CORE CURRICULUM IN NEPHROLOGY

Urinalysis: Core Curriculum 2008

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INTRODUCTION

Urinalysis is one of the most ancient and basic tests to evaluate the presence, severity, and course of diseases of the kidney and urinary tract. Therefore, when a patient is first seen by a nephrologist, a complete basic investigation of the urine should always be requested.

In most instances, this is done by means of dipstick, a widely accepted method for screening purposes because of its quick, simple, and inexpensive use. However, clinicians too often are unaware of the principles and limits of this approach, which allows one to detect and obtain an approximate estimation of concentrations of a number of analytes, including albumin, blood, leukocytes, and bacteria.

Clinicians, and nephrologists among them, also often are unaware of the valuable information that can be obtained with examination of urinary sediment. Today, this usually is performed in central laboratories, where too many samples are analyzed every day (several hundreds in many situations), far from the bedside of the patient, and without the correct methods, equipment, and professional qualification.

It is our firm belief that nephrologists should regain possession of the examination of the urine sediments of their patients. This would result in more correct diagnoses, as shown by a recent study in which examination of the urine of 26 patients with acute renal failure carried out by the personnel of clinical laboratories missed significant particles (renal tubular epithelial cells [RTECs] and various types of casts) that were identified correctly by trained nephrologists.

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However, to achieve this goal, the following requirements are mandatory: (1) use of a correct method for patient preparation and urine collection and handling; (2) capability to identify the most important particles of the urinary sediment; (3) knowledge of their clinical meaning; and (4) capability to arrange urinary sediment findings into a clinical context.

It must be remembered that all nephrological conditions take advantage of a urinalysis of good quality, and negative urinary findings also help in the correct evaluation of a renal patient.

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COLLECTION OF SPECIMENS

Results are influenced greatly by the way urine is collected and handled in the laboratory. Thus, written, simple, and clear instructions should be given to the patient for correct urine collection (Table 1).

The same procedures also can be used for children. For small infants, bags for urine often are used, although these carry a high probability of contamination.

Urine particles can lyse rapidly after collection, especially when urine pH is alkaline and/or specific gravity or osmolality is low. Thus, it is recommended that the sample be analyzed within 2 to 4 hours from collection. Otherwise, samples can be kept at a temperature of +2°C to +8°C; however, this procedure favors precipitation of phosphates or urates, which makes examination of the sample difficult and inaccurate. Alternatively, formaldehyde, glutaraldehyde, and "cell-FIX" (a formaldehyde-based fixative [Becton Dickinson and Company, Erembodegem, Bel-

Table 1. Example of Instructions for Urine Collection

Avoid strenuous physical exercise (eg, running or a soccer match) in the 72 hours preceding the collection to prevent exercise-induced proteinuria and/or hematuria or cylindruria.
Avoid urinalysis during menstruation because blood contamination can occur, which can erroneously lead to a diagnosis of hematuria.
In case of mild genital discharge (eg, leukorrhea), use internal tampons to prevent contamination.
Wash your hands.
Wash the urethral meatus after spreading the vulvar labia (female) or withdrawing the foreskin of the glans (male) and wipe with a towel.
Collect the urine after discarding the first portion of micturition (midstream technique) to reduce contamination from urethral and/or vaginal cells and secretions.
Close the container completely and write your name clearly and in full on the label.

gium]) can be used as preservatives of urine particles. However, preservatives can alter the appearance of particles. Thus, every effort should be made to examine samples within 2 to 4 hours from collection. For this reason, in our laboratory, we usually examine samples within 2 to 3 hours from collection. We only use formaldehyde or cellFIX to preserve particles for teaching purposes.

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PHYSICAL PARAMETERS

Color

In normal conditions, the color of urine ranges from pale to dark yellow and amber. Abnormal color changes can be caused by pathological conditions, drugs, and foods (Table 2).

Turbidity

Normal urine usually is transparent. Urine can be turbid because of an increased concentration of any urine particle, but especially erythrocytes, leukocytes, bacteria, squamous epithelial cells, or crystals. In our experience, urinary infection and contamination caused by genital secretions are the most frequent causes of urine turbidity.

It must be remembered that pathological samples can be perfectly clear.

Odor

Infection also is the most frequent cause of abnormal pungent odor of urine, which is caused by the production of ammonia by bacteria. The following rare pathological conditions confer a specific odor to the urine: maple syrup urine disease (maple syrup odor), phenylketonuria (musty or mousy odor), isovaleric acidemia (sweaty feet odor), and hypermethioninemia (rancid butter or fishy odor). Ketones may confer a sweet or fruity odor.

Relative Density

Relative density can be measured by using different methods:

Specific Gravity

This is a function of the number and weight of dissolved particles. It usually is measured by using a urinometer, which is a weighted float marked with a scale from 1.000 to 1.060. The urinometer is simple and quick to use, but today, it is outdated.

Osmolality

This represents the gold-standard method. It depends on the number of particles present and is

Table 2. Main Causes of Abnormal Color Changes in Urine

Pathological conditions	Gross hematuria, hemoglobinuria, myoglobinuria (pink, red, brown, black)
	Jaundice (yellow to brown)
	Chyluria (white milky urine)
	Massive uric acid crystalluria (pink)
	Porphyrinuria, alkaptonuria (red to black upon standing)
Drugs	Rifampin (yellow-orange to red)
	Phenitoin (red)
	Chloroquine, nitrofurantoin (brown)
	Triamterene, blue dyes of enteral feeds (green)
	Metronidazole, methyldopa, imipenem-cilastatin (darkening upon standing)
Foods	Betroot (red)
	Senna, rhubarb (yellow to brown, red)

measured by using an osmometer. High glucose concentrations significantly increase osmolality (10 g/L of glucose = 55.5 mOsmol/L).

Refractometry

This is based on measurement of the refractive index, which depends on the weight and size of solutes per unit volume. Today, refractometry is used widely because it is simple, requires only 1 drop of urine, and has good correlation with osmolality.

