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Practical Procedures in Pediatric Nephrology



Editors
Subal Kumar Pradhan
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CHAPTER 4

Urinalysis

Urmila Anandh, Varsha Vadera, Shiva Priya Eswaran

INTRODUCTION

Urinalysis is the oldest of all clinical laboratory examinations. It was practiced centuries ago by the "Pisse prophet" who claimed to divine the mysteries of disease by examining the yellow liquid. With the application of proper care and attention, it is one of the most valuable of all clinical laboratory examinations and provides a large amount of medical information in a cost-efficient way. Traditionally, urine examination is divided into a routine examination and a microscopic examination. Besides, urine is also subjected to various biochemical examinations and is often cultured to obtain the type of organisms responsible for urinary tract infections.

METHODS OF URINE COLLECTION

- Random collection taken at any time of the day with no precautions regarding contamination. The sample may be dilute, isotonic, or hypertonic and may contain white cells, bacteria, and squamous epithelium as contaminants. In females, the specimen may contain vaginal contaminants such as trichomonads, yeast; and during menses red cells.
- Early morning collection of the sample before ingestion of any fluid. This is usually hypertonic and reflects the ability of the kidney to concentrate urine during dehydration, which occurs overnight. If all fluid ingestion has been avoided since 6 pm, the previous day, the specific gravity usually exceeds 1.022 in healthy individuals.
- Clean-catch, midstream urine specimen collected after cleansing the external urethral meatus. A cotton sponge soaked with benzalkonium hydrochloride is useful and non-irritating for this purpose. A midstream urine is one in which the first half of the bladder urine is discarded and the collection vessel is introduced into the urinary stream to catch the last half. The first half of the stream serves to flush contaminating cells and microbes from the outer urethra prior to collection. This sounds easy, but it is not (try it yourself before criticizing the patient).

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- Catheterization of the bladder through the urethra for urine collection is carried out only in special circumstances, i.e. in a comatose or confused patient. This procedure risks introducing infection and traumatizing the urethra and bladder, thus producing iatrogenic infection or hematuria.
- Suprapubic transabdominal needle aspiration of the bladder. When done under ideal conditions, this provides the purest sampling of bladder urine. This is a good method for infants and small children.

Another important factor is the interval of time which elapses from collection to examination in the laboratory. Changes which occur with time after collection include: (1) decreased clarity due to crystallization of solutes, (2) rising pH, (3) loss of ketone bodies, (4) loss of bilirubin, (5) dissolution of cells and casts, and (6) overgrowth of contaminating microorganisms. Generally, urinalysis may not reflect the findings of absolutely fresh urine if the sample is >1 hour old. Therefore, get the urine to the laboratory as quickly as possible.

For culture prompt fresh urine or refrigerate at 4° can be used if loop method of colony count is to be done. For dipstick method, a fresh urine sample within 2 hours should be examined or the sample should be refrigerated immediately for 24 hours.

ROUTINE ANALYSIS OF THE URINE

Urine is physically examined for its clarity and color. Turbidity of the urine is caused by excessive cellular material or protein in the urine. It may also develop from precipitation of its salts and crystals. The chemical analysis of the urine is usually done by the dipstick **(Figs 4.1 and 4.2)**.

The chemical constituents of the urine analyzed by the dipstick are:

pH

The glomerular filtrate is usually acidified by renal tubules from a pH of 7.4 to about 6 in the final urine. However, depending on the acid-base status, urinary pH may range from as low as 4.5 to as high as 8.0. Failure to acidify the urine in a child with normal renal functions is seen in renal tubular acidosis.

Specific Gravity

Specific gravity between 1.002 and 1.035 on a random sample should be considered normal, if kidney function is normal. A fixed specific gravity of 1.010 is a marker of lack of concentrating ability and is called isosthenuria.

Any urine having a specific gravity over 1.035 is either contaminated, contains very high levels of glucose, or the patient may have recently received high-density radiopaque dyes intravenously for radiographic studies or low molecular weight dextran solutions.

