Chapter 2
Indication, Collection, and Laboratory Processing of Cytologic Samples

Keywords Indications for urine cytology • Follow up of bladder cancer • Methods of collection • Voided urine • Catheterized urine • Direct sampling techniques • Bladder washings • Bladder barbotage • Direct brushings • Ileal conduit • Retrograde catheterization • Liquid-based preparations • ThinPrep • SurePath • Advantages and disadvantages of various preparations • Laboratory processing techniques for liquid-based preparations • Fixatives for liquid-based preparations • Space-occupying lesions • Occult malignant tumors

Urine is an acellular liquid product of renal excretory function. As liquid passes through the renal tubules, renal pelvis, ureter, urinary bladder, and urethra, it picks up desquamating cells derived from the epithelia of these organs. Inflammatory cells, erythrocytes and macrophages are frequently seen. Voided urine has an acid pH and a high content of urea and other organic components; therefore it is not isotonic. Consequently, urine is not a hospitable medium for desquamated cells, which are often poorly preserved and sometimes difficult to access microscopically.

General

Urinalysis is a standard procedure on all patients. Detecting the presence of albumin, glucose, ketone, bilirubin, occult blood, nitrate, leucocyte esterase, and a superficial analysis of urinary sediment are routine. Machines capable of automated procedures in the assessment of these factors are in wide use and are helpful in the diagnosis and follow-up of a wide variety of diseases. In patients whose lower urinary tract is intact the epithelial sediment is very scanty.

The principal indications for use of cytology in disorders of the lower urinary tract (bladder, urethra, ureters, and renal pelves) are as follows:

1. The diagnosis of high grade urinary cancers, most importantly, flat carcinoma in situ; the cytologic techniques are of a very limited value in the identification of well-differentiated (low grade) papillary tumors.
2. Routinely used in the follow-up of patients with a history of bladder cancer because close monitoring of patients is essential for the early detection of recurrences. The current standard of care consists of cystoscopy and cytology every 3–6 months for the first year and at reduced intervals subsequently.

For the upper urinary tract, cytologic techniques serve to may identify the nature of space-occupying lesions.

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Methods of Specimen Collection

The principal methods of specimen collection are:

- Voided urine
- Catheterized urine
- Direct sampling techniques
  - Bladder washings or barbotage
  - Cell collection by retrograde catheterization of ureters
  - Direct brushings

The selection of method of specimen collection and processing depends on clinical circumstances and the goal of the examination. Advantages and disadvantages of the various methods are summarized in Table 2.1 and in Chap. 3, Table 3.1.

Voided Urine

This is by far the easiest and the least-expensive method of cytologic investigation of urinary tract. The technique is valuable as preliminary assessment of broad spectrum of abnormalities of the urethra, bladder, ureters, and renal pelves.

Table 2.1 Principle advantages and disadvantages of various cytologic preparatory techniques for urinary specimens

Method	Advantages	Disadvantages
Cytocentrifugation	Simple, large-sized clusters, better-preserved architecture	Air-drying artifact; multiple slides need to be prepared due to cell loss; more unsatisfactory or less than optimal specimens
Membrane Filter	Good morphology	Difficult to prepare, rapid drying makes storage difficult, cells are distorted by pores, cells that are placed in various planes of focus makes screening tedious, background may not be clean, requires fresh specimens as prefixation coagulates proteins that clog the filter; longer screening time
ThinPrep (TP)	Standardized and easy preparation, monolayer, increased cellularity, better preservation, decrease in less-than-optimal specimens, uniform cell distribution, clean background, shorter and easy screening, multiple slides can be prepared, additional cost is offset by improved specimen quality	Some alteration of key nuclear morphologic features and background elements; fragmentation of cell clusters, cell shrinkage; more expensive than conventional preparations
SurePath (SP)	Standardized preparation, excellent cell yield, preservation and morphology, multiple slides can be prepared, slides are stained on the processor	Cells that are in various planes of focus make screening and focusing at high magnification tedious

Collection

Usually the best specimen is the morning's second voiding. Three specimens obtained on 3 consecutive days are diagnostically optimal. Unless the urine is processed without delay, the addition of a fixative is recommended. Small volume of urinary specimen can also be submitted in one of the fixatives described in the liquid-based specimen processing technique section below.

Specimen Fixation

Fresh urine samples that are delivered to the laboratory for processing within 6–12 h of voiding do not require fixation. These specimens should be promptly processed. Specimens fixed in 50% ethanol do not require refrigeration for 24–48 h. Specimens fixed in liquid collection media do not require refrigeration and are described below.

Catheterized Urine

The specimens are collected via a catheter and processed like voided urine as described below.