Dry Chemistry

This is the method incorporated into dipsticks. In the presence of cations, a complexing agent releases protons, which produce a color change of the indicator bromthymol blue. Because of its simplicity, this method is the most used. However, it should be remembered that underestimation occurs with urine pH greater than 6.5, whereas overestimation is found with urine protein concentration greater than 7.0 g/L. In addition, it is not sensitive to nonionized molecules, such as glucose and urea. Thus, it is not surprising that it has poor correlation with results obtained by using osmolality and refractometry.

CHEMICAL PARAMETERS

pH

In routine practice, pH most commonly is measured by means of dipstick. This is based on an indicator that covers the pH range 5.0 to 8.5 to 9.0. With this method, significant deviations from true pH are observed for values less than 5.5 and greater than 7.5. Therefore, a pH meter with a glass electrode is mandatory when accurate measurement is necessary.

In addition to its application in clinical practice, measurement of urine pH is needed for correct interpretation of urinary microscopy findings (see microscopy).

Hemoglobin

Hemoglobin also usually is detected by means of dipstick. This is based on the pseudoperoxidase activity of the heme moiety of hemoglobin, which catalyzes the reaction of a peroxide and a chromogen to produce a colored product. The presence of hemoglobin produces either green spots, which are caused by intact erythrocytes, or a homogenous diffuse green pattern. The latter may be caused by marked hematuria because of the high number of erythrocytes that cover the entire pad surface or by lysis of erythrocytes, which can occur on standing or because of alkaline urine pH and/or low relative density.

The most important false-positive results occur for the presence of hemoglobinuria (from intravascular hemolysis), myoglobinuria (from rhabdomyolysis), or high concentration of bacteria with pseudoperoxidase activity (*Enterobacteriaciae* species, *Staphylococci* species, and *Streptococci* species).

False-negative results are mainly caused by ascorbic acid, a strong reducing agent, the presence of which can result in a low-grade microscopic hematuria being completely missed. In such cases, although dipstick results are negative, microscopy shows hematuria.

Detection of hemoglobin by means of dipstick has 95% to 100% sensitivity and 65% to 93% specificity.

Glucose

With a dipstick, glucose is first oxidized to gluconic acid and hydrogen peroxide. Then, through the catalyzing activity of a peroxidase, hydrogen peroxide reacts with a reduced colorless chromogen to form a colored product. This test is sensitive to concentrations of 0.5 to 20 g/L. When more precise quantification of urine glucose is needed, such enzymatic methods as a hexokinase must be used.

False-negative results occur in the presence of ascorbic acid and bacteria, whereas false-positive findings may be observed in the presence of oxidizing detergents and hydrochloric acid.

Protein

Three different approaches can be used for the evaluation of proteinuria (Table 3).

Dipstick

It is based on the principle of the protein error: the presence of protein in a buffer causes a change in pH that is proportional to the concentration of protein itself. Thus, dipstick changes its color from pale green to green and blue according to pH changes induced by the protein. This method is sensitive to albumin (detection limit, ~0.020 to 0.025 g/dL [0.20 to 0.250 g/L]),

Table 3. Different Approaches for the Evaluation of Proteinuria and Their Features

Dipstick		24-Hour Protein Excretion		Protein-Creatinine Ratio	
Advantages	Limitations	Advantages	Limitations	Advantages	Limitations
Easy to use	Very low sensitivity to tubular proteins and light chain immunoglobulins	Reference (gold-standard) method	Requires detailed instructions	Requires only 1 spot urine sample	Requires the measurement of 2 analytes
Low cost	High threshold for albumin	Universally known and used	Inconvenient for patients	Practical	Greater risk of analytical errors and higher costs
Possibility to detect other analytes in the same sample (eg, hemoglobin, leukocyte esterase, nitrites)	Rough semiquantitative method	Averages the circadian rhythm of protein excretion	Possibility of contamination during collection	The same sample also usable for microscopy	Inaccurate with proteinuria > 1 g/L
Accessible also to developing countries	Inaccurate for monitoring proteinuria	Most accurate for monitoring proteinuria during treatment	Frequent preanalytic errors	Reduced preanalytic errors	Not suitable for monitoring proteinuria during treatment

whereas it has very low sensitivity to other proteins, such as tubular proteins and light chain immunoglobulins.

In addition, dipstick allows only an approximate quantification of urine albumin, expressed on a scale from 0 to +++ or ++++. This is confirmed by the following data obtained in our laboratory: in 30 samples with + albumin by means of dipstick, we found a protein concentration, measured by using benzetonium chloride, that ranged from 100 to 1,600 mg/L (10 to 160 mg/dL; mean, 800 ± 300 mg/L [80 ± 30 mg/dL]); in 30 samples with ++ albumin, protein concentration ranged from 600 to 3,330 mg/L (60 to 333 mg/dL; mean, 1,445 ± 665 mg/L [144.5 ± 66.5 mg/dL]); and in another 30 samples with +++ albumin, protein concentration ranged from 1,060 to 8,680 mg/L (106 to 868 mg/dL; mean, 3,074 ± 1,479 mg/L [307.4 ± 147.9 mg/dL]).

Thus, for reliable quantification of total protein excretion, other methods are necessary, such as turbidimetric or dye-binding techniques (eg, benzetonium chloride or pyrogallol red-molybdate colorimetric method).

Twenty-Four–Hour Protein Excretion

This approach represents the reference (gold-standard) method. It is used universally, averages the variation in proteinuria caused by circadian rhythm, and is the most accurate for monitoring proteinuria during treatment. However, it is influenced largely by the rate of diuresis, requires detailed instructions for urine collection, and can be impractical in some circumstances (eg, outpatient setting and elderly patients). Moreover, during collection, urine can undergo contamination and rough preanalytic errors can occur (eg, incorrect collection and incorrect calculation of urinary volume).

Protein-Creatinine Ratio on a Random Urine Sample

This is a recommended alternative to 24-hour urine collection. It is easy to obtain, is not influenced by variation in water intake and rate of diuresis, greatly reduces preanalytic errors, and the same sample can be used for microscopic investigation.