Protein

Dipstick screening for protein is done on whole urine, but semi-quantitative tests for urine protein should be performed on the supernatant of centrifuged

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Fig. 4.1: Urine dipstick analysis

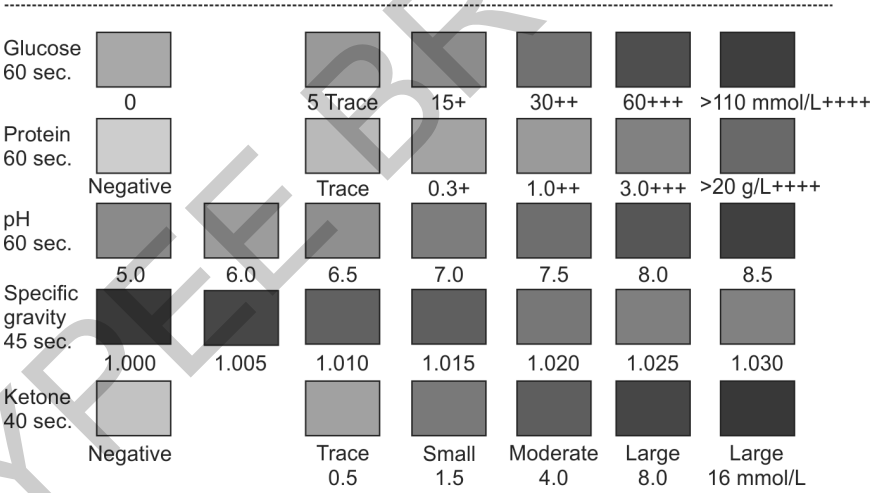

Fig. 4.2: Various tests done by dipsticks *(For color version see Plate 1)*

urine since the cells suspended in normal urine can produce a falsely high estimation of protein. Dipstick detects albumin but is insensitive to Bence-Jones proteins.

False-positive results occur in alkaline urine (pH more than 7.5), when the dipstick is immersed too long, with highly concentrated urine, with gross hematuria, in the presence of penicillin, sulfonamides or tolbutamide, and with pus, semen or vaginal secretions.

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False-negative results occur with dilute urine (specific gravity more than 1.015) and when the urinary proteins are nonalbumin or of low molecular weight.

The results are graded as negative (<10 mg/dL), trace (10–20 mg/dL), 1 + (30 mg/dL), 2 + (100 mg/dL), 3 + (300 mg/dL) or 4 + (1,000/per dL).

Dipsticks also detect microalbumin by an immunological test strip called Micral test.

Glucose

Dipsticks employing the glucose oxidase reaction for screening are specific for glucose but can miss other reducing sugars such as galactose and fructose. For this reason, most newborn and infant urines are routinely screened for reducing sugars by methods other than glucose oxidase (such as the Clinitest, a modified Benedict's copper reduction test).

Ketones

Ketones (acetone, acetoacetic acid, beta-hydroxybutyric acid) result from either diabetic ketosis or some form of caloric deprivation (starvation) and are easily detected using dipsticks. Acetoacetic acid, an intermediate product of fat metabolism, is excreted in the urine and gets slowly converted to acetone if the urine is left at room temperature.

Nitrite

A positive nitrite test indicates that bacteria (Gram negative) may be present in significant numbers in urine. *Enterococcus, Staphylococcus* and *Pseudomonas* are partial nitrite formers. The detection rate increases if the urine is held in the bladder for at least 4–6 hours. Thus, a first void sample is used. High doses of ascorbic acid produces false-negative results.

Leukocyte Esterase

A positive leukocyte esterase test results from the presence of white blood cells either as whole cells or as lysed cells. A negative leukocyte esterase test means that an infection is unlikely.

MICROSCOPIC EXAMINATION OF URINE

Microscopic examination of the urine consists of examining the urine for the presence of cells, casts, crystals, and bacteria. Urine microscopy should be performed on any patient who has persistent hematuria or proteinuria and may be useful if the urine dipstick is suggestive of urinary tract infection (UTI).

