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29 Urinalysis

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Routine urinalysis is one of the oldest clinical laboratory tests. One can imagine the primitive shaman making observations about a patient's urine in order to gain prognostic information. Certainly, ancient Greek physicians (later known as Pisse prophets) routinely examined the urine as part of their test repertoire. Early physicians were able to observe the appearance of urine and thereby detect the presence of bilirubin or blood, indicating liver disease or glomerulonephritis, respectively. Tasting urine or pouring it on the ground to see if insects were attracted were techniques employed to assess for the presence of sugar. Protein was identified by the coagulum that appears when urine is boiled. More sophisticated ancients apparently used sulfur to suggest the presence of bile in the urine.

Unfortunately, in spite of its distinct value, the humble urinalysis has fallen into a state of semi-disrespect. Today's physicians sometimes neglect the proven value of the urinalysis for the diagnosis of liver abnormalities, urinary tract diseases, or metabolic diseases such as diabetes, given its effectiveness in both monitoring chronic problems or screening for asymptomatic conditions. The knowledgeable laboratory director will attempt to raise the urinalysis technique to a position where it receives the attention that it deserves.

ROUTINE URINALYSIS

What is a routine urinalysis?

Routine urinalysis has been defined by the National Committee for Clinical Laboratory Standards (NCCLS) as "the testing of urine with procedures commonly performed in an expeditious, reliable, and cost effective manner in clinical laboratories." The term "routine" is not meant to suggest the indiscriminate performance of urinalysis because this investigation, like any test, should be used in a cost-effective manner. Although individual laboratories should customize their own protocols, today's routine urinalysis commonly consists of a visual examination of the urine to note its color and consistency. This may be followed by a measurement of specific gravity. Next, a series of semi-quantitative chemical screening tests are conducted on the urine. This is most conveniently done using "dipsticks." Dipsticks are thin, plastic strips on which are fixed chemically impregnated squares of porous material. These chemicals are able to rea with various components of the urine. With chemical te completed, the urine is centrifuged and the heavier parti ulate matter is concentrated and isolated onto a microsco slide. This is then examined microscopically to identify t specific nature of the particulate matter and determi whether or not it is made up of cells, casts, or debris. Typic values for the normal urinalysis are provided in Table 29- A synthesis of the facts provided supplies a vast amount information at a very low price.

The routine and microscopic urinalysis therefore consi of:

1. Macroscopic observation (color, clarity, odor)
2. Physical measurements (volume, specific gravity)
3. Dipstick or tablet chemical analysis
4. Microscopy of the centrifuged solid debris

Purposes for which a urinalysis is performed include:

1. To aid in the diagnosis of specific diseases.
2. To monitor disease progress.
3. To monitor therapy (effectiveness or complication
4. As a population screening for congenital, hereditar or asymptomatic diseases.

Facilities

Urinalysis should be carried out in a clean, well-illuminate well-ventilated space. At least one sink with running wa should be close at hand. A separate area for microsco work should be available with a conveniently centered ce trifuge capable of holding the number of tubes appropri for the laboratory workload and spinning them at a relati centrifugal force (RCF) of 400 to 450 for 5 minutes. Relati centrifugal force rather than revolutions per minute (rp is used as this value can be corrected for differences centrifuge rotor radius. The formula for the calculation

$$RCF = 1.118 \times 10^{-5} \times \text{radius (cm)} \times (\text{rpm})^2$$

A regular cleaning routine must be employed in the l oratory. This should involve disposing of completed ur samples down the drain, followed by flushing with copi amounts of water, and the disinfection of bench tops a sinks. Freshly voided urine is usually almost odorless a

Table 29-1 Typical urinalysis results*

Assay	Findings
Specific gravity	1.003-1.040
pH range	4.8-7.5 (mean, 6)
Protein	Negative to trace
Glucose	Negative
Ketones	Negative
Urobilinogen	Less than 1 mg/dL
Bilirubin	Negative
Occult blood	Negative
WBC esterase	Negative
Nitrite	Negative
MICROSCOPIC EXAM	
RBC	0-3/HPF
WBC	0-5/HPF
Bacteria	Few/HPF or less than 1+
Epithelial cells	Renal tubular, 0-1/HPF; transitional, 0-2/HPF; squamous variable/HPF
Mucus	Less than 1+
Crystals	Few calcium oxalate, few amorphous urates or phosphates
Casts	0-2 Hyaline casts/LPF 0-1 Granular casts/LPF
Spermatozoa	May be present in both men and women
Yeast	Negative
Trichomonas	Negative

*WBC, white blood cell; RBC, red blood cell; HPF, high power field; LPF, low power field.

therefore a disagreeable smell emanating from the urinalysis area may indicate a lack of hygiene (see "Specimen Collection").

Specimen collection

Urinalysis is ideally carried out with an aliquot of a single voiding of urine within 2 hours of collection. The minimum sufficient quantity to permit adequate macroscopic and microscopic exam is considered to be 12 mL, but a volume of 50 mL is preferable. The first morning specimen is considered the most valuable diagnostically because it is standardized and also reflects the kidney's ability to concentrate urine after an overnight period.

The ideal specimen is collected as a clean-catch midstream urine. This is obtained by having the patient begin to void, then interrupt the stream to catch the midportion. The first portion of the voiding is undesirable as it may contain surface debris from the genitalia, such as degenerated epithelial cells that can provide a false result. Providing the urine is collected in a sterile container, it is possible to follow up the routine urinalysis with a culture if initial tests warrant this procedure. A variety of other urine specimens are sometimes collected, including random samples and timed, 24-hour collections.

All urine samples should be collected into absolutely clean, though not necessarily sterile, containers. Features of a desirable collection container (NCCLS recommendations) are listed in Box 29-1. The responsible laboratory will ensure that any containers used are biodegradable.

Urinalysis samples should be examined within 2 hours. If they cannot be examined within this timeframe, they should be refrigerated at 4°C. Unfortunately, refrigeration may precipitate amorphous urates or phosphates, which can obscure subsequent microscopic examination. Undue delay in performing urinalysis may also allow bacteria to proliferate, cells and casts to degenerate, the pH to change, certain chemical substances (glucose and ketones) to disappear, and other substances (bilirubin, urobilinogen) to degrade. Even with refrigeration, microscopic examination should still be carried out within 4 hours of collection. Samples that have been maintained for 24 hours are only suitable for albumin, and hemoglobin testing.

Box 29-1 Desirable features for urine collection devices (NCCLS recommendations)

1. The container should be labeled with: patient's name, identification code, location, time and date of collection. (The label should be on the side, not on the lid, and should adhere to the container even if the specimen is refrigerated.)
2. Collection devices should be free from interfering chemicals (e.g., detergent).
3. Sterile containers should be used (if culture specimen).
4. Sufficient volume (approximately 50 mL is preferred).
5. Collection devices should have a wide opening (minimum 4.0 cm) to facilitate collection yet avoid contamination.
6. There should be a leakproof closure that is easily applied and removed.
7. Collection devices should have a wide base to avoid accidental spillage.
8. Specialized smaller containers should be available for pediatric specimens.
9. The reuse of collection containers is not recommended.

