smooth muscle may be assisted by IHC. Second, grading of dysplastic changes in flat urothelium and of papillary tumours, and the distinction of reactive change from neoplastic requires thorough examination through the tissue block and these distinctions may also be assisted by IHC, particularly if there is traumatic or diathermy artefact.

Further investigations on biopsy
IHC for cytokeratins and smooth muscle actin may be used to assist staging in biopsy and TUR material. It is also useful in ensuring that eosinophilic spindle cell tissue is smooth muscle and not fibroblastic reaction to previous surgery.

A combination of CK20 and p53 may help in distinguishing reactive atypia from dysplasia and carcinoma in situ (CIS). Normal urothelium expresses CK20 in the umbrella cell layer, and p53 is only focally present in normal urothelium. In dysplasia and CIS, there is full-thickness cytoplasmic positivity for CK20 and nuclear positivity for p53 throughout the urothelium.

More recently, smoothelin has been described as an antibody that stains the smooth muscle fibres of muscularis propria but not those of the muscularis mucosa, hence it holds promise in confirming tumour invasion into the former in difficult cases. Invasion of the muscularis propria is a key consideration in making the decision for cystectomy.

Examination of cystectomy specimens
The pathologist reporting the cystectomy specimen should review any preceding relevant histological and cytological material. If radical surgery and pathological examination are to be performed at a centre different from where the diagnostic resection was performed, the relevant material should be forwarded to the centre. Review of slides at the centre should be recorded in the form of a report, a copy of which should be sent to the consultant who originally reported the case. Slides and any blocks borrowed should be promptly returned following review and discussion at the MDM.

The pathologist may receive a number of variations of a cystectomy specimen. These may be radical or partial cystectomy or diverticulectomy. Radical cystectomy may be open, laparoscopic or robot-assisted and may include a partial or total urethrectomy. The purpose of examination is the same for each—to confirm the presence of tumour, its histological type, stage and completeness of excision. In addition, following chemo/radiotherapy, the effect of treatment may be assessed, and with new operating modalities, there is a role in assessing the quality of surgery.

Collection of fresh tissue
Where facilities exist, specimens should be collected fresh and selected samples of tumour and normal tissues stored in order to enable ongoing and future research. It is important that sampling does not compromise the histopathological assessment of tumours and should be carried out under the direct supervision of or by the histopathologist. This would also enable a role for either a BMS or other trained staff in collecting and sampling of specimens alongside pathologists.

Procedures for consent for tissue retention must be in place, and there should be a strong drive to develop tissue banks alongside cancer centres to best avail this resource.

Macroscopic description and sampling
Currently accepted methods of examining the bladder as described in standard texts are to fix either open (pinned out) or by distension (with formalin), to examine intact and after bisection and to take blocks of tumour and other abnormal areas identified by visual examination of the luminal surface. An alternative method is proposed here that offers the following advantages: it is algorithmic and ensures complete macroscopic examination of the full thickness of the bladder wall so that the site of deepest penetration of the tumour is reliably identified. Furthermore, the slices may be compared with transverse CT or MRI scans allowing correlation with radiology. It is strongly recommended that reports of imaging and previous resections be reviewed immediately before cut-up. This guides sampling of relevant margins especially in areas where extra-vesical spread is suspected.

Distension with formalin
The bladder may be fixed by distension with 150–250 ml of formalin in the operating theatre allowing some fixation of the mucosa to take place before the specimen reaches the pathology laboratory. The bladder is distended through a catheter, with
leaks sealed with soft clamps or loosely tied ligature or stitches (figure 1). The whole bladder is then suspended in formalin.

**Inking the specimen and separation of prostate**

On receipt in the lab, the formalin is drained and the catheter removed. The non-peritonealised margins overlying the tumour may be inked. The prostate gland and seminal vesicles may also be inked. The prostate may be severed at the bladder neck and examined in slices as described below (figure 2).

**Sampling ureteric and urethral margins**

Identify the ureteric margins and block a single slice of each. This is not necessary if ureteric margins have been submitted for frozen section and circumferential sections are available. Sections should be taken from attached segments of ureters on the cystectomy specimen; if sent separately, orientation of proximal and distal ends as indicated by sutures or clips should be recorded. The urethral shave margin may be sampled at this stage after removing the catheter. These margins may be put into labelled cassettes and left in the container until the remainder of the specimen is examined. In female patients, the urethra is almost always included with the cystectomy. In the male patients, the prostatic apical margin is the distal urethral margin.