Procedure

Microscopic examination is to be done if possible in 1–2 hours; and if it is not done within 4 hours, it is preferable to refrigerate the urine at 2–8 ° centigrade.

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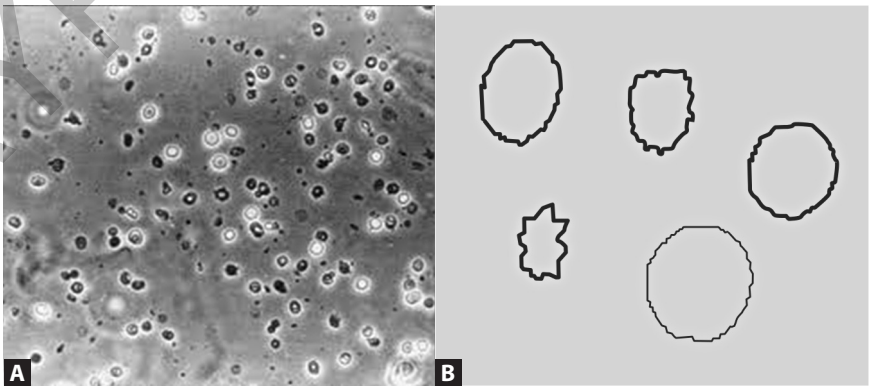
A sample of well-mixed urine (usually 10–15 mL) is centrifuged in a test tube at relatively low speed (about 2–3,000 rpm) for 5–10 minutes until a moderately cohesive button is produced at the bottom of the tube. The supernatant is decanted and a volume of 0.2–0.5 mL is left inside the tube. The sediment is examined on a clean slide as a wet mount. In infants and neonates, the ideal sample is a suprapubic aspirate. If not feasible, a catheter/sterile collection may be used to collect the urine.

The sediment is first examined under low power to detect most crystals, casts, squamous cells, and other large objects. The number of casts seen is usually reported as the number of each type found per low-power field (LPF). Since the number of elements found in each field may vary considerably from one field to another, several fields are averaged (5–10). Next, examination is carried out at high power to identify crystals, cells, and bacteria. The various types of cells are usually described as the number of each type found per average high-power field (HPF).

Microscopic Elements of Urine

Red Blood Cells (Figs 4.3A and B)

Usually, two or less red blood cells (RBCs) per high-power field are considered normal. RBCs may appear normally shaped, swollen by dilute urine (in fact, only cell ghosts and free hemoglobin may remain), or crenated by concentrated urine. Both swollen, partly hemolyzed RBCs and crenated RBCs are, sometimes, difficult to distinguish from WBCs in the urine. In addition, red cell ghosts may simulate yeast. When a patient has persistent urine dipstick positive for blood, the presence of increased RBCs should be confirmed by microscopy. In dilute urine of normal color, a heme-positive dipstick and little or no RBCs seen on microscopic examination is more likely due to lysis of RBCs. In a patient who has urine that appears grossly bloody, however, a strongly positive dipstick with few RBCs visualized on microscopic examination is suggestive of rhabdomyolysis or intravascular hemolysis. Urine microscopy is useful in determining the etiology of hematuria. The presence of dysmorphic RBCs