Although the use of chemical preservatives is not recommended by the NCCLS, the Metropolitan Life Insurance Company has developed a unique preservative tablet (potassium-acid-phosphate, 100 mg; sodium benzoate, 50 mg; benzoic acid, 65 mg; methenamine, 50 mg; sodium bicarbonate, 10 mg; and red mercuric oxide, 1 mg). The use of this tablet is said to allow the preservation of urine for routine chemical tests for a period of weeks at room temperature.

Unacceptable specimens should be rejected rather than tested. Criteria of unacceptability are:

1. Sample age
   - Unrefrigerated samples older than 2 hours
   - Refrigerated samples older than 4 hours
2. Collection container used
   - Unclean specimen containers (e.g., pickle jars); containers supplied by the laboratory are preferred.
3. Sample volume
   - Less than 2.5 mL. In the case of children or elderly patients, satisfying this criterion may be difficult. Patients with urinary tract infections often produce very small volumes. In this instance, the sample should be accepted. Volume should always be recorded.
4. Sample label
   - Improperly labeled samples should be rejected.

Dipsticks

Until the late 1950s, chemical tests were carried out on urine specimens using a variety of liquid chemical reagents. Current urinalysis depends on the use of the dipstick. The dipstick is a thin strip of plastic with one or more cellulose pads affixed. Each pad is impregnated with buffer and certain chemicals. The specific combination of materials in the dipstick pad is activated when water from the urine sample soaks into the pad. Reagents in the pad are then able to react with components of the urine sample. By this means the presence of various substances in the urine can be detected and their relative amounts can also be estimated. There are a number of important considerations involving the successful use of dipsticks.

1. The expiration date on the bottle is vital and materials must never be used beyond this date.
2. The container should be stored in a cool, dark place but not in the refrigerator.
3. Moisture must be avoided in the storage area as this may inactivate the pads.
4. Contamination of the test area by fumes or chemicals must be avoided to prevent false interpretation of the strip.
5. The lid of the container should be screwed on tightly when dipsticks are not in use. The potency of many of the chemical reagents in the pads declines with air exposure.
6. Reagent strips from different containers should never be combined. When strips are removed from a container but not used, they should not be placed back in the container.
7. The test strip should be applied to the sample briefly, but completely, so that the cellulose pads are completely immersed. If the immersion period is too long, chemicals may be allowed to dissolve or wash out of the pads. Following immersion, the dipstick is generally tapped lightly to remove excess moisture.
8. The clinical test sites on reagent strips should not be physically touched.
9. The developed color must be read at exactly the time specified by the manufacturer for the individual dipstick pad. A timing device with a second hand is required for visual reading. If used, automated strip readers should be adjusted to read the reaction pads at the times specified by the manufacturer.
10. All color readings should be conducted in a quiet, well-lit (illumination 500 lux) room where the light has a color temperature corresponding to that of daylight.
11. The instructions for dipsticks are all somewhat similar but sufficient differences exist such that the product monograph should be carefully reviewed for each product used.
12. The specificity and limitations of each test must be understood.
13. Quality control procedures must be incorporated.

There are a variety of different commercial products available for use in urinalysis. Because these undergo constant evolution, only the basic principles of individual tests will be reviewed in detail. A summary of the different dipstick reactions is provided in Table 29-2.

Automation

There are a number of instruments that have been deve to mechanize part or all of the routine urinalysis exa tion. The most common and most useful of these ar atively inexpensive devices that automate the reading dipstick. When using such a system, the dipstick i mersed in urine in the usual fashion, excess urine moved, and the stick is placed on or in the machine machine then reads the pads at the appropriate time lizing reflectance photometry.

These instruments are supposed to offer increase cision over visual reading, but studies have not a proven this to be the case. Depending on the instru the work throughput may be no greater than (and ma tually be less than) a manual/visual approach. The mac do offer a convenient way to perform stat analyses be timed readings can be performed without waiting. S cant time and labor savings can be realized when it i sible to interface these instruments with a laboratory puter system. At least one manufacturer has deve an instrument that performs routine urinalysis, che testing, and specific gravity in a totally "walk mode.

Developments currently underway that allow sem mated microscopic examinations to be performed sho cellent promise. To date, because of the expense c technology, any potential labor saving (particularly croscopic examinations are performed selectively) h hibited widespread introduction.

Quality assurance

A quality assurance program for urinalysis should porate continuous monitoring of every aspect of thi cedure. Quality control represents only a single asp quality assurance. Quality assurance programs invol ordination and communication between the patient, th oratory, and the clinician. According to NCCLS r mendations, components of urinalysis quality ass should include specimen collection and handling, r keeping, technical competence, standardization, tinuing education, and a scheduled, documented r process.

Recordkeeping

Recordkeeping is a fundamental part of quality ass and all records must be reviewed on a daily basis urinalysis supervisor or designee. Records, control instrument checks should be available for each shift. V procedures for detection and correction of errors, control results, and review of test results are require tient results should be reported accompanied by ref ranges.

A workbench record or log book should be availabl the following information:

1. Reagent strip lot number and expiration date
2. Date of opening of reagent strip containers (also be written on the bottle)
3. Patient and control results
4. Specimen collection time and laboratory time rival
5. Identification of technical staff who perform test(s)

Table 29-2 Summary of dipstick reagent testing

Test	Principle	Reaction interference: False-positive	Reaction interference: False-negative	Correlation with other tests
Specific gravity	pK change of polyelectrolyte	Protein	Alkaline urine	None, extremes may affect some pad results
pH	Double-indicator system	None	None	Microscopic exam, nitrite
Protein	Protein error of indicators	Highly alkaline urine, quaternary ammonium compounds, detergents	High salt concentration, very dilute urine, does not detect Bence Jones protein	Blood, leukocytes, nitrite, microscopic exam
Glucose	Glucose oxidase, double sequential enzyme reaction	Peroxide, oxidizing detergents, and hypochloride	Ascorbic acid, 5-HIAA, homogentisic acid, aspirin, levodopa, ketones, high specific gravity with low pH	Ketones
Ketones	Sodium nitroprusside reaction	Levodopa, phthalein dyes, phenylketones	—	Glucose
Blood	Pseudoperoxidase activity of hemoglobin	Oxidizing agents, vegetable and bacterial peroxidases	Ascorbic acid, nitrite, protein, pH below 5.0, high specific gravity	Protein, microscopic exam
Bilirubin	Diazo reaction	Medication color	Ascorbic acid, nitrite	Urobilinogen
Urobilinogen	Ehrlich's reaction	Ehrlich-reactive compounds (Ames), medication color	Nitrite, formalin	Bilirubin
Leukocytes	Granulocytic esterases reaction	Oxidizing detergents	Glucose, protein, high specific gravity, oxalic acid, gentamicin, tetracycline, cephalexin, cephalothin	Nitrite, protein, microscopic exam
Nitrite	Griess's reaction	Medication color	Ascorbic acid	Protein, leukocytes, microscopic exam
Ascorbate*	Tilmann's reaction	Pad is sensitive to ascorbate concentrations 10 mg/dL or greater. Approximately 2.5% false-positive reactions occur	—	Glucose, blood
Micro albumin*	Conjugated antibodies and enzyme	Pad is sensitive to micro albumin concentrations 10 mg/dL or greater, no known substances		Glucose, microscopic exam

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*Available as special dipstick pad or separate dipstick.