A review of the literature showed sufficient evidence of a strong correlation between protein-creatinine ratio in a random urine sample and

24-hour protein excretion. However, it should be remembered that a normal protein-creatinine ratio is sufficient to rule out the presence of pathological proteinuria (which decreases the number of unnecessary 24-hour urine collections), whereas in the case of a protein-creatinine ratio greater than the cutoff value, a full 24-hour quantification is indicated. Moreover, correlation between protein-creatinine ratio and 24-hour protein excretion may be not accurate at protein levels greater than 1g/L (>0.1 g/dL), and the reliability of protein-creatinine ratio for monitoring proteinuria during treatment is still not proven.

Leukocyte Esterase

This dipstick evaluates the presence of leukocytes on the basis of indoxyl esterase activity released from lysed neutrophils and macrophages. This explains why, in urine with alkaline pH and/or low relative density, which favors the lysis of leukocytes, there frequently is a positive dipstick result, but negative microscopy findings. Conversely, high relative density values decrease the sensitivity of this dipstick because of the prevention of leukocyte lysis.

False-negative results also occur in the presence of high glucose or protein concentrations (≥20 g/L and ≥5 g/L [≥2 g/dL and ≥0.5 g/dL], respectively) or in the presence of cephalotine and tetracycline (strong interference), cephalexine (moderate interference), or tobramycin (mild interference).

False-positive results are very rare (eg, when formaldehyde is used as urine preservative). Sensitivity varies from 76% to 94%, and specificity, from 68% to 81%.

The detection limit of dipstick is 20×10^6 leukocytes/L.

Nitrites

This dipstick test shows the presence of bacteria that have the capability of reducing nitrates to nitrites because of nitrate reductase activity. This is present in most gram-negative uropathogenic bacteria, but is low or absent in others, such as *Pseudomonas* species, *Staphylococcus albus*, and *Enterococcus* species.

Test positivity also requires a diet rich in nitrates (vegetables), which form the substrate for nitrite production, and sufficient bladder incubation time. Thus, it is not surprising that the sensitivity of this test is low, whereas specificity is greater than 90%.

Bile Pigments

With the introduction of liver enzyme measurement in blood, detection of urinary urobilinogen and bilirubin has lost its clinical utility.

Ketones

This dipstick is based on the reaction of nitroprusside with acetoacetate and acetone. These are excreted into urine during diabetic acidosis, fasting, vomiting, or strenuous exercise.

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MICROSCOPY

Methods

Microscopy is an integral part of basic urinalysis and adds irreplaceable information to the physicochemical investigation, especially when performed by a trained nephrologist. However, reliable results can be achieved only with standardized methods for the handling of urine, one example of which is shown in Table 4.

Notes

Second Urine of the Morning

We prefer this urine to the first urine of the morning (produced overnight) because pro-

Table 4. Example of Standardization for Preparation of Samples for Urine Microscopy

Written instructions to patients for urine collection
Collection in disposable containers of the second urine of the morning after discarding the first few milliliters of urine (midstream technique)
Sample handling and analysis within 2-3 hours from collection
Centrifugation of a 10-mL aliquot of urine at 400*g* (2,000 rpm) for 10 minutes
Removal by suction of 9.5 mL of supernatant urine
Gentle, but thorough, resuspension with a pipette of the sediment in the remaining 0.5 mL of urine
Transfer by pipette of 50 μL of resuspended urine to a slide
Covering sample with a 24 × 32-mm coverslip
Examination of all samples by a phase contrast microscope at original magnifications ×160 and ×400
Use of polarized light to identify doubtful lipids and crystals
Match of microscopic findings with dipstick for pH, specific gravity, hemoglobin, leukocyte esterase, and albumin
For routine work, cells expressed as lowest to highest number seen/high-power field, casts as number/low-power field, all the other elements on a scale from 0 to ++++
For scientific work, cells expressed as total number counted on 20 high-power fields

Note: This method is used in the authors' laboratory.

longed standing of urine in the bladder favors the lysis of urine particles.

Centrifugation

Use of noncentrifuged samples, advocated by some investigators to avoid the loss/lysis of particles caused by centrifugation itself, greatly reduces sensitivity because particles are not concentrated in the bottom of the tube. This is important, especially for such rare, but important, particles as erythrocyte casts.

Type of Microscope

International guidelines recommend the use of phase contrast microscopy to improve the identification of particles. Compared with traditional bright field microscopy, phase contrast has much greater sensitivity for hyaline casts and erythrocytes with low hemoglobin content (the so-called "ghost cells"). In addition, it allows the best examination of morphological details, an important feature for differentiation of cells. Filters to polarize light also are mandatory for the correct identification of lipids and crystals with doubtful/unusual appearance.

Examination of the Sample

We currently examine no fewer than 20 microscopic fields at original magnification ×160 in different and random areas of the sample. Then, we pass to original magnification ×400 for careful analysis of particles shown by the overview at low magnification. For such special conditions as isolated microscopic hematuria of unknown origin, we always examine 50 microscopic fields at original magnification ×160 to evaluate whether erythrocyte casts are present.

Match With Dipstick Results

For correct interpretation of the findings, both pH and specific gravity of the sample must be known. Alkaline pH and/or low specific gravity, especially less than 1.010, favor the lysis of erythrocytes and leukocytes, which can cause false-negative results by means of microscopy. The knowledge of pH also is useful for the correct identification of crystals (discussed later). The knowledge of albumin results is of help in the evaluation of patients with a glomerular disease.

Counting of Particles

The use of counting chambers, which allows precise quantification of particles (number/milliliter), is recommended by international guidelines. However, this method is rarely used in everyday practice. We find the semiquantitative method (lowest to highest number or average number of cells/high-power field) workable for routine practice. When precise quantification of cells is needed, we count them as total number found over 20 microscopic fields at original magnification ×400.

Cells

Two groups of cells can be found in urine: cells deriving from the circulation (ie, erythrocytes, leukocytes, and macrophages) and cells deriving from epithelia (ie, renal tubular cells, urethelial cells, and squamous cells; Table 5).