Figs 4.3A and B: Red blood cells in urine

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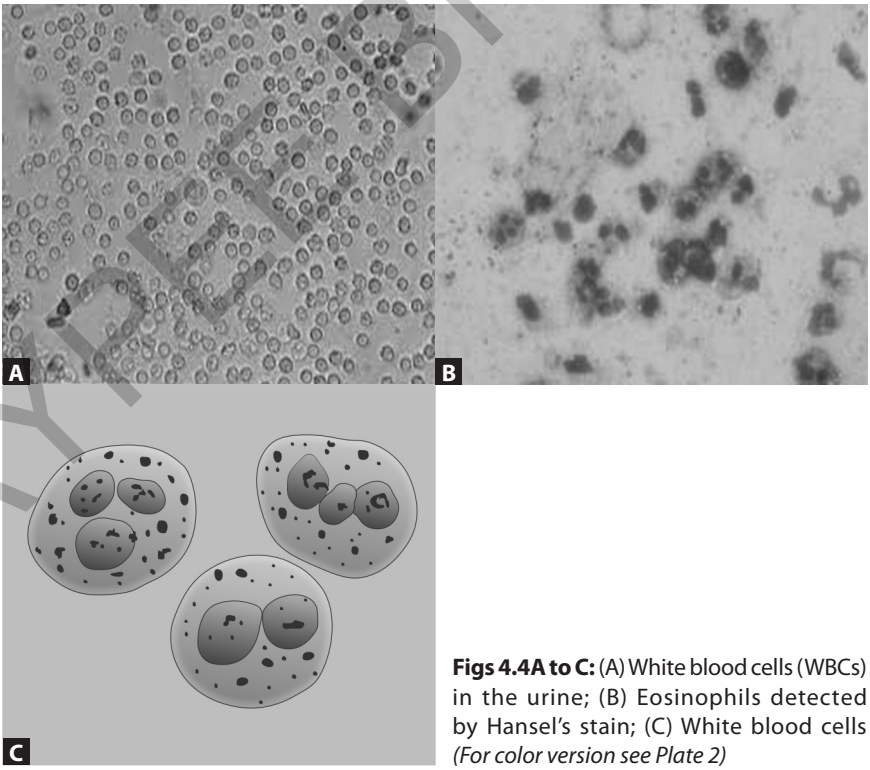
(using phase-contrast microscopy) and RBC casts suggests a renal source of the hematuria, most likely glomerular disease. Normal-appearing RBCs and the lack of casts suggest lower urinary tract bleeding.

White Blood Cells (Figs 4.4A to C)

Greater than five white blood cells (WBCs) per HPF are generally considered abnormal. Pyuria usually signifies UTI, although it is not specific for UTI. Other conditions that can result in pyuria, include fever, glomerulonephritis, and other inflammatory processes, whether in the kidney (interstitial nephritis) or pelvic region, e.g. appendicitis. The presence of pyuria does not add to and may not be as good a screen for UTI as leukocyte esterase and nitrites from the urine dipstick. The urine should be cultured to confirm UTI. White blood cells from the vagina can contaminate urine specimens. The presence of eosinophils in the urine is suggestive of allergic interstitial nephritis. Wright, Giemsa and Hansel stains are used to detect eosinophiluria. Hansel stain is most effective. Prolonged standing of hypotonic urine results in lysis of white blood cells.

Epithelial

Renal tubular epithelial cells, usually larger than granulocytes, contain a large round or oval nucleus and normally slough into the urine in small numbers



Figs 4.4A to C: (A) White blood cells (WBCs) in the urine; (B) Eosinophils detected by Hansel's stain; (C) White blood cells *(For color version see Plate 2)*

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cells **(Fig. 4.5)**. However, with nephrotic syndrome and in conditions leading to tubular degeneration, the numbers increase.

Transitional epithelial cells from the renal pelvis, ureter, or bladder have more regular cell borders, larger nuclei, and smaller overall size than squamous epithelium **(Fig. 4.6)**.

Squamous epithelial cells from the skin surface or from the outer urethra can appear in urine **(Fig. 4.7)**.