Procedure and instrument manuals

Urinalysis procedure and instrument manuals, including test directions, should be available at the workbench and include the following:

1. Acceptability and rejection criteria for specimens
2. Reference ranges
3. Panic values
4. Information regarding controls
5. Procedures for confirmatory testing

Instruments and other mechanical equipment should have:

1. A written procedure manual and manufacturer's instructions manuals
2. A written maintenance routine and regular maintenance schedule
3. Service and repair records

Proficiency testing

Some type of external proficiency testing program should be employed with results recorded on a regular basis. The 35-mm transparencies included with some external proficiency surveys are useful to assess technologists' recognition capacities but do not check urine handling or slide preparation. In-house and commercial controls for microscopic examination should only serve as precision checks.

Continuing education and training

Only qualified, properly trained personnel should perform a complete urine microscopic examination. Workshops and self-study programs for technical personnel should be provided. All technical personnel should have regular skill updates. Up-to-date reference texts, atlases, charts, and posters should be readily available. In-house conferences and seminars should be periodically held and any changes in procedures or reference ranges must be published and circulated to all appropriate staff and clients.

Quality control

Quality control is an important element of quality assurance and constitutes an accepted absolute requirement for the performance of clinical laboratory tests. For some reason, this principle is often less rigorously implemented in the area of urinalysis. There are four fundamental methods for establishing quality control in the urinalysis laboratory:

1. Maintaining an awareness of the daily distribution of positive results

2. Recognizing internally inconsistent results on a given patient's sample and following up results that strongly suggest improper collection or some form of unusual sample
3. Using quality control materials designed to mimic abnormal urines
4. Organizing repeat samples and introducing split samples into the laboratory

Urinalysis technologists should be well acquainted with possible combinations of abnormalities so that they will be alerted when illogical results occur.

Quality control of the chemical urinalysis

Multiconstituent controls to validate the performance of each chemical test at two distinct levels are recommended. Controls should be tested each day a test is performed, and whenever a fresh bottle of reagent strips is opened. When dipsticks are changed, parallel testing with old and new lots on controls and patient specimens is desirable. Commercial materials may be used as multiconstituent controls. However, commercial lyphilized materials are often very expensive. It is this expense that frequently prohibits the routine use of these materials. It is easy for any laboratory to prepare its own control samples. One formulation is as follows:

To make 300 mL of solution:
pH: Use phosphate buffers to create the desired pH
Protein: 0.3 g Bovine albumin (100 mg/dL)
Glucose: 750 mg Dextrose (0.014 m/L or 14 mmol/L)
Specific gravity: 15.0 g NaCl per 300 mL (1.022)
Ketones: 0.09 g Acetoacetic acid per 300 mL (pinch)
Nitrite: Sodium nitrite (one minute grain)
Color: Clayton yellow (small pinch)
Method:
Add phosphate buffer solutions in required amount.
Add remaining reagents in amounts required.
Add demineralized water to bring volume to 300 mL.
Add small stirring bar and mix until all reagents are dissolved.
Dispense into 7.0-mL serum tubes labelled "Urine QC."

The supervisor may split several samples each day and reintroduce them into the analytical area on a blind basis during each shift. After results are recorded, the supervisor is then able to identify the samples and access consistency.

Some important points to consider regarding quality control of the chemical urinalysis are: (1) if confirmatory chemical tests are carried out after dipstick analysis, these should be consistent with dipstick results; (2) brown urine should usually test positive for bilirubin, (3) bloody appearing urine or urine with erythrocytes on the microscopic examination should test hemoglobin-positive; (4) in most instances, urine with a large number of white cells on microscopic examination should test positive for esterase and nitrite; (5) urine with a high glucose content should have an increased specific gravity.

Freis has defined overall goals for quality control samples in chemical urinalysis. He has suggested that not more than 50% of positive results be more than one color block away from the assigned value. Obviously, a negative result is not acceptable for a positive control. Similarly, a negative reaction is the only acceptable result for a negative control. Participation in an external urinalysis proficiency survey to evaluate the performance of the chemical urinalysis desirable.

Quality control of refractometers and speci gravity determinations

A quality control program for refractometers and s gravity reagent strips must include the daily use of ref solutions or commercial control urine. Specific gravit standards can be created to calibrate this measureme

Result:	Amount of sodium chloride
SG 1.009:	1 g NaCl per 100 mL
SG 1.014:	3 g NaCl per 100 mL
SG 1.022:	5 g NaCl per 100 mL

Make up in 1 L amounts and store refrigerated.

Refractometers can be checked with deionized or d water (SG = 1.000) and with the aforementioned sol The calibration and daily performance checks of refr eters and specific gravity reagent strips must be docur and retained.

Quality control of the microscopic examina

During the microscopic portion of the urinalysis, sonnel should follow the same procedure using the id equipment and report results in a standard format us same terminology. Commercial control products are n erally available for all sediment elements. However, c containing red blood cells (RBCs) and white bloo (WBCs) are commercially available. Replicate tes fresh patient specimens can establish the reproducib microscopic analysis of casts, renal cells, and other elements, both within the laboratory and between laboratories. For any microscopically detected eleme result should agree within ± 1 reporting range.

If a disagreement exists regarding the presence o tity of a microscopic element, the examination n repeated. When necessary, the urinalysis supervisor oratory director should resolve discrepancies. Eacl ratory should establish appropriate criteria for rev abnormal sediment results.

QUALITATIVE CHEMICAL EXAMINATION

Physical appearance

Normal urine is usually a pale, straw-yellow color the presence of the pigment urochrome and small a of uroerythrin and urobilin. Urochrome is a product dogenous metabolism, and under normal conditio produced at a constant rate. Urochrome production in in thyroid conditions or when urine stands at roo perature. However, normal urine may also appear de ber to almost colorless secondary to the wide variati sible in urine fluid volume. When reporting urinary a good light source should be used and the specimen be examined against a white background.

Whereas normal urine may be almost any color, th ence of certain colors may be the result of pathology be secondary to the presence of a drug or food. It is im to be able to recognize a possible cause of urine color because, besides constituting a disconcerting symp the patient, it may be mistaken for a pathological p In addition, the intensity of the color change may

the ability to read dipstick results properly. This is especially true in the case of the drug phenazopyridine (Pyridium), which is frequently used as a urinary antiseptic.