Erythrocytes

Erythrocytes, which have a diameter of 4 to 7 μm, are a frequent finding in patients with kid-

Table 5. Cells of the Urinary Sediment

Cell	Subtype	Main Clinical Associations
Erythrocytes	Dysmorphic	Glomerular disease
	Isomorphic	Nonglomerular disease
Leukocytes	Polymorphonuclear	Urinary infection and contamination, interstitial nephritis, urological diseases
	Eosinophils	Acute interstitial nephritis, prostatitis, cholesterol embolism, etc
	Lymphocytes	Acute cellular rejection of kidney allograft
Macrophages	Fatty, granular, phagocytic, vacuolar	Marked proteinuria, active glomerulonephritis, immunoglobulin A nephropathy
Renal tubular epithelial cells	Ovoidal to columnar, depending on the tubular segment they come from	Acute tubular necrosis, acute interstitial nephritis, acute cellular rejection of kidney allograft, proliferative glomerulonephritis
Uroepithelial	Deep	Severe urological diseases
	Superficial	Urinary tract infection, urological disorders
Squamous	—	Contamination of urine from genital secretions

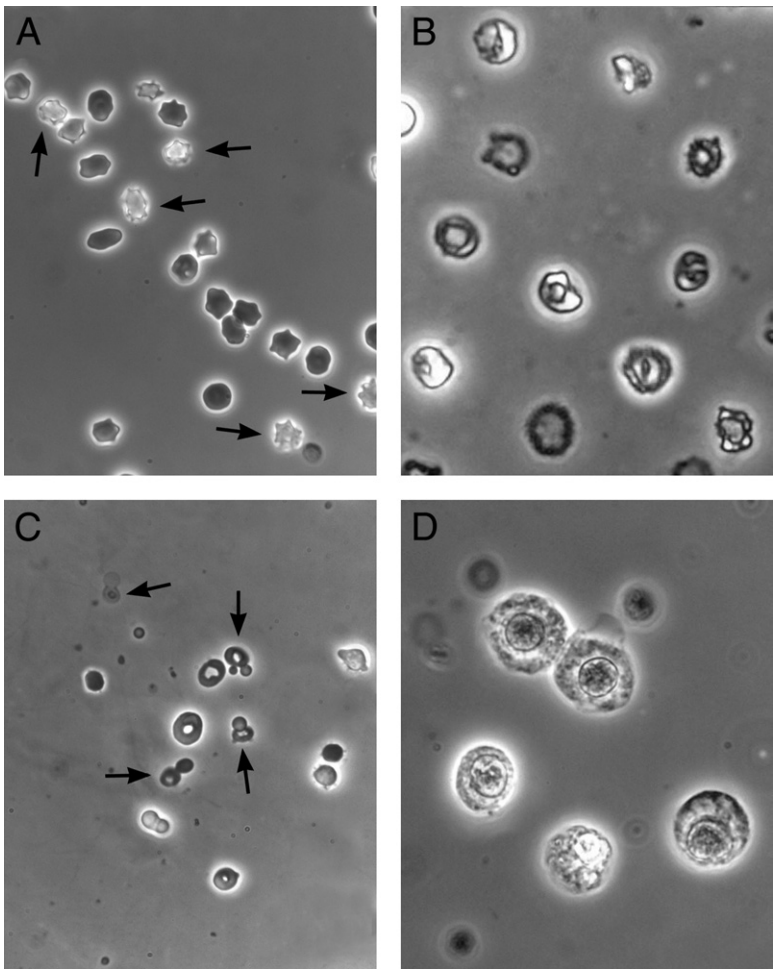

Figure 1. (A) Isomorphic erythrocytes, some with a "crenated" appearance (arrows); (B) different types of dysmorphic erythrocytes; (C) acanthocytes or G1 cells with their typical shape (arrows); and (D) proximal renal tubular cells with round shape, large nucleus, and granular cytoplasm.

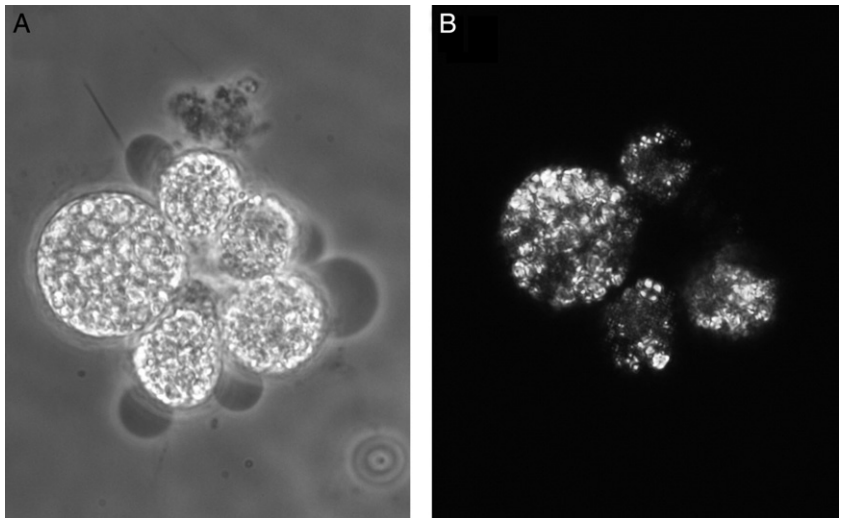

Figure 2. (A) "Oval fat bodies" or macrophages packed with lipid droplets. (B) The same particles seen by polarized light. Note the Maltese crosses with symmetrical arms.

ney diseases. From the clinical standpoint, it is useful to be able to distinguish the so-called isomorphic erythrocytes, which are similar to erythrocytes found in the bloodstream (Fig 1A), from dysmorphic erythrocytes, which are characterized by irregular shapes and contours (Fig 1B). The former are suggestive of hematuria of urological origin, whereas the latter are typical of patients with a glomerular disease. This distinction is of special value for patients with isolated microscopic hematuria of unknown origin because it allows one to orientate the diagnosis toward a nephrological cause of hematuria, rather than a urological one, which can spare the patient inappropriate or invasive procedures.

Unfortunately, the lack of unequivocal criteria for this type of analysis has hampered the diffusion of this test.

In our laboratory, we consider hematuria to be glomerular when 40% or greater of erythrocytes are dysmorphic and/or 5% or greater of erythrocytes examined are acanthocytes (also known as G1 cells). These are a subtype of dysmorphic erythrocytes that are easily identifiable because of their peculiar appearance; namely, a ring-shaped body with 1 or more protruding blebs of variable shape and size (Fig 1C).