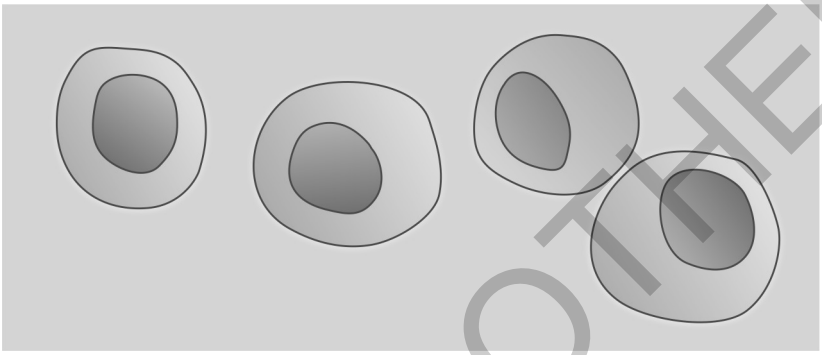

Fig. 4.5: Renal tubular epithelial cells

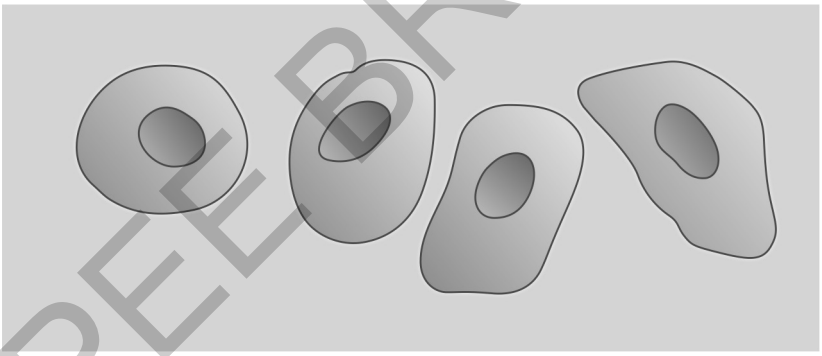

Fig. 4.6: Transitional epithelial cells

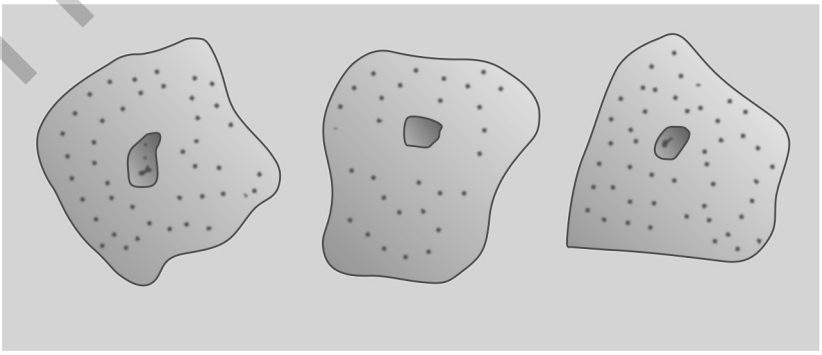

Fig. 4.7: Squamous epithelial cells

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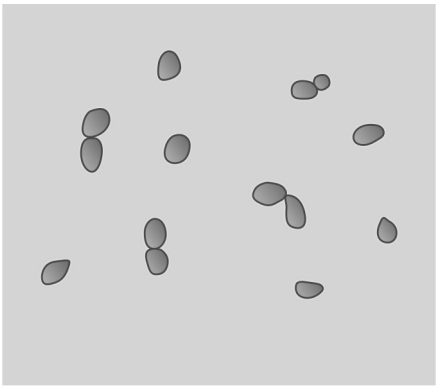

Fig. 4.8: Yeast

Bacteria

The presence of bacteria in an asymptomatic patient is most likely due to contamination. Well-mixed uncentrifuged urine may be examined with Gram's stain. If bacteria are identified under oil-immersion lens, it corresponds with significant bacteriuria (>100000 organisms/mL).

Yeast (Fig. 4.8)

Yeast cells may be contaminants or represent a true yeast infection. They are often difficult to distinguish from red cells and amorphous crystals but are distinguished by their tendency to bud. Most often they are *Candida*, which may colonize bladder, urethra, or vagina.

Casts

Casts are formed as translucent colorless gels in the lumen of distal convoluted tubules and collecting ducts and consist of an organic matrix composed of Tamm–Horsfall protein with or without additional elements. There are many different types of urinary casts that may be observed on urine microscopy.

Hyaline casts are composed primarily of secreted by the tubule cells. The Tamm–Horsfall protein secretion (green dots) is illustrated in the **Figure 4.9**, which forms a hyaline cast in the collecting duct **(Fig. 4.10)**.

Hyaline casts are the most common and can be seen in normal individuals. They may be increased with concentrated urine, diuretics, renal disease, fever, and exercise.