Orange urine

Orange urine may very rarely be due to bile pigments. Their detection is outlined later in this chapter. Usually, anisindione, gantrisin (azogantrisin) ethoxazene, indanediones, mannose, phenothiazines, nitrofurantoin, phenazopyridine (Pyridium), rifampin, rhubarb, carrots, or senna is the cause.

Yellow urine

Yellow urine may also be due to bilirubin or urobilin but is probably the result of carrots, rhubarb, cascara, fluorescein, nitrofurantoin, phenacetin, picric acid, or quinacrine.

Green, blue-green, or blue urine

Bilirubin may become oxidized to biliverdin and generate green urine. Bacteria may also tinge the urine green, as will acriflavine, amitriptyline, methocarbamol, anthraquinone, azuresin, creosote, Doan's pills, Clorets, methylene blue, nitrofurans, phenols, phenyl salicylate, resorcinol, tetralin, thymol, tolonium, triamterene, and vitamin B complex. Blue or blue-green urine is exclusively due to medications such as dithiazanine, Doan's pills, Evans blue, methylene blue, and nitrofurans.

Red urine

Urine may be red as a result of the presence of red cells, hemoglobin or myoglobulin. Each of these substances can activate the dipstick's hemoglobin pad. Myoglobin can be identified using the special tests indicated later in this chapter. Porphyrins may also, on occasion, produce a red hue. Other reported causes of red urine include acetophenetidin, acrolein, aminopyrine, anisindione, antipyrine, beets, rhubarb, benzene, bromsulfophthalein (Bromsulphalein), cascara, chincophen, chrysarobin, Congo red, crayon pigment, danthron, deferoxamine, emodin, ethoxazene, indanediones, merbromin (Mercurochrome), methyldopa, phenazopyridine, phenindione, phenolphthalein, phenothiazines, phenolsulfonphthalein (PSP), phenytoin, and senna.

Brown or black urine

Biliary pigments, hematin, and myoglobin (positive hemoglobin on dipstick) color the urine brown. In addition, brown urine may be due to aloin, cascara, chloroquine, cresol, furazolidone, iron salts, metronidazole, nitrobenzene, nitrofurants, nitrofurantoin, rhubarb, sulfamethoxazole, and sulfonamides. The discovery of black urine is often an ominous sign. Of particular interest is a clear or dark urine that becomes even darker when left to stand. This phenomenon is observed in the presence of homogentisic acid, indican, melanin, metranidazole, and urobilinogen. Other nonpathologic causes are cascara, iron-sorbitol-citric acid complex, methyldopa, levodopa, methocarbamol, nephthol, phenols, and pyrogallol.

A summary of a number of the most common agents that cause different colored urines is provided in Table 29-3, along with possible additional tests that may facilitate identification.

Turbidity

Turbid urines are very common and are usually due to crystal formation in the bladder secondary to concentration or pH changes. Turbidity may also occur in the collection vessel as urine cools to room temperature. Acid urine will encourage the formation of uric acid crystals, whereas amorphous phosphates form in alkaline urine. Besides amorphous crystals, other common causes of turbidity in the urine are the presence of white blood cells, epithelial cells, and bacteria. Other possible causes include the presence of lipids, semen, mucus, yeast, fecal material, or possible extraneous contamination with substances such as talcum powder or X-ray contrast material. The presence of turbidity on the macroscopic examination of the urine should be followed up during the microscopic examination. The degree of turbidity should correspond with the types and amounts of material observed under the microscope.

Odor

Fresh urine has a mild but characteristic odor. The breakdown of urea in the urine is responsible for the characteristic ammonia odor. A pungent aroma may be the result of urinary tract infections, a high ammonia concentration, failure to deliver the specimen to the laboratory in a fresh state, or an unclean collection vessel. Some unusual conditions may be associated with very characteristic odors. For example, maple syrup urine disease is often first detected by a maple-syrup–like smell observed in a patient's urine. Other metabolic diseases that can result in a characteristic urine odor include phenylketonuria, isovaleric acidemia, and methenamine malabsorption. The ingestion of particular foods (such as asparagus) may impart a characteristic smell from the amino acid asparagine.

Evaluation

The clarity of the urine should be reported as clear, slightly cloudy, or cloudy. Other descriptive words can be used but should be employed sparingly.

Next, the color should be stated. Acceptable terminology includes colorless, pale yellow, dark yellow, amber, brown, red, or green. Words such as straw, dark, coffee, and so forth should be avoided if possible.

When present, other unusual findings should be mentioned (blood clots, fatty layers, or mucous clots). Any unusual odor should also be noted on the report form.

In some instances it may be impossible to read the dipstick because of the presence of Pyridium, other chemical contamination, or dark-colored urine samples. In this case, report: "Unable to read because of color interference on dipstick."

Specific gravity

Clinical significance

Specific gravity is the weight of a measured volume of a substance expressed in relation to the same volume of pure water. The specific gravity of urine increases as the concentration of material dissolved in it increases. In normal urine the main constituents that lead to an increase in specific gravity are salts (such as sodium and chloride) and nitrogenous wastes (such as urea and creatinine). In abnormal situations glucose or protein may be excreted in large

Table 29-3 Potential factors affecting urine color

Color	Possible cause	Laboratory correlations
Dark yellow Amber orange	Concentrated specimen	May be normal after strenuous exercise or in a first-morning spec men
	Bilirubin	Dehydration from fever or burns; yellow foam when shaken; and positive chemical tests for bilirubin
	Acriflavine	Negative bile tests and possible green fluorescence
	Carrots or vitamin A	Soluble in petroleum ether
	Phenazopyridine (Pyridium)	A drug commonly used for urinary tract infections; may have ora foam and thick orange pigment that can obscure or interfere w dipstick readings
	Nitrofurantoin	An antibiotic used for urinary tract infections
Yellow-green	Bilirubin oxidized to biliverdin	Colored foam in acidic urine and negative chemical test for bili- rubin
Yellow-brown	Rhubarb	Seen with acidic urine
Green/blue-green/blue	*Pseudomonas* infection	Positive urine culture
	Amitriptyline	An antidepressant
	Methocarbamol	A muscle relaxant
	Clorets	Breath freshener
	Indican	Confirm with Obermayer's test
	Phenol	When oxidized
	Methylene blue	None
Pink/red	Red blood cells	Cloudy urine with positive chemical tests for blood and RBCs vis microscopically
	Hemoglobin	Clear urine with positive chemical tests for blood; plasma may b red
	Myoglobin	Clear urine with positive chemical tests for blood; plasma will b colorless; specific tests are available
	Porphyrins	Negative chemical tests for blood; confirm with Watson-Schwart screening test or fluorescence under ultraviolet light
	Beets	With alkaline urine in genetically susceptible persons
	Phenolsulfonphthalein	With alkaline urine after *PSP test for renal function
	Bromsulphalein	With alkaline urine after *BSP test for liver function
	Rhubarb	Seen with alkaline urine
	Phenindione	An anticoagulant
Brown/black	Red blood cells oxidized to methemoglobin	Seen with acidic urine after standing; positive chemical test for blood
	Myoglobin	Positive chemical test for blood
	Homogentisic acid (alkaptonuria)	Seen with alkaline urine after standing; specific tests are availabl
	Melanin or melanogen	Urine darkens upon standing and reacts with nitroprusside and f chloride
	Phenol derivatives	Interfere with copper reduction tests
	Argyrol (antiseptic)	Color disappears with ferric chloride
	Methyldopa	An antihypertensive
	Levodopa	An anti-Parkinson drug
	Metronidazole (Flagyl)	Urine may darken on standing