Direct Sampling techniques

Bladder Washings and Barbotage

This technique may be applied during cystoscopic examination or via a catheter to obtain well-preserved cells from clinically high-risk patients. It is the specimen of choice for DNA ploidy analysis of the urinary epithelium. Bladder washings have significantly better diagnostic yields. The bladder should first be emptied by catheter. Bladder barbotage is then best performed during or prior to cystoscopy by instilling and recovering three to four times 50–100 ml of normal saline or Ringer's solution. The procedure can also be performed through a catheter but is uncomfortable, particularly for male patients, and the results are less satisfactory.

Retrograde Catheterization of Ureters or Renal Pelves

This procedure is used to establish the nature of a space-occupying lesion of ureter or renal pelvis, observed by radiologic techniques. Differential diagnosis of a space-occupying lesion includes a stone, a blood clot, or a tumor. Other rare space-occupying lesions of the renal pelves are inflammatory masses, angiomas, and congenital aberrations of the vascular bed. In the ureters, other causes include a stricture or extraneous pressure.

Another important application of this technique is the localization of an occult malignant tumor diagnosed in voided urine sediment but not found in the bladder. The purpose is to determine whether the tumor can be localized in the left or right kidney or ureter. For urine collection, separate catheters must be used for each side to avoid cross-contamination. Although, the procedure may be tedious to the patient, it is quite efficient in localizing the lesion.

The Direct Brushing Procedure

This procedure is used in the investigation of space-occupying lesions in the ureters or renal pelves. Brushing is performed through a ureteral catheter. The indications are same as listed for retrograde catheterization.

Ileal Bladder Urine

After cystectomy for malignant tumors of the urinary bladder an artificial bladder is often constructed from segments of the small intestine, notably the ileum. Because of the propensity of urothelial tumors to sequentially affect various segments of the lower urinary tract (and sometimes segments of the intestinal tract), the status of these organs must be monitored after treatment. Collection of urine from the ileal bladder serves this purpose well.

Laboratory Processing of Urinary Specimens

Several techniques are available for processing urinary specimens for microscopy including liquid-based preparations (LBP), cytocentrifugation, and membrane filters. Only the two currently available LBP, including ThinPrep [TP (Hologic, Bedford, MA)] or SurePath [SP (BD Diagnostics, Burlington, NC)] will be described in this chapter. Details on semiautomated Bales' Method, cytocentrifugation, and membrane filters preparatory techniques have previously been documented in detail in Koss's Diagnostic Cytology and Its Histologic Bases, by Koss LG and Melamed MR, 5th edition, 2006 and Diagnostic Cytology of the Urinary Tract by Koss LG, 1996. Liquid-based preparations are gaining popularity in the last few years for processing non-gynecologic specimens, particularly, urinary specimens. It is designed to prepare uniform monolayer cells on a glass slide with minimal background blood and cell debris. Table 2.1 lists the various preparatory techniques, their advantages and disadvantages. Liquid-based urinary samples can be collected fresh or in the proprietary liquid preservative media for processing.

Liquid-Based Processing Techniques

Two LBP currently in use include SurePath (SP) and ThinPrep (TP). Both LBP techniques reduce debris and cell clumps and also homogenize the specimen. Due to smaller screening area the slide screening time is reduced and the cleaner background makes it easier to determine whether the cells are normal or abnormal. Immediate liquid fixation prevents air-drying. Despite the difference in preparatory techniques, both TP and SP are similar in morphologic appearance. Technical details for the two LBP are provided in Figs. 2.1 and 2.2 and Tables 2.2, 2.3, and 2.4 outline technical, general cytologic, and specific cellular features for the LBP.

SurePath

Principle

The principle of SP is density gradient-based cell enrichment process. It is a semi-automated technique. Specimen is processed on the PrepStain processor.