Cellular casts are of greater significance. Cellular casts can dissolve within 30 minutes in acidic urine and within 10 minutes in alkaline dilute urine and can be missed if the microscopic examination is not performed soon after voiding. Cellular casts include RBC casts (seen with glomerulonephritis), WBC casts **(Fig. 4.11)** (seen with pyelonephritis), renal tubular epithelial cell casts (viral illnesses, ATN, heavy metal poisoning, acute graft rejection), mixed cellular casts and broad casts.

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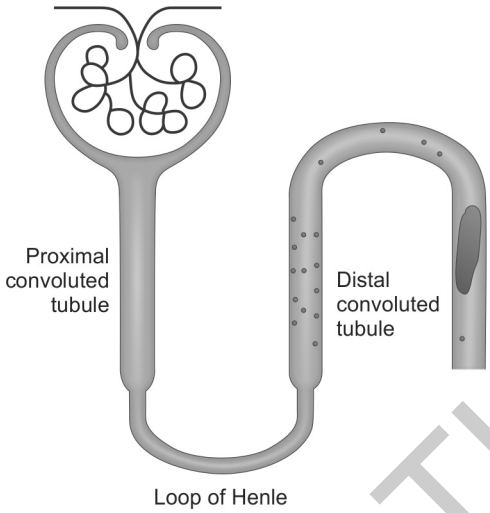

Fig. 4.9: Loop of Henle

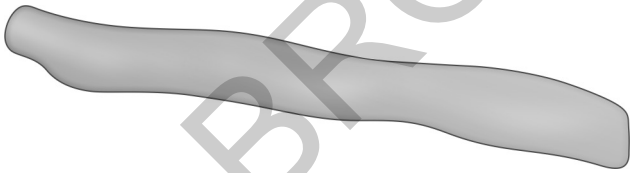

Fig. 4.10: Hyaline cast

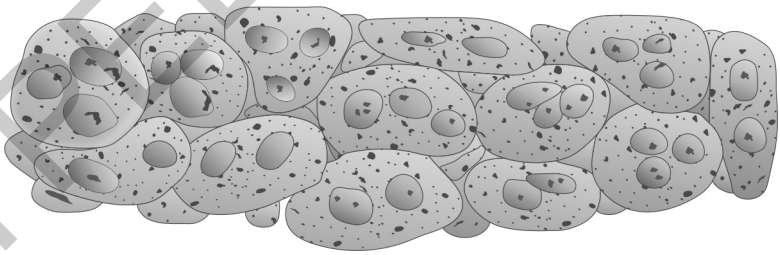

Fig. 4.11: White blood cell cast

Inclusion casts include granular, fatty, hemosiderin and crystal casts.

Pigmented casts include hemoglobin, myoglobin and bilirubin casts.

When cellular casts remain in the nephron for some time before they are flushed into the bladder, the cells may degenerate to become a coarsely granular cast, later a finely granular cast, and ultimately, a waxy cast. Broad casts are believed to emanate from damaged and dilated tubules and are, therefore, seen in end-stage chronic renal disease **(Figs 4.12A to C)**.