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*PSP, Phenolsulfonphthalein; BSP, Bromsulphalein.

amounts. These substances can have a significant influence on urinary specific gravity. Also, contaminants in the urine may cause an increase in the specific gravity. In general, however, the specific gravity is a reflection of the state of the patient's hydration, combined with the functional ability of the kidney tubules. A normal individual who has been deprived of water for a period of time will concentrate urine, resulting in a high specific gravity. Large amounts of fluid given to a normal individual will naturally result in a dilute urine with a low specific gravity.

The normal spectrum of urinary specific gravity results for random specimens ranges from 1.003 to 1.040. The highest values are found with first-morning specimens following an overnight fast. The lowest values are observed several hours after a large amount of water has been sumed.

Very high values (greater than 1.040) are almost alv secondary to the presence of a contaminant in the u One of the most common causes is the presence of molecular weight radiopaque dyes given for radiogra imaging of the kidneys. Another possibility is sucrose (t sugar) added to the urine by individuals attempting to lead their physicians. Very high values may be see diabetes mellitus when large amounts of glucose are pre Serious proteinuria will also cause a raised specific gra The specific gravity of a first-morning specimen shoul greater than 1.015. An inability to concentrate the u above this value after fasting overnight provides strong

idence that kidney tubules are losing their ability to concentrate urine, that the body is greatly overloaded with fluid, that antidiuretic hormone is not being released (diabetes insipidus), that a diuretic is being administered, or that early glomerular disease is leading to an excess of fluid in the tubules.

Very dilute urine with specific gravity between 1.001 and 1.005 may be associated with extremely high fluid intakes, diuretic administration, and diabetes insipidus. In general, the urinary specific gravity is lower in persons on low-protein diets.

Measurement of specific gravity

Traditionally, specific gravity measurements were performed by using a modified hydrometer known as a urinometer. This consists of a glass vial with a thin top and weighted bottom. The urinometer is floated in the urine in such a way that the higher the specific gravity of the urine, the higher the device will rise. A dilute urine will allow the flotation device to float very low in the sample. The specific gravity is read directly from a calibrated scale fixed to the urinometer.

Currently, the specific gravity is usually evaluated using a commercial refractometer or total solids meter (TS meter). Two basic types of refractometers are commonly used; a hand-held model requiring only a single drop of urine and pour-through models (often incorporated with automated instruments). The principle involved with refractometry is the measurement of the refractive index. Refractive index is a comparison of the velocity of light in air to light velocity in a solution (urine). The velocity depends on the concentration of dissolved particles in the solution, which in turn affects the angle at which light passes through the solution. With different urine densities (different specific gravities), light will be bent to different degrees in an ocular device and this deflection can be calibrated using an appropriate scale.

Recently, a dipstick pad has been developed that approximates the specific gravity. This strip contains a polymeric acid that releases hydronium (H^+) ions when reacting with positive ions in the urine. The released hydronium ions cause a pH change, which in turn results in a color change. The higher the acidity, the higher the ion-concentration, and thus the higher the specific gravity result. This evaluation should be repeated using a refractometer if there is any amount of glucose or protein present when the dipstick pad is used for specific gravity measurements.

Cleaning, calibration, and maintenance of the refractometer is important and should be carried out carefully as follows:

1. Always check calibration daily. Raise the daylight plate and place a few drops of distilled water on the prism face.
2. Close the daylight plate gently. Look through the eyepiece and bring the scale into focus by turning the eyepiece. If the scale is correct, the boundary line should fall on the Wt and UG line. If it does not, adjust the boundary line to make it coincide with the zero line by turning the scale-adjusting knob. If the zero reading is correct for distilled water, it is unnecessary to check the instrument with the three specific gravity standards.
3. Wipe off the refractometer with a soft tissue. Place a few drops of urine on the prism and read the results on the UG scale.
4. Wipe off the sample with a soft tissue. It is important that the face of the prism is not scratched during cleaning.
5. It is important to keep all areas near the hinges and adjusting screws clean and dry at all times.

Interfering substances

As noted earlier, dipstick determinations of specific gravity depend on hydronium ion generation. High concentrations of nonionic substances such as glucose, protein, or X-ray contrast media may result in an elevated true specific gravity but will not affect the dipstick result.

When to perform specific gravity measurements

The choice of which specific gravity measuring technique to employ or, indeed, whether or not this measurement should be performed at all is a topic of debate. Physiologically, the only specimen that has clinical significance is one in which the hydration of the patient is well known prior to collection of urine for specific gravity measurement. In most routine instances, only a first-morning urine specimen will provide any valuable clinical information. Specimens collected randomly and without knowledge of the patient's state of hydration probably do not warrant performing urine specific gravity measurements. However, there is additional useful information obtained from urine specific gravity that can be helpful in the interpretation of other dipstick pad results. For example, a very dilute urine could cause a dilution of substances such as protein and glucose, rendering the detection of trace values of these substances clinically significant.

It is widely considered that specific gravity measurements performed by an accurate device, such as a TS meter, should use the first-morning voided specimen. The dipstick pad, which estimates the specific gravity, is all that is necessary under other clinical circumstances.

Ultimately, it must be remembered that in complex solutions such as urine, there is only a very general relationship between specific gravity and osmolarity. When more elaborate hydration studies need to be carried out, the formal measurement of urinary osmolality is a far more accurate tool.

pH

Clinical significance

The human body produces acid as a by-product of metabolism. A portion of the acid is volatile and can be eliminated through the lungs as CO_2. Some acid remains in the form of fixed organic acid (phosphoric, citric, oxalic), which must be removed by the kidneys. In general, urinary pH reflects the status of the pH of the blood. Under normal circumstances, urine is slightly acidic with a pH ranging from 4.8 to 7.5 (mean, 6.0). This pH is a function of the need to eliminate fixed acids.

A strongly acidic urine may be found in patients with systemic acidosis, when the body has an undue burden of hydrogen ions to remove. If there is acidemia, but the urine is neutral or just barely acidic (pH greater than 6), then a condition known as renal tubular acidosis must be consid-

ered to be present. In this condition the ability of the kidneys to secrete hydrogen ions into the urine is impaired.