Leukocytes

Neutrophils are the leukocytes most frequently found in urine. They have a diameter of 7 to 13 μm and are easily identifiable because of their granular cytoplasm and lobulated nucleus (Fig 4 online).

Urinary tract infection and contamination of urine from genital secretions are the most frequent conditions associated with leukocyturia (and bacteriuria). However, this also is found in patients with acute or chronic interstitial nephritis, proliferative glomerulonephritis, and urological disorders.

Eosinophils, once considered a marker of acute allergic interstitial nephritis caused by antibiotics, are now regarded as nonspecific elements. By use of the highly sensitive and specific Hansel stain, it was shown that eosinophiluria can be found in a wide spectrum of conditions, such as rapidly progressive glomerulonephritis, prostatitis, chronic pyelonephritis, urinary schistosomiasis, and renal cholesterol embolism.

Lymphocytes are seen as an early and reliable marker of acute cellular rejection in renal allograft recipients. However, their identification requires cytological techniques, and today this approach is very rarely used in clinical practice.

Macrophages

Macrophages are cells of variable size (15 to $>$100 μm) and appearance: fatty (the so-called "oval fat bodies"; Fig 2A and B), phagocytic, vacuolar, or granular (Fig 7 online). Recent studies performed with monoclonal antibodies specific for macrophages showed that these cells can be found in urine of patients with nonselective proteinuria, active glomerulonephritis, or immunoglobulin A nephropathy. However, further investigation is necessary to

clarify the diagnostic importance of these cells in the urine.

Renal Tubular Epithelial Cells

RTECs derive from the tubular epithelium. They differ in size (diameter, 11 to 15 um) and shape (roundish to rectangular or columnar) according to the tubular segment they come from (Fig 1D). A morphological feature common to all RTECs is a well-evident nucleus with nucleoli.

Tubular cells are a marker of tubular damage. Therefore, they are found in acute tubular necrosis (in which they often are damaged and/or necrotic, isolated, or as fragments of the tubular epithelium, and associated with RTEC casts), acute interstitial nephritis, and acute cellular rejection of a renal allograft. In smaller numbers, they also are found in proliferative glomerular diseases.

Urothelial (transitional) Cells

These cells derive from the multilayered epithelium that lines the urinary tract from renal calyces to the bladder in women and to the proximal urethra in men. In urine, 2 main types of urothelial cells can be found; those deriving from the deep layers of the uroepithelium, which are small (diameter, ~13 to 20 μm) and oval to club-like in shape (Fig 9 online), and those from the superficial layers, which are larger (diameter, ~20 to 40 μm; Fig 10 online).

In our experience, finding 1 or more transitional cells from the deep layers/high-power field indicates the presence of a urological disease, such as bladder carcinoma, ureteric stones, or hydronephrosis.

Cells of the superficial layers are a common finding, especially in urinary tract infection.

Table 6. Classification, Appearance, and Clinical Associations of Casts

Cast	Appearance	Main Clinical Associations
Hyaline	Colorless, easily missed with bright field microscopy	Normal subject and renal disease
Hyaline-granular	Variable amounts of granules plunged in the colorless matrix of the cast	Normal subject and renal disease
Granular (finely and coarsely granular)	Fine granules caused by lysosomes containing ultrafiltered proteins Coarse granules caused by degenerated RTECs or leukocytes entrapped within the cast	Renal disease of whatever nature
Waxy	Large, with hard and indented contours and a "melted wax" appearance	Renal insufficiency, either acute or chronic
Fatty	Containing various amounts of lipid droplets, rarely cholesterol crystal	Marked proteinuria Nephrotic syndrome
Erythrocytic	Containing erythrocytes (from few to uncountable, sparse or packed), occasionally with a brownish hue	Proliferative/necrotizing GN Glomerular bleeding (of particular value in patients with isolated microscopic hematuria of unknown origin)
Hemoglobin	With a brownish hue; often with a granular appearance caused by the degradation of erythrocytes	The same as the erythrocytic casts Hemoglobinuria
Leukocytic	Containing leukocytes	Acute interstitial nephritis Acute pyelonephritis
RTEC (epithelial) casts	Containing RTECs	Acute tubular necrosis Acute interstitial nephritis GN (especially of proliferative type)
Myoglobin	Similar to hemoglobin casts	Rhabdomyolysis
Bilirubin	Yellow	All conditions associated with bilirubinuria
Containing microorganisms	Containing bacteria or yeasts	Bacterial or fungal infection of the kidney
Containing crystals	Containing uric acid, calcium oxalate, etc	Crystalluria with/without renal function impairment
Mixed	Waxy-granular, fatty-granular, granular-erythrocytic, etc	See waxy, granular, fatty, erythrocytic casts

Abbreviations: GN, glomerulonephritis; RTEC, renal tubular epithelial cell.