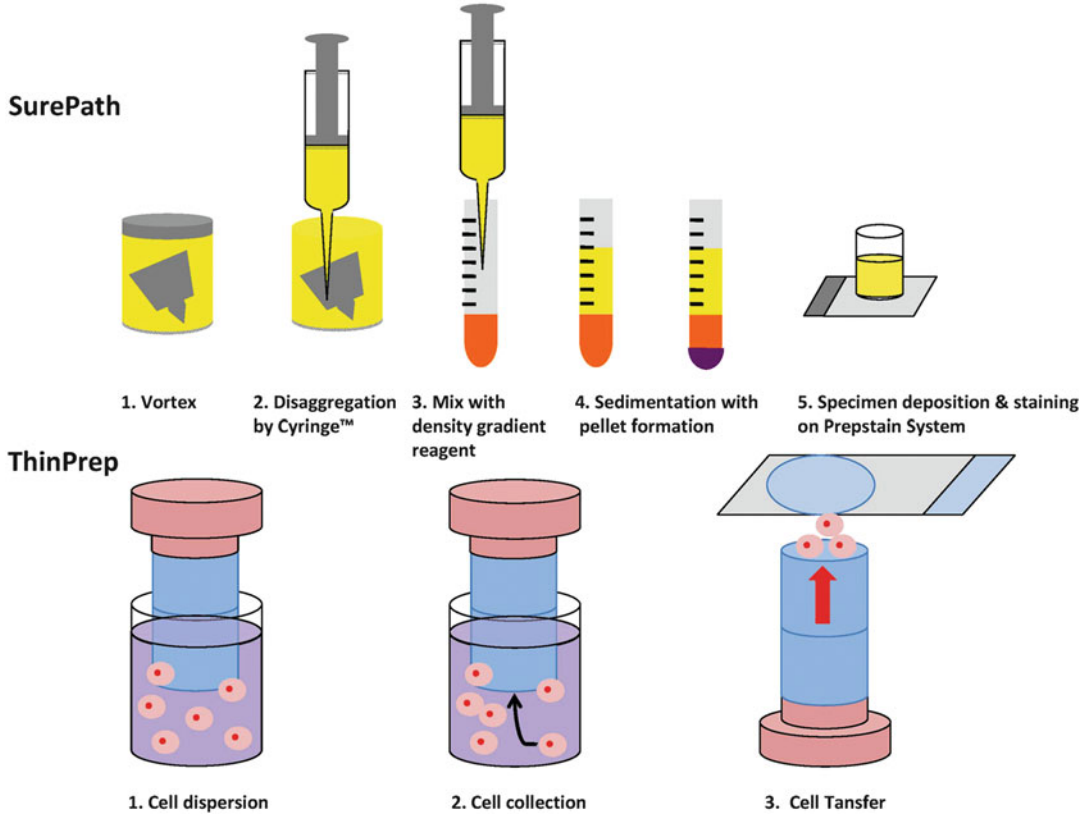


Fig. 2.1 SurePath™ Processing technique: (1) Sample is collected in CytoRich®-(ethanol-based SP preservative fluid) filled collection vial and vortexed; (2) A SP proprietary device, CyRinge, is inserted into the collection vial to disaggregate larger cell fragments; (3) The CyRinge is then inverted over a labeled 15-ml centrifuge tube filled with 4 ml of SP density gradient reagent, a polysaccharide solution that acts to trap small particulates and debris. The samples flow through the drainage tube on the CyRinge onto the density gradient; (4) Specimen is transferred to a sedimentation tube and centrifuged to form a cell pellet, which is resuspended and the sedimentation is repeated; (5) Sample processing is completed using the PrepStain™ slide processor, where a robotic arm transfers the fluid to a settling chamber, which sits atop a modified poly-L-lysine-coated glass slide. Robotic arm then stains the slides on the PrepStain™. ThinPrep™ Processing technique: (1) Cell dispersion. A cylinder with a polycarbonate thin filter attached to one end is introduced into the specimen vial and gently rotated creating a current that disaggregates mucus, blood, and other debris; breaks up large cell clusters; and mixes and homogenizes the cell suspension; (2) Cell Collection: A gentle vacuum is applied to the cylinder that causes most of the broken erythrocytes and debris to pass through the filter pores, while the cells adhere to the filter. The instrument monitors cell density across the filter and the flow rate decreases when cells are evenly distributed on the filter with minimal overlap; (3) Cell Transfer: The cylinder moves out of the specimen and is lightly pressed against a positively charged slide. A slightly positive air pressure is applied to transfer the cells to the slide. The slide is immediately dropped into 95% ethanol fixative. Slide is removed from the processor and may be stained either manually or by an automatic stainer (modified from: Cibas ES and Ducatman BS. Liquid-based preparation methods. In Cytology. Diagnostic Principles and Clinical Correlations. 3rd ed., Saunders, Philadelphia, PA, 2009, p. 5. Drawing by Raza Hoda, Columbia University College of Physicians and Surgeons, New York, NY)

Specimen Collection and Fixation

Non-gynecologic specimens are collected in one of the following: CytoRich® Preservative Fluid (an ethanol-based medium, which also lyses blood), in 50% alcohol, or as fresh, unfixed specimens. Specimen is labeled and transported to the cytology laboratory.