An alkaline urine is seen in patients with systemic alkalosis as the body attempts to conserve hydrogen ions and remove fixed base. Following meals, all patients will experience a certain degree of alkalosis as a result of the stomach's production and secretion of hydrochloric acid. Vegetarians tend to have a more alkaline urine than persons who ingest meat. This is because ingested meat contributes to the fixed acid load that must be removed by the kidneys. With urinary tract infections, microorganisms in the urine may, through enzymatic action, split urea into ammonia. This will cause a distinct increase in urinary pH. Thus, with severe urinary tract infections or if urine is allowed to sit resulting in bacterial overgrowth in vitro, there will be a high pH. Increased alkalinity of the urine may destroy red blood cells and casts, especially if the specific gravity is also low. A knowledge of urinary pH is helpful for the identification of crystals noted during microscopic exam.

As part of the therapy for certain conditions, there may be a need to manipulate urinary pH to affect the solubility of inorganic substances that may be precursors of crystals and calculi. For instance, an acidic urine may be beneficial in the prevention of calcium carbonate and magnesium-ammonia-phosphate kidney stones. Maintaining an acidic urine may also benefit the treatment of certain urinary tract infections, because urea-splitting organisms cannot multiply as readily at an acidic pH. An alkaline urine may be induced to prevent stones due to oxalate, uric acid, and cystine. Alkalinization of the urine is occasionally used for the treatment of drug overdose (such as salicylate) or hemolytic transfusion reactions. Alkalinization of the urine also encourages the optimum action of some antibiotics.

Measurement

The most accurate measurement of urinary pH is performed using a pH meter. This degree of accuracy is seldom clinically necessary but may be useful as part of the formal evaluation of suspected renal tubular acidosis.

For patients who need to maintain their urine at either an acidic or alkaline pH, there are a variety of pH papers that can be used for frequent monitoring purposes. The dipstick pad that is part of routine urinalysis generally combines indicators such as methyl red and bromthymol blue. This provides a visual range of between pH 5 to 9. Urinary pH should be reported to the nearest whole number.

The routine measurement of urinary pH has little clinical significance. Its most important role is that, if very alkalotic, it may suggest that the specimen was not stored properly prior to testing. This observation suggests that bacteria may have been allowed to proliferate in urine left to stand too long at room temperature or at warmer temperatures. A strongly acid urine may suggest contamination of the collection vessel. Because several of the pads on the dipstick depend upon some aspect of pH, it is important that other dipstick pad results from urine specimens with extremes of pH be interpreted with caution.

Reaction interference

No known substances interfere with pH measurements performed by using dipsticks. However, care must be taken that no runover takes place between the adjacent l acidic protein testing pad, or false acidic results m reported.

Follow-up testing

A follow-up test is generally not required for urinar As noted earlier, if very high or very low values ar covered, then it may be wise to collect another spe and ensure its integrity before other dipstick measure are recorded. A neutral or alkaline urinary pH obser a patient with known acidemia merits consideration o sible renal tubular acidosis and may prompt a formal up for this condition.

Protein

Clinical significance

Although the kidney glomerulus may be consider be an efficient filter, in reality, the situation is conside more complex. As a result of hydrostatic pressure, s proteins below a certain molecular weight routinely through the glomerulus and into the proximal tubule bular proteins are mostly reabsorbed. Some protein, ever, normally passes through the tubules and enter urine in trace amounts. A normal individual may be t to have between 50 and 150 mg of protein in the urine day. Between 20% and 50% of this protein is albumin remainder consists of high molecular-weight, uromu Tamm-Horsfall proteins emanating from the renal tu cells; small amounts of serum and tubular microglob and proteins from vaginal, prostatic, and seminal secret

There are five different categories of proteinuria should be considered when evaluating protein found i urine. These are prerenal, glomerular, tubular, lower ur tract, and asymptomatic proteinuria.

Prerenal proteinuria is caused by disorders that elsewhere in the body rather than in the kidneys themse They can be further divided into two different types. I first type, an abnormal low molecular-weight protein its way into the circulation and, because of its small easily passes through the glomerulus into the urine. T the case with myoglobinuria (seen after crush injury or inflammatory myopathy), hemoglobinuria, or the light proteinuria associated with multiple myeloma. The form of prerenal proteinuria is secondary to a chan hydrostatic pressure in the kidney glomerulus. Followi increase in pressure, proteins that would normally n filtered are forced through the filtration bed into the urine. For this reason, a mild degree of pro uria in the absence of primary kidney disease ma associated with hypertension, congestive heart failur dehydration.

Proteinuria due to glomerular disease represents the common pathological form. A wide variety of agents cluding toxins, infections, vascular disorders, and in nological reactions, may produce damage to the glome filtration device. This allows an excessive protein le occur. In the earliest stages of glomerular damage, pro uria is selective and the urinary proteins are primaril lowest molecular weight proteins found in the bloodstr such as albumin and transferrin. Later, if glomerular dar progresses, virtually all of the proteins found in the s

may appear in the urine in approximately the same distribution as they occur in the bloodstream. When very severe, proteinuria can lead to nephrotic syndrome. In this situation, so much albumin is lost via the urine that the liver cannot keep up with synthesis and serum albumin concentration is ultimately reduced. This results in a variety of hydrostatic problems throughout the body, producing tissue swelling and edema. Generally, for the nephrotic syndrome to be present, there must be a minimum of 2 g of proteinuria a day. However, in severe cases, 10 g or more of protein may be present in a 24-hour urine collection.

Tubular proteinuria is generally mild, resulting in less than 2 g of proteinuria per day. In this situation, the proteins are low molecular weight (between 14,000 and 50,000 daltons). Thus, it may be possible to distinguish tubular proteinuria from glomerular proteinuria by using electrophoretic techniques to segregate the proteins on the basis of size. Tubular proteinuria is usually secondary to damage to the proximal tubules, which prevents the normal reabsorption of some of the smaller proteins. Some causes of tubular proteinuria include heavy metal intoxication, phenacetin damage, vitamin D intoxication, hypokalemia, Wilson's disease, galactosemia, Fanconi's syndrome, post-transplantation syndrome, pyelonephritis, acute tubular necrosis, and polycystic kidney disease.

Lower urinary tract diseases may result in exudation of protein through the mucosal layer of the lower urinary tract. This is almost always secondary to infection of either the ureters or bladder.

Asymptomatic proteinuria is generally discovered by accident. The most curious variant is orthostatic proteinuria. Individuals with this condition do not excrete protein into the urine after they have been lying down (first-morning specimen) but, after standing for 2 hours or more, will routinely display a small amount of proteinuria. About 50% of such individuals will be found to have minor glomerular disorders if subjected to kidney biopsy. The long-term significance of asymptomatic proteinuria is not fully known. However, it is recognized that a few of these individuals progress to more serious disorders, while the majority remain without any subsequent renal problems.

Another form of asymptomatic proteinuria is exercise proteinuria. Following severe exercise, many individuals will excrete a small amount of protein. This is of limited clinical significance providing it does not continue during times of rest.