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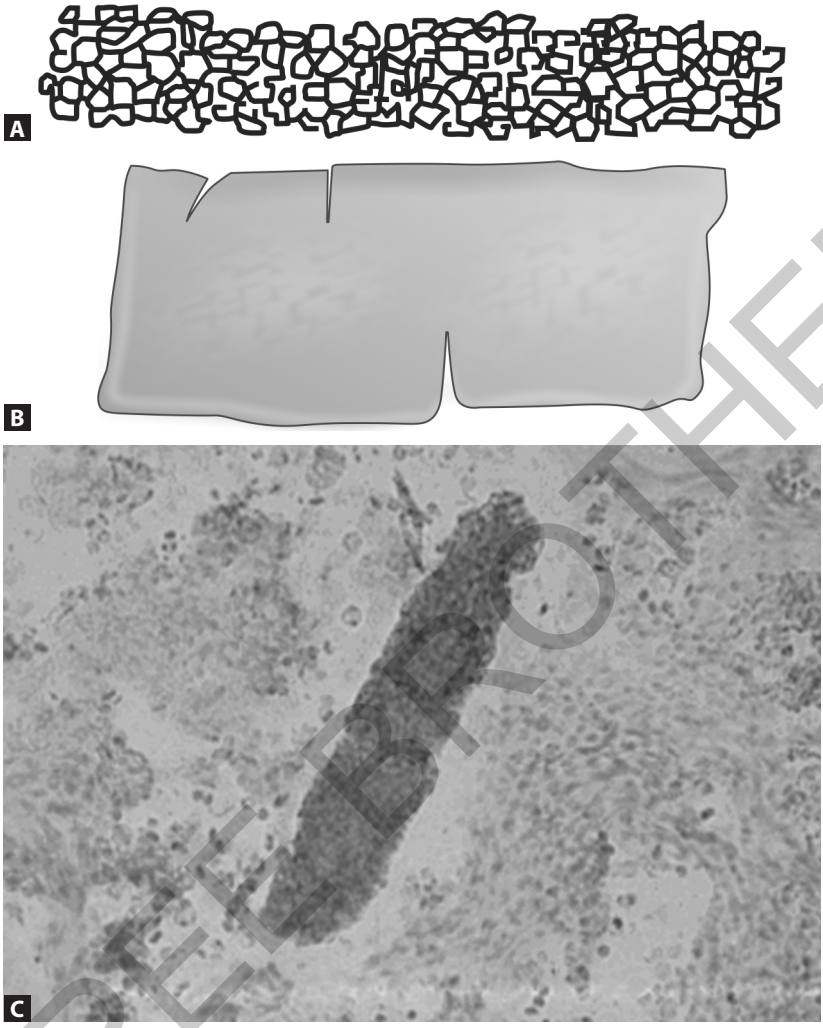

Figs 4.12A to C: (A) Granular cast; (B) Waxy cast; (C) Finely granular cast
(For color version of Fig. C see Plate 2)

The so-called telescoped urinary sediment is one in which red cells, white cells, oval fat bodies, and all types of casts are found in more or less equal profusion. The conditions which may lead to telescoped sediment are: (1) lupus nephritis, (2) malignant hypertension, (3) diabetic glomerulosclerosis, and (4) rapidly progressive glomerulonephritis.

Crystals

It is common to find crystals on microscopic examination of the urine. Crystal formation depends on a number of factors, and the presence of crystals may or may not be pathologic **(Fig. 4.13)**.

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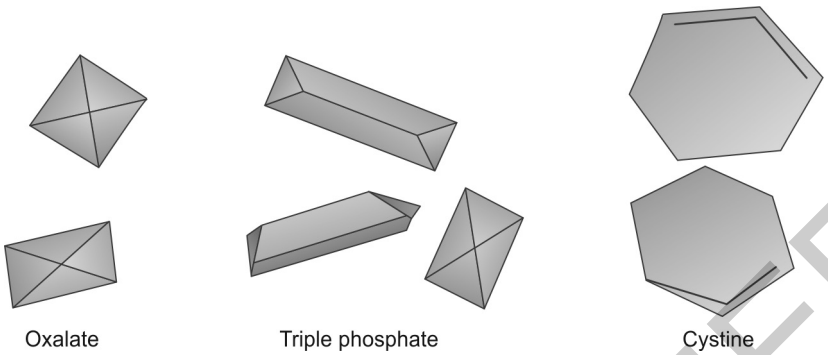

Fig. 4.13: Commonly seen crystals in patient urine

Common crystals seen even in healthy patients include calcium oxalate, triple phosphate crystals and amorphous phosphates **(Fig. 4.13)**.

Very uncommon crystals include: cystine, tyrosine and leucine crystals.

BIOCHEMICAL ANALYSIS OF URINE

Chemical analysis of the urine is usually done for further confirmation of results obtained by dipstick.