**Dimensions of the specimen**

The dimensions of the specimen including those of the bladder, prostate, seminal vesicles and any other attached organs (eg, urethra, uterus and adnexa) should be recorded. Any gross abnormality such as tumour penetrating the serosa or at a surgical margin should be noted.

**Bisection of the bladder and description of tumour**

The bladder with attached organs and structures is then bisected (figure 5). Bisection may be carried out in the sagittal plane for lateral tumours or in the coronal plane for posterior tumours. Photographs of the luminal surfaces may be taken at this stage. The macroscopic description of the internal surface should include the size, site and appearance of any macroscopic lesions and the state of the background mucosa.

**Slicing and sampling**

Each bisected half of the bladder is then sectioned at 5 mm intervals and individual slices laid out from bladder neck to fundus (figure 4). The direction of slicing is important; in most cases, slicing in the transverse plane will give the best orientation and will match tomographic scans. If the tumour is situated at the fundus, slicing the bladder perpendicular to the surface...
of the tumour might provide better orientation. Blocks recommended from a cystectomy specimen are shown in box 2. Sites from which the blocks are taken may be marked on photographs. A line diagram with the block key would also suffice. In bladder resections without evident tumour (increasingly seen as tumour may be focal), extensive sampling is recommended, as the residual mucosa haemorrhagic, granular, ulcerated, firm white (squamous) appearance. Normal appearing mucosa.

Prostate—one slice in large or small blocks as a minimum; consider further sampling if significant prostatic carcinoma found on initial sampling.

Seminal vesicles—in continuity with any overlying bladder tumour or one block from each seminal vesicle.

Uterus, cervix and vaginal margin—in continuity with bladder tumour; representative blocks from the uterus and cervix, vaginal margin, fallopian tubes and ovaries.

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Assessment of ureteric margins is sometimes difficult due to freezing artefact but also has significant clinical limitations. Skip-
Partial cystectomy/diverticulectomy and resection of the urachus
Specimens from these procedures are not common, and each one will present its own set of questions and problems. There is little substitute for discussing the approach to the specimen with the referring surgeon/oncologist before dissection. Orientation of the specimen may be assisted by the use of sutures by the urologist and by inking the relevant margins at cut-up.

REPORTING
Reporting of histological grade and stage
RCPath cancer datasets recommend the use of WHO 1975 and the optional use of WHO 2004 classifications for the grading of urothelial carcinoma. Despite its limitations, there is a wealth of follow-up data on the WHO 1975, and the WHO 2004 is currently not widely used in the UK, there being no evidence to suggest that it is superior when used in clinical management. The current TNM stating the year should be used in reporting the stage of the tumour.

Quality assurance
As well as assuring themselves that they are providing a high-quality service, pathologists need to provide evidence of quality to clinicians and ultimately to patients. Quality assurance is thus an essential part of best practice. Documentation of external schemes of accreditation such as CPA is appropriate for the laboratory as a whole. Participation in appropriate External Quality Assurance (EQA) schemes by pathologists is recommended by NICE in relation to urological cancer services and may be important for revalidation. There is currently a national slide-based uropathology EQA scheme in the UK. Histopathologists with an interest in uropathology may seek information for participation by emailing eqadmn@uhl-tr.nhs.uk.

Further evidence of quality may include documenting routine double reporting of appropriate cases according to a rule base, for example, cases that include treatment thresholds such as the presence of possible microinvasion or muscle invasion.

Clinical audit is also an essential part of quality assurance. As outcome data are not likely to be valuable in assessing quality of this complete process, regular audit of both the process and reports should be used as a tool to monitor and improve the quality of the service. Subjects suitable for audit include turnaround times of reporting, conformity of reports to college datasets, number of radical resection specimens with appropriate photographs, presence of muscularis propria in resected specimens, etc.

Data collection
Data should be collected prospectively to allow periodic review of cancer services. The MDM should allow centralised collection of data for audit, research as well as recruitment into clinical trials.

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REFERENCES
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