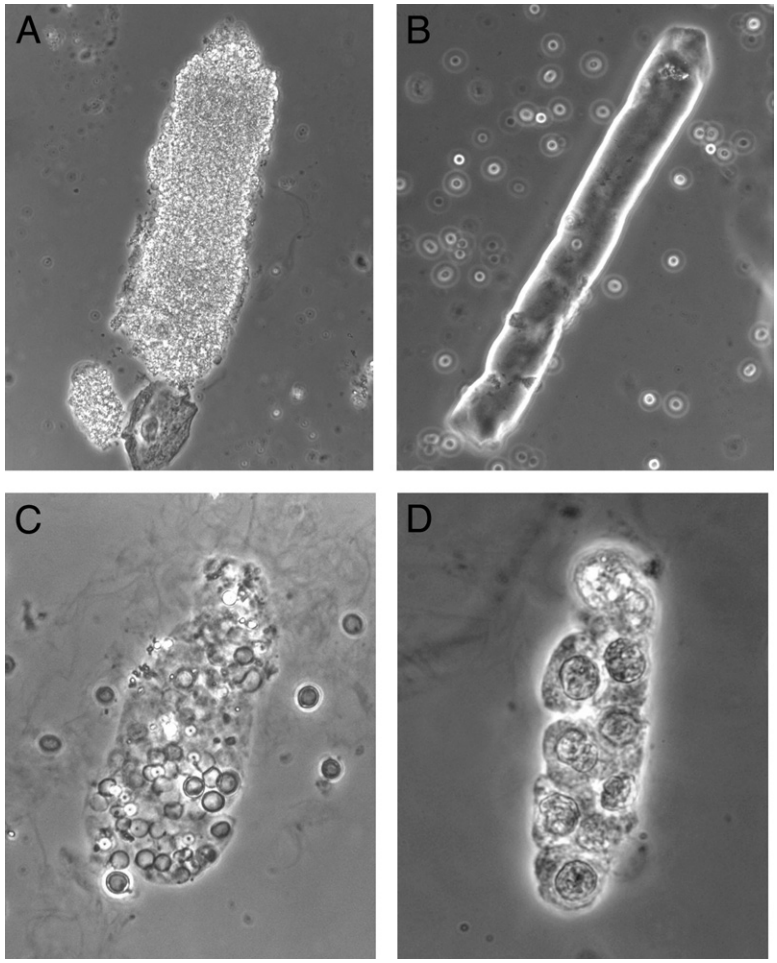

Figure 3. (A) A finely granular cast; (B) a waxy cast with the typical "melted wax" appearance, irregular edges, and high refractive index; (C) an erythrocyte cast; and (D) a renal tubular epithelial cell (RTEC) cast with RTECs easily identifiable because of their large nucleus.

Squamous Cells

These are the largest cells of the urinary sediment (diameter, 45 to 65 μm), have an irregular shape (Fig 11 online), and derive from the urethra or from the external genitalia. Not rarely, they are found in massive amounts in association with leukocytes and bacteria, a pattern that indicates urine contamination from

Table 7. Common Crystals (Figs 22 to 27 online)

Crystal	Urine pH	Birefringence	Most Frequent Appearance
Uric acid	≤5.8	+ (polychromatic)	A wide spectrum (most typical: lozenges), amber color common to all appearances
Amorphous urates	≤5.8	+	Irregular granules
Monohydrated calcium oxalate (Whewellite)	5.4-6.7	+	Ovoids, dumb-bell, biconcave disks
Bi-hydrated calcium oxalate (Wedellite)	5.4-6.7	—	Bipyramidal
Calcium phosphate	≥7.0	+	Prisms, star-like particles, or needles of various sizes
		—	Plates
Triple phosphate	≥7.0	+	"Coffin lids"
Amorphous phosphates	≥7.0	—	Irregular granules

Table 8. Pathological Crystals (Fig 4A and B)

Crystal	Urine pH	Birefringence	Most Frequent Appearance
Cholesterol	5.4-6.7	—	Transparent and thin plates, often clumped together, with sharp edges
Cystin	≤5.8	Variable (− to +)	Hexagonal plates with irregular sides, often heaped 1 upon the other
2,8-Dihydroxyadenine	≤5.8	+ (Maltese cross)	Spherical, brownish crystals with radial striations from the center

genital secretions because it occurs especially in women with vaginitis.

Lipids

Lipids appear as spherical, translucent, and yellow drops of different size. They can be free in urine, either isolated or in clumps, or within the cytoplasm of RTECs or macrophages (the so-called oval fat bodies), or within the matrix of casts (fatty casts). Under polarized light, lipid drops appear as "Maltese crosses" (Figs 2B and 17 online). Urinary lipids also include cholesterol crystals (discussed later).

Lipiduria is typical of glomerular diseases associated with marked proteinuria, usually, but not invariably, in the nephrotic range. In addition, lipids can be found in such sphyngolipidoses as Fabry disease. In this condition, they appear as particles that differ from typical lipid drops caused by the presence of an irregular membrane protrusion and the presence of "myelin bodies" under electron microscopy. These particles also form Maltese crosses under polarized light.

Casts

Casts are elements with a cylindrical shape of renal origin that form from the aggregation of fibrils of Tamm-Horsfall glycoprotein (uromodulin), which is secreted by cells of the thick ascending limb of the loop of Henle under a wide range of both physiological and pathological circumstances. Trapping of various particles (cells, lysosomes, lipids, pigments, crystals, microorganisms) within the cast matrix, as well as degenerative processes, result in casts with different appearances and clinical significance (Table 6; Fig 3; and Figs 12, 13, 16, 17, 20, and 21 online).

From the diagnostic standpoint, it is important to remember that whatever particle is contained in a cast is of renal origin.

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CRYSTALS

There are several types of urinary crystals. The combined knowledge of: (1) crystals most common appearances, (2) urine pH, and (3) birefringence features under polarized light allows the identification of most crystals. However, in rare cases, such sophisticated techniques as infrared spectroscopy are needed. Urinary crystals can be classified as: "common" (Table 7), pathological (Table 8), and crystals caused by drugs (Table 9).

Table 9. Main Crystals Caused by Drugs (Fig 4C and D)

Drug	Crystal Appearance	Clinical Manifestations
Sulfadiazine	Birefringent "shocks" of wheat or "shells" with striations	Isolated crystalluria, hematuria, ARF, stones
Acyclovir	Birefringent fine needles	Isolated crystalluria, ARF
Indinavir	Birefringent plate-like rectangles, star-like forms, irregular plates	Isolated crystalluria, stones, ARF
Piridoxylate	Asymmetrical hexagons or rectangles with rounded extremities	Crystalluria and stones
Primidone	Birefringent hexagons	Isolated crystalluria, transient hematuria
Felbamate	Needles, cat-tail configuration	Macroscopic hematuria, ARF
Amoxicillin	Birefringent needles, shocks of wheat	Isolated crystalluria, hematuria, ARF
Ciprofloxacin	Birefringent needles, sheaves, stars, fans, butterflies, etc	Isolated crystalluria, ARF
Naftidrofuryl oxalate	Birefringent monohydrate calcium oxalate	ARF
Vitamin C	Birefringent monohydrate calcium oxalate	ARF
Orlistat	(No better defined) calcium oxalate	ARF

Abbreviation: ARF, acute renal failure.