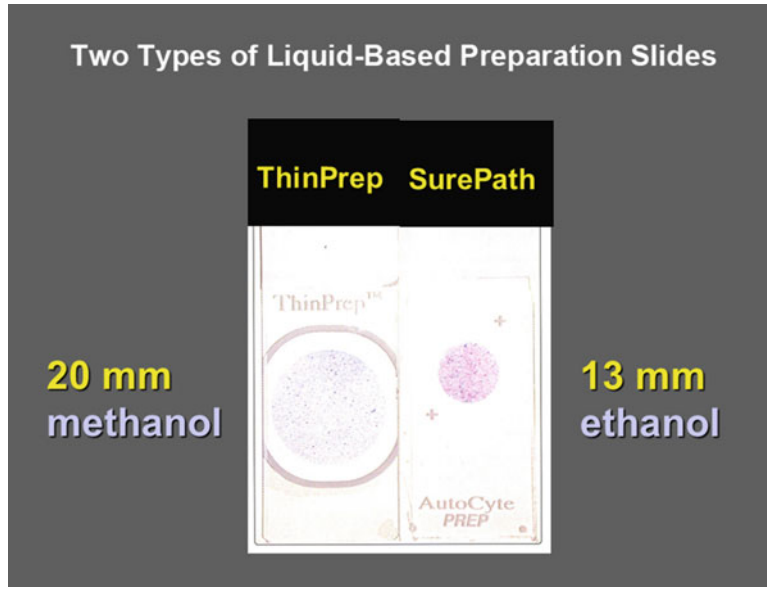


Fig. 2.2 ThinPrep (TP) and SurePath (SP) Slides: For the TP slide, the circle where the cytologic material is deposited has a diameter of 20 mm. The specimen collection preservative medium is methanol based. For SP slide, the diameter of the circle is 13 mm, and the specimen collection preservative medium is ethanol based

Table 2.2 Technical differences between LBP preparations

Features	ThinPrep	SurePath
Cost	Expensive	Less expensive
Sample collection	Uniform	Uniform
Sample transfer	Entire	Entire
Fixation	Immediate	Immediate
Transport	Easy	Easy
Slide preparation	Fully automated	Partial automation
Slide evaluation	Easier	Easy
Cells deposition	Well defined 20-mm-diameter area	Well defined 13-mm-diameter area
Cell preservation	Good	Good
Obscuring factors	None	None
Air-drying	None	None
Screening time	Reduced	Reduced
Reproducibility	Yes	Yes
Ancillary studies	Possible	Possible

Table 2.3 General cytologic features on LBP preparations

Features	ThinPrep	SurePath
Quality	Enhanced	Enhanced
Background		
Clean	Yes	Yes
RBCs	Reduced	More reduced
Neutrophils	Reduced	Reduced
Necrosis	Clumped	Clumped
Cellularity	Lower	Higher
Cell distribution	Uniform, one plane of focus	Uniform, thick, different planes of focus
Cell size	Smaller	Small
Architecture	Less well-preserved	Better preserved
Cytomorphology	Preserved	Preserved
Extracellular material		
Quantity	Reduced	Less reduced
Appearance	Altered	Less altered

Table 2.4 Specific cellular features of LBP preparations

Features	ThinPrep	SurePath
Architecture		
Fragmentation	Present ++	Present +
Monolayer cells	+	−
Cell clusters	Present, 3D	Present, thick, 3D
	Flat, smaller	>Depth of focus
	Cohesive	Cohesive
	Minimal overlap	More overlap
Flattening	More	Less
Cellular morphology		
Shape	More rounded	Rounded/elongated
Nucleus		
Detail	Enhanced	Enhanced
Nucleoli	More prominent	Preserved
Inclusions	Less apparent	Preserved
Cytoplasm		
Detail	May be denser	May be denser
Shape	Retained	Retained
Elements[a]	Preserved	Preserved

+ present, − not present

[a] Cytoplasmic elements include: vacuolations, pigment, PMNs

Slides

Pre-coated slides are provided by the company and are marked with a 13-mm-diameter circle. The slides can also be freshly prepared in the laboratory for use for 48 h. The slides are coated with a modified poly-L-lysine and air-dried. These positively charged slides allow diagnostic cells to settle out of solution and adhere to the surface.