Measurement

The first-line measurement of proteinuria is via the dipstick. Methods for measuring protein by a dipstick rely on a well-known phenomenon called the "protein error of indicators." In this situation, pH indicators change color in the presence of protein as ion transfer with the positive and negative groups on the protein molecule takes place. This protein error effect has been exploited to determine the amount of protein present in a sample. It should be recognized that only proteins that are capable of producing this phenomenon will participate in a measurement. For this reason, urine proteinuria determined by dipstick is almost entirely due to albumin. In general, dipsticks will not detect abnormal proteins such as myeloma light chains.

Typical reagent combinations for dipstick technology include: a citrate buffer (pH 3), tetrabromophenol blue, and a protein absorbent. Another combination uses tetrabromophenolphthalein ethyl ester.

There are other tests for performing semiquantitative measurements of protein in the urine, which include the heat-and-acid method. With this technique, the combination of heat and acid denatures protein and allows the semiquantitation of values down to 25 mg/dL. Phosphates and a variety of drugs may interfere with this technique. A somewhat more specific approach uses sulfosalicyclic acid. This method is sensitive to protein concentrations down to 20 mg/dL. Some antibiotics have been reported to interfere with this method. The protein area of the dipstick is one of the most difficult to interpret, especially in regard to "trace readings."

Protein should be reported as trace, 0.3 g/L, 1 g/L, 3 g/L, 20 or more g/L, or some other number as provided by the individual strip. "Plus" values should not be used. If the protein concentration is greater than 3.0 but less than 20 g/L, report "greater than 3.0 g/L." When the SG is greater than 1.025, trace protein results should be reported as negative.

Reaction interference

False-positive dipstick reactions may occur when urine is highly concentrated (specific gravity >1.030), whereas alternatively false-negative reactions may be associated with very dilute specimens (specific gravity <1.010). However, the major source of error with protein dipstick pads results when highly alkaline urine overrides the buffer system, producing a rise in pH and color change that is unrelated to protein concentration. The technical error of allowing the reagent pad to remain in contact with the urine over a prolonged period can strip the buffer, producing a false-positive reaction. Container contamination with quaternary ammonium compounds and detergents may also cause false-positve reactions.

Follow-up testing

If protein is discovered in the urine as part of a routine urinalysis or on a random specimen, it is generally superfluous to evaluate another urine specimen using a different semiquantitative method. It is far better to proceed directly to a 24-hour urine collection with quantitation of total protein and protein electrophoresis. This will provide an accurate quantitation of the amount of protein in order to properly assess the severity of the proteinuria. The electrophoresis will also help by indicating the specific nature of the disorder. In patients strongly suspected of excreting small amounts of Bence Jones proteinuria, either an early morning specimen or 24-hour specimen for concentration and protein electrophoresis may be used. However, both should ultimately be performed if either is initially negative because there are individual patients who will be negative by one collection technique but positive by another.

Microalbumin

The introduction of radioimmunoassay (RIA) methods for the measurement of albumin in urine has resulted in an awareness that urinary albumin excretion in amounts below

the usual limit of detection (<200 mg/L) can have clinical significance especially in diabetes mellitus. The range for microalbuminuria has been defined as 20 to 200 μg/min (30 to 300 mg/day) or >20 but <200 mg/L in a random sample. Subclinical albumin excretion in this range has been given the misnomer "microalbuminuria." It has been noted that the detection of microalbuminuria precedes the onset of clinical proteinuria associated with diabetic retinopathy and nephropathy. For example, insulin-dependent diabetics who excrete more than 15 μg of albumin per minute have been recognized to have an almost 90% chance of developing diabetic retinopathy, while those producing less than 15 μg per minute have less than a 4% chance of developing this complication. Trials are currently underway in diabetic patients with microalbuminuria to see if aggressive management of diet, blood sugar, and arterial blood pressure can reverse microalbuminuria and prevent progression to full-blown retinopathy or nephrotic syndrome with proteinuria. It is likely that measurement and monitoring of microalbuminuria will become an integral part of the assessment and management of diabetes mellitus.

There is much debate as to the best urine sample to test for microalbumin. Most data have been collected using 24-hour specimens or timed overnight samples. The actual measurement of microalbuminuria can be performed by radioimmunoassay or enzyme-linked immunoassay. For screening purposes, random urine samples may be tested. Recently, a manufacturer has developed a dipstick with good sensitivity for screening for microalbuminuria. Other companies are certain to follow.

Sugar

Clinical significance

Sugars are a normal component of urine. As small molecules, glucose (and other sugars) is easily filtered through the glomerulus, following which they enter the proximal tubule. In order to prevent the loss of valuable carbohydrate energy, the body ordinarily reclaims this filtered glucose. For this purpose, an active transport reabsorptive mechanism is located in the cells of the proximal kidney tubule. This transport mechanism is very efficient and removes almost all of the glucose originally filtered at the glomerulus. When the plasma glucose concentration exceeds 10 mmol/L, the reabsorptive capacity of the tubules is exceeded and unabsorbed sugar passes into the urine. Even with normal concentrations of blood glucose, some sugar may be found in urine because it is not possible for the proximal tubules to be 100% efficient in reabsorption.

Significant amounts of glucose will therefore be detectable in the urine when there is a high concentration of glucose in the bloodstream, as occurs in diabetes. Glucose will also be found in the urine in the case of certain proximal tubular diseases that can impair the ability of the reabsorptive mechanism.

Measurement

Dipstick. The dipstick measurement of glucose utilizes the enzyme glucose oxidase, which is highly specific for glucose. When mixed with atmospheric oxygen (a normal component of urine), glucose is acted upon by glucose oxidase to produce gluconic acid and hydrogen peroxide. The hydrogen peroxide thus generated can then be coupled an oxygen-accepting indicator with an associated change. The color change is proportional to the amou glucose present. This enzyme is highly specific and wi react with other sugars such as galactose, lactose, levu maltose, or pentose. However, contaminants such as b or peroxide in the collection vessel can cause a false-po reaction by producing the indicator color.

Glucose dipstick results should be reported as: neg 6 mmol/L, 15 mmol/L, 30 mmol/L, or greater thai mmol/L rather than using the + designations.

Reducing substances. Another technique for estin the amount of glucose present is to measure reducing stances. This test is begun by heating alkalinized urine glucose present under these conditions will reduce ions. This process is nonspecific and any reducing subs present in urine can potentially cause this color chan

An elegant example that utilizes this approach Clinitest tablet. This tablet is a combination of cupri fate, citric acid, sodium carbonate, and anhydrous so hydroxide. When urine is added to one of these tabl a test tube, a heat-generating reaction that also lib carbon dioxide occurs as the sodium hydroxide and acid are combined. The carbon dioxide generated pr the tablet from room air and allows the reaction to pr in an anaerobic environment. If reducing substance present in the urine, the cupric ion is changed to cu ion, producing a bright orange color.

Benedicts' reaction is similar in principle to the che changes involved with the Clinitest tablet.