Crystals Caused by Drugs

An increasing number of drugs can cause crystalluria (Table 9). Precipitation of crystals is favored by various factors, such as drug overdose, rapid intravenous bolus administration, hypoalbuminemia, dehydration, or urine pH (for example, indinavir crystallization occurs at pH > 6.0, amoxicillin at pH ~ 4.0 or >7.0 with "U"-shaped behavior, ciprofloxacin at pH > 7.3).

Most drugs cause atypical and/or pleomorphic crystals, which differ remarkably from common or pathological crystals, whereas others (naftidrofuryl oxalate, vitamin C, and orlistat) cause calcium oxalate crystalluria.

Clinical Significance of Crystals

In our laboratory, the finding in urine of uric acid, calcium oxalate, or calcium phosphate crystals occurs in about 8.0% of samples. In most instances, it is an occasional finding without clinical importance because it reflects transient supersaturation of urine caused by ingestion of some foods, mild dehydration, or even precipitation of crystals in the interval between urine collection and urine examination. However, persistence of calcium oxalate or uric acid crystalluria in repeated samples of the same subject should raise the suspicion of a possible metabolic disorder, such as hypercalciuria, hyperoxaluria, or hyperuricosuria.

Moreover, uric acid crystalluria may be associated with acute renal failure caused by acute uric acid nephropathy, whereas calcium oxalate crystalluria may be associated with acute renal failure from ethylene glycol intoxication, some drugs (Table 9), or ingestion of the exotic star fruit.

Some crystals are always pathological. Cholesterol crystals are found in patients with marked proteinuria, cystine crystals are pathognomonic of the inherited condition cystinuria, and 2,8-dihydroxyadenine crystals are a highly sensitive marker of homozygotic deficiency of the enzyme adenine phosphoribosyltransferase, found in about 96% of untreated patients. Crystalluria is associated with other clinical manifestations, such as recurrent radiolucent urinary stone formation ($\sim$65%), acute renal failure ($\sim$26%), or chronic failure ($\sim$17%).

When crystalluria is caused by drugs, this may be the only urinary abnormality or it may be associated with hematuria (either gross or microscopic), obstructive uropathy caused by drug stones, or acute tubular necrosis caused by precipitation of crystals within renal tubules. As a general rule, one should always suspect drug crystalluria when finding atypical crystals. If confirmed, this should always prompt the check of renal function because acute renal failure can occur, especially in patients with chronic kidney disease and impaired renal function or those exposed to other potentially nephrotoxic drugs. Moreover, it is advisable to withdraw the drug or decrease the dosage and remove the predisposing factors (eg, reestablish euvolemia, stimulate a high urine flow, and manipulate urine pH).

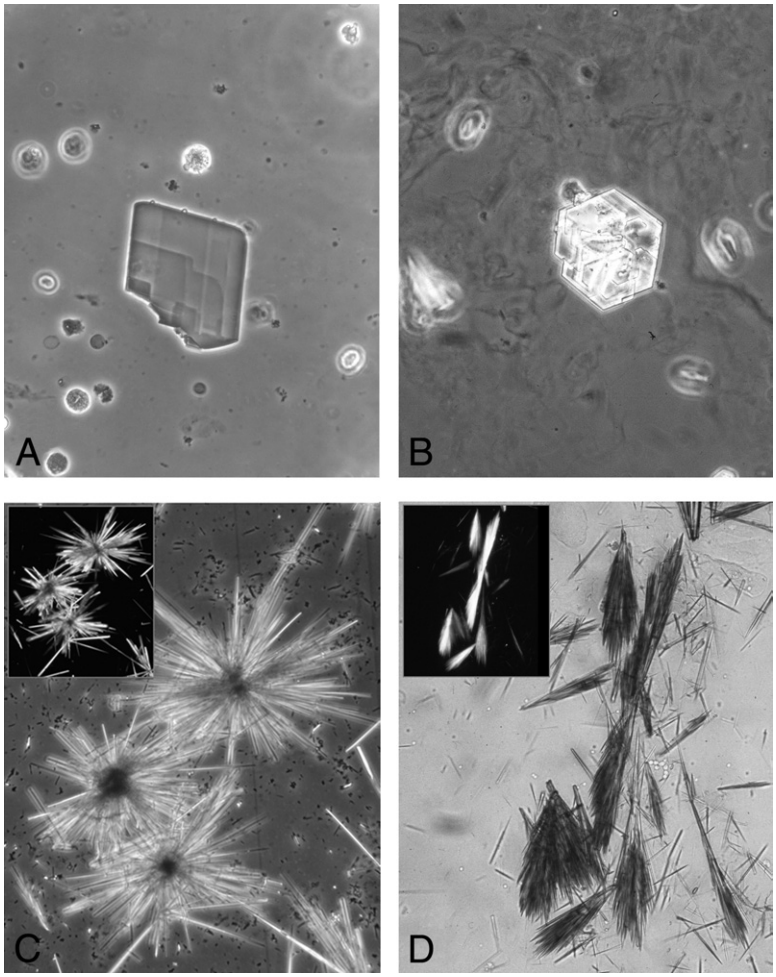

Figure 4. (A) A cholesterol crystal made up of plates heaped 1 upon another. Note the clear-cut edges and corners. (B) Cystine crystals heaped 1 upon another. Note the irregular hexagonal shape. (C) Birefringent star-like crystals of ciprofloxacin. (D) Amoxicillin crystals appearing as needles, shocks of wheat, and "broom bush," all strongly birefringent under polarized light.

Organisms

Bacteria

Bacteria are a frequent finding, caused by either infection or contamination from genital secretions. In both instances, bacteriuria is associated with leukocyturia, but in our experience, contamination only usually is associated with massive amounts of squamous epithelial cells of vaginal origin.

Candida *Species*, Trichomonas vaginalis, *and* Enterobius vermiculari

These are mostly contaminants deriving from genital secretions.

Schistosoma hematobium

This parasite is responsible for urinary schistosomiasis. Examination of urinary sediment is the most widely used method to diagnose this condition, which causes recurrent bouts of macroscopic hematuria and obstructive uropathy and favors both bladder cancer and glomerulonephritis. The diagnosis is based on finding parasite eggs, with their typical terminal spikes (Fig 32 online). The eggs especially are found between 10:00 AM and 2:00 PM, when the female worm lays the eggs in the bladder mucosa, and after physical exercise, which favors detachment of the eggs from the bladder wall.