The most accurate quantitative estimation of protein is by the 24-hour collection which may be cumbersome for children. Also the hour-to-hour inconsistency of protein excretion particularly in daytime has questioned the rationale of using a timed urine sample. If a timed urine sample for quantification is considered, then a 12-hour overnight sample appears more accurate. This collection also takes care of the presence of orthostatic proteinuria in children/adults. Hence, over the years, spot urine collections (first morning/early morning) are becoming more acceptable. Quantification is calculated by using the protein-creatinine ratio.

The detection of Bence-Jones protein involves the heat precipitation test which is based on demonstrating a protein, which precipitates at 40–60°C and re-dissolves on cooling. It lacks specificity and sensitivity and needs to be confirmed with electrophoretic tests.

Testing for microalbumin is done by various methods **(Table 4.1)** and should be used for diabetic children and not the routine protein estimations.

Bilirubin is chemically checked by Fouchet's test and urobilinogen by Ehrlich's test. Presence of hemoglobin in urine is tested by the Guaiac test.

Table 4.1: Methods of estimating urinary albumin

Precipitation	Boiling, sulphosalicylic acid
Dye binding	Biuret, tetrabromophenol, albumin blue 580
Antibody binding	Test strips, nephelometry, radioimmunoassay
Molecular size/shape	HPLC, spectrophotometry

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Neonatal screening for inherited aminoaciduria is done using urine which is collected at least 48 hours after birth so that a sufficient build up of the amino acid occurs after a diet of proteins. The urine needs to be free of drugs, e.g. antibiotics or other endogenous substances, which interfere with the testing. Antibiotics can be inactivated by autoclaving the sample. This, however, does not destroy the amino acid to be tested. In prematures all positive results must be viewed with caution as the mild elevation can occur due to relative immaturity of the liver.

URINE CULTURE

Urine is often cultured with the midstream clean catch sample. It is necessary to estimate the approximate number of bacteria in urine so as to determine significant bacteriuria. This is done by using a calibrated loop which has a known quantity of urine (usually 0.002 mL or 1/500 mL) and this amount is placed on a quarter plate of cystine lactose electrolyte deficient (CLED) agar. As each colony is derived from a single bacterium, if twenty colonies are formed after 48 hours of incubation it means that the bacterial count of the sample is 10,000 colonies/per mL (20 × 500/mL). A count of 100,000 is considered significant. In suprapubic aspirates from children, a count of 1000 may represent UTI. The morphological appearance of common bacteriae is shown in **Table 4.2**.

AUTOMATED URINALYSIS METHODOLOGIES

Two technologies that are currently used in urine particle recognition systems are flow imaging and flow cytometry. Both systems force the particles to flow in a single plane.

Flow Imaging Technology

With flow imaging, the particles pass through a microscope, are photographed, and then analyzed and classified by computer as to size, shape, and texture. Particle concentration is then calculated based on the number of images and the volume that is scanned.

Table 4.2: Appearance of various colonies of common uropathogens

Organism	*Appearance*
E. coli	Yellow opaque colonies with deeper center
Klebsiella	Large mucoid yellow/yellow white colonies
Proteus	Translucent blue-grey colonies
Pseudomonas	Green colonies
E. fecalis	Small yellow colonies
Staph. aureus	Deep yellow colonies of uniform color
S. saprophyticus	Yellow to white colonies

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Flow Cytometry Technology

With the flow cytometry method, light scatter and the height and width of fluorescent signals are measured. The data is displayed in scatterplots and presented to the operator for further particle classification, if necessary.

Automated instruments classify and quantitate microscopic elements within the known parameters of the systems. When elements are unknown or outside of the defined parameters, operator intervention is needed. Non-squamous epithelial cells require operator review with some systems.

Manual microscopy is always needed to identify and classify cellular casts.

If trichomonas vaginalis is suspected, the sediment must be examined microscopically so that flagella and motility can be confirmed.

SUMMARY

The evaluation of the urine by physical, microbiologic and biochemical examination reveals a wealth of information regarding the clinical status of the child. The oldest laboratory investigation still is the most cost effective investigation.

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