Processing

1. Mix entire specimen to resuspend cellular material. In large-volume samples, pour off one or two aliquots into 50-ml centrifuge tubes, cap securely, and centrifuge for 10 min at 1,800 rpm to concentrate the specimen.
2. Decant off the supernatant and add 10 ml of distilled water.
3. Vortex the cell button to form a homogeneous cell slurry. Transfer 5–10 drops of the cell slurry to 10 ml of CytoRich® Red Preservative Fluid and mix to an even cell suspension. Sample may also be received in a vial with CytoRich collection fluid.
4. Specimen collection vials are vortexed. CytoRich fluid lyses most of the red blood cells.
5. A SurePath proprietary device, CyRinge®, is inserted into the collection vial to disaggregate larger cell fragments. CyRinge is a syringe-like device consisting of a double-layered mesh screen with 18-gauge holes in its base and a drainage tube extending from its top.
6. The CyRinge is then inverted over a labeled 15-ml centrifuge tube filled with 4 ml of SurePath density gradient reagent, a polysaccharide solution that acts to trap small particulates and debris. The samples flow through the drainage tube on the CyRinge onto the density gradient reagent.
7. Specimen is transferred to a sedimentation tube to concentrate the cellular components of the specimen by centrifuging the specimen on the Hettich Centrifuge $600 \times g$ for 10 min. A cell pellet is formed, which is resuspended and the sedimentation is repeated.

8. Load the centrifuge racks onto the PrepStain Processor and run Program 2 for Non-GYN specimens. A robotic arm transfers the fluid to a settling chamber, which sits atop a modified poly-L-lysine-coated glass slide. Robotic arm then delivers sequential stains to each settling chamber to stain the specimen. The result is a 13-mm circular smear. PrepStain System processes and stains up to 48 specimens per run.

ThinPrep

Principle

ThinPrep preparation is a filter-based cell concentration technique. TP-2000 processor is a semiautomated device, which processes one specimen at a time. A newer version, TP-5000, is a fully automated bench-top instrument that processes specimens in batches of 20. Multiple preparations can be made from a single vial.

Specimen Collection and Fixation

Sample is collected by the clinician in one of the following: CytoLyte® solution (methanol-based fixative, which is both hemolytic and mucolytic), in saline, or as fresh, unfixed specimens. Specimen is labeled and transported to the cytology laboratory.

Slides

The microscopic slides are provided by the company and are marked with a 20-mm diameter circle.

Steps in Preparation

Urine is first centrifuged at 1,500 rpm and cell pellet is then resuspended in 30 ml of Cytolyte and again centrifuged. Two to three drops of the cell pellet are transferred to PreservCyt (methanol-based preservative solution). The vial and a labeled slide are placed into the ThinPrep processor. Preparatory steps include specimen dispersion, collection, transfer, and staining.

(a) *Dispersion*. A disposable cylinder with a polycarbonate filter attached to one end is introduced into the vial. The pore size of the filter is 5.5 μm (pore size for Pap test specimen is 8 μm). The instrument is rotated creating a current that disaggregates blood, mucus, and other debris and breaks up large cell clusters, mixes and homogenizes the cell suspension.
(b) *Collection*. A gentle vacuum is applied to the cylinder, which aspirates the cell suspension through the filter. Most of the broken red blood cells and debris is allowed to pass through while the diagnostic cells attach to the external surface of the filter. The instrument monitors cell density across the filter and the flow rate decreases when cells are evenly distributed on the filter with minimal overlap.
(c) *Transfer*. the cylinder moves out of the specimen, is inverted 180°, gently pressed against a positively charged slide and with slight positive pressure transfers the cells (~70,000) to the glass slide. The result is a 20-mm circular smear with even distribution of cells and minimal overlap.

The slide is immediately dropped into 95% ethanol fixative. Preparation time ranges between 30 and 90 s depending on cell concentration.

(d) *Staining*. Papanicolaou staining is either performed manually or in an automatic stainer. The staining process takes 30 min. Papanicolaou stain of fixed samples offers the best option of judging the fine details of cell structure. All illustrations in this book are stained with this method.

Residual LBP Specimen

The shelf life of the residual specimen for SP and TP is 3 weeks and 3 months, respectively, at room temperature. Residual specimen can be used for immunochemistry, molecular tests such as UroVysion (Chap. 7) or processed as a cell block. Cellient (Hologic, Bedford, MA) is a new automated cell block machine based on centrifugation and filtration and can capture cells from a low cellularity specimen such as a urinary specimen.

Liquid-based preparations (LBP) are increasingly being used for urinary cytology. The LBP have several advantages over the conventional preparatory techniques including standardized preparation, uniform (monolayered) cell distribution, lack of obscuring elements, better cell preservation with enhanced nuclear detail due to immediate wet fixation, and decreased rate of unsatisfactory specimens. These features of LBP have resulted in increased diagnostic accuracy compared to conventional preparations.

Residual material from LBP fixed in the proprietary fixatives may also be used for ancillary studies such as immunocytochemistry and fluorescence in situ hybridization.

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