Table 10. Main Urinary Sediment Profiles

Clinical Condition	Urine Sediment Hallmark	Associated Urine Sediment Findings
Nephrotic syndrome	Lipiduria	RTECs
	Marked cylindruria	RTEC casts
		Microscopic hematuria: absent (ie, minimal change disease) to mild (ie, membranous nephropathy) or moderate (ie, focal segmental glomerulosclerosis)
Nephritic syndrome	Moderate to severe dysmorphic hematuria	Mild leukocyturia
		RTECs
	Erythrocytic/hemoglobin cylindruria	RTEC casts
		Waxy casts
Acute tubular necrosis	Necrotic/damaged RTECs	Variable according to cause (eg, high numbers of erythrocytes and erythrocytic casts in proliferative/necrotizing GN, myoglobin casts in rhabdomyolysis, uric acid crystals in acute uric acid nephropathy, calcium oxalate crystals in ethylene glycol intoxication)
	Tubular fragments	
	Cylindruria with RTEC casts and muddy brown granular casts	
Urinary tract infection	Leukocyturia	Superficial transitional cells
	Bacteriuria	Triple phosphate crystals (for infections due to urease-producing bacteria)
		Leukocyte casts (in renal infection)
Urinary contamination from genital secretions	Leukocyturia	Massive amounts of squamous epithelial cells
	Bacteriuria	*Candida* and/or *trichomonas vaginalis*
Urologic disorders	Isomorphic hematuria	Deep urothelial cells
	Leukocyturia	Superficial urothelial cells

Abbreviations: RTECs, renal tubular epithelial cells; GN, glomerulonephritis.

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INTERPRETATION OF THE URINARY SEDIMENT FINDINGS

On the basis of the urinary sediment findings, it is possible to identify some urinary profiles that, integrated with measurement of proteinuria and serum creatinine, allow a first evaluation of the renal patient. Table 10 lists the main urinary sediment profiles.

Notes

Nephritic Syndrome

Under the effect of treatment, nephritic sediment may clear, whereas its reappearance indicates a relapse of the disease. Therefore, in patients with lupus nephritis or systemic vasculitis, examination of urine sediment over time is of special value in identifying recurrence of the renal disease. However, it should not be forgot-

ten that in rare cases, there may be an active renal disease in the absence of nephritic sediment.

Urinary Tract Infection

False-positive results may occur as a consequence of urine contamination from genital secretions or bacterial overgrowth upon standing. False-negative results may be caused by misinterpretation of bacteria (especially with cocci) or lysis of leukocytes, favored by alkaline pH and/or low specific gravity, or delayed urine sediment examination.

In addition, nonspecific urinary abnormalities can be found. In many instances, the urinary findings can be nonspecific and not arranged in one of the urinary profiles described (a few hyaline or hyaline-granular casts with or without mild erythrocyturia or leukocyturia, a few common crystals, a few superficial transitional cells, etc). In such cases, the correct interpretation of urinary findings requires adequate clinical information and results of other diagnostic tests.

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AUTOMATED ANALYSIS OF THE URINE SEDIMENT

In recent years, instruments for automated analysis of urinary sediments have been put on the market, obtaining widespread diffusion.

One instrument is based on flow cytometry, and the other on digital imaging software. Flow cytometry supplies results as both "scattergrams" and numeric data. The other instrument supplies results as both black and white images and numeric data.

Both these instruments, compared with manual microscopy, achieve acceptable results for erythrocytes, leukocytes, squamous epithelial cells, some types of crystals, bacteria, yeasts, and sperms. However, they do not recognize such particles of nephrological importance as lipids and RTECs and give too many false-negative results for casts (flow cytometry, 15% to 40%; digital imaging system, ~60%).

Today, these instruments are used in large laboratories to screen large numbers of mostly normal samples in a short time. In our opinion, this approach is not adequate for renal patients, for whom manual microscopy coupled with a motivated and well-educated examiner represents the gold standard.

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SUPPLEMENTARY MATERIALS

Figure S1: Isomorphic erythrocytes.

Figure S2: Different types of dysmorphic erythrocytes.

Figure S3: Acanthocytes or G1 cells.

Figure S4: Polymorphonuclear leukocytes with granular cytoplasm and lobated nucleus.

Figure S5: "Oval fat bodies" or macrophages packed with lipid droplets.

Figure S6: Oval fat bodies under polarized light.

Figure S7: A granular macrophage.

Figure S8: Proximal renal tubular cells.

Figure S9: Deep urothelial cells.

Figure S10: A clump of superficial transitional cells.

Figure S11: Squamous cells.

Figure S12: A colorless hyaline cast with Tamm-Horsfall glycoprotein fibrils.

Figure S13: A hyaline-granular cast.

Figure S14: A finely granular cast.

Figure S15: A waxy cast with typical "melted wax" appearance.

Figure S16: A fatty cast with packed lipid droplets.

Figure S17: A fatty cast under polarized light.

Figure S18: An erythrocyte cast.

Figure S19: A renal tubular epithelial cell cast with large nucleus.

Figure S20: A hemoglobin cast.

Figure S21: A bilirubin cast.

Figure S22: An aggregate of rhomboidal uric acid crystal under polarized light.

Figure S23: Birefringent amorphous urates.

Figure S24: Ovoidal monohydrated calcium oxalate crystals under polarized light.

Figure S25: Bihydrated calcium oxalate crystals.

Figure S26: A star-like calcium phosphate crystal birefringent under polarized light.

Figure S27: Two triple phosphate crystals.

Figure S28: A cholesterol crystal.

Figure S29: Cystine crystals.

Figure S30: Amoxicillin crystals under polarized light.

Figure S31: Birefringent star-like crystals of ciprofloxacin.

Figure S32: An egg of *Schistosoma hematobium* with typical terminal spike.

Note: The supplementary material accompanying this article (doi:10.1053/j.ajkd.2007.11.039) is available at www.ajkd.org.