Reaction interference. ***Dipsticks.*** As noted earlie glucose oxidase method is specific for glucose and positive reactions will not occur from other sugars. ever, false-positive reactions may occur when the spe container has been contaminated with peroxide, hypo rite, or strong oxidizing detergents. Similarly, subst that interfere with the enzymatic reaction or reducing a that prevent oxidation of the chromogen will produce negative results. Such substances include ascorbic aspirin, levodopa, and homogentisic acid. Bacteria tabolize glucose so specimens that are allowed to room temperature for several hours may also have negative results. High levels of ketones may inhib dipstick test pad when accompanied by low urinary gl concentrations.

Clinitest and copper reduction tests. The copper red test is relatively nonspecific and a variety of reducing stances that may be present in the urine can produce positive results. These include aspirin, vitamin C, levo probenecid, and a variety of antibiotics such as the alosporins, tetracyclines, and nalidixic acid.

Sugars other than glucose. Sugars other than gl may also be found in the urine. The most common is tose. Fructose will appear in the urine after the ingest large amounts of fruit. It can also be found in the ur a harmless condition known as essential fructosuria, results from an inability to efficiently remove fructos other condition, hereditary fructose intolerance, du deficiency of fructose-1-phosphate aldolase deficien more severe. With this enzyme deficiency excess fr distorts the normal glycolytic pathway, resulting in

glycemia, vomiting, jaundice, amino-aciduria, cirrhosis of the liver, and renal tubular acidosis.

Pentoses (five-carbon sugars) may also be present in the urine following massive fruit ingestion. Ribose can be found in the urine of individuals with muscle-wasting conditions. A benign variant known as essential pentosuria has also been described.

Galactosuria is an inherited condition resulting in liver disease secondary to a deficiency of the enzyme galactose-1-phosphate-uridyl-transferase. Infants with this condition fail to thrive and eventually develop cataracts due to the unusual deposition of the converted sugar alcohol, galactol, in the lens.

Mannoheptulose has been noted in the urine of people who have eaten large amounts of avocado. Lactose may be detected in the urine during the latter part of pregnancy and is also seen in lactating women as a result of the production of breast milk.

Sucrose can be found in urine following massive ingestive of cane sugar. However, a more common cause of sucrosuria follows its factitious addition to urine samples by individuals attempting to cause a false-positive result who are unaware that this sugar will not be identified by routine urinary dipstick analysis.

The best approach for measuring an unusual sugar in the urine is to perform a quantitative reducing method to determine the amount of material present. This should be followed by thin-layer chromatography to actually identify the nature of the individual sugar.

There are some methods that can be used to specifically identify sugar, such as Seliwanoff's fructose test, which utilizes resorcinol and heat. There is little call to perform these tests in the routine clinical laboratory.

Ketones

Clinical significance

Ketone bodies are short-chain organic acids produced as an end-product of fat breakdown. Fat metabolism will occur in starvation and in insulin-deprivation. Therefore, when a person starves or follows a severe diet, ketones will routinely appear. Indeed, after a 14-hour fast, many persons will show a small amount of urinary ketone. Insulin-deprivation (diabetes mellitus) prevents normal metabolism of glucose, so the body must metabolize fat stores to provide energy. An end-product of this process is the production of ketones.

There are three main ketones. With human ketosis, 78% is comprised of β-hydroxybutyric acid, 20% is acetoacetic acid, and 2% is acetone. These ratios may vary slightly but are usually relatively constant. It is important to recognize that analytical methods that detect a single species correlate well with total ketones present, but, to truly quantitate the total amount of ketones, the distribution between species must be taken into consideration.

Measurement

The dipstick measurement of ketones employs sodium nitroprusside, glycine, and buffer incorporated into a reagent pad. This chemical combination is very sensitive to the presence of acetoacetic acid, producing 15 times as much color with this material vs. acetone. The combination does not react at all with β-hydroxybutyric acid. There are other tests available for the measurement of ketones, such as Gerhardt's ferric chloride reagent. This reagent acts in the presence of acetoacetic acid but it is neither sensitive nor specific. Urinary ketones should be reported as negative, trace (0.5 mmol/L), small (1.5 mmol/L), moderate (4 mmol/L), or large (8 or 16 mmol/L).

Reaction interference

False-positive results have been reported with levodopa, phenazopyridine, phenformin, and with paraldehyde ethanol combinations. Specimens collected after diagnostic procedures employing phthalein dyes can generate an interfering red color in the alkaline test medium. Ketones are volatile and will disappear from a sample over time. Thus, false-negative results may occur if the urine sample is allowed to stand for any length of time, particularly in a warm environment. Aspirin has also been reported as a cause of false-negative results. Paradoxically, aspirin in large amounts may also cause the body to produce ketones.

Follow-up

The presence of ketones in the urine can be accepted at face value and will usually correlate with the patient's clinical condition. Therefore, there is little need to follow up with any further urinary tests, although, depending on the patient's condition, a variety of blood measurements for glucose may be required.

Hemoglobin

Clinical significance

Hemoglobin can be present in the urine following intravascular hemolysis, as the result of red cells being filtered through the glomerulus, or secondary to red cells entering the lower urinary tract. There are many possible causes of hemolysis (see Chapter 11). When an intravascular hemolytic disorder occurs, hemoglobin is released from red cells into the bloodstream. Here, it is picked up by hemopexin and haptoglobin and recycled to the liver. When the capacity of these proteins to bind and remove hemoglobin is exceeded by the rate of hemoglobin release, the free hemoglobin is left to pass through the glomerulus and into the urine.

Red cells may pass through the glomerulus as a component of glomerular disease. In this case the hemoglobinuria may be associated with the presence of red cells and red-cell casts in the urine.

Bleeding from the lower urinary tract (particularly the bladder) is a frequent cause of hemoglobinuria. Such bleeding can be accompanied by red cells but never red-cell casts.

When small amounts of hemoglobin are detected in the urine of an asymptomatic person, follow-up is often required to determine if this finding is clinically significant. It has been shown that almost half of all patients with asymptomatic hematuria have no discernible cause for this condition. However, the degree of diagnostic yield on follow-up is definitely age- and sex-related. For instance, the incidence of pathology found may be as low as 2% in persons under the age of 30 years. In the older age group, investigation of asymptomatic hematuria in individuals (especially males) over the age of 60 has revealed significant pathology in up to 50% of the cases. Diseases found in this population include prostatic disease, urinary tract infections, or renal

calculi. Urinary tract tumors are rarer but obviously constitute a very significant finding. A cost benefit analysis for all ages showed that the large increase in life expectancy for younger vs. older patients to some extent balanced out the lower prevalance of disease discovered in the younger group. Thus, the repeated detection of hematuria probably warrants a thorough investigation of the urinary tract regardless of a patient's age.

Measurement

Hemoglobin is a peroxidase and this chemical feature is exploited for its detection capability. The peroxidase effect is used to catalyze O-toluidine to form a blue complex. Myoglobin will also cause a positive reaction with this enzyme system.

A recent feature in dipstick technology has been the inclusion of red cell lysing agents with the hemoglobin pad. These agents cause the lysing of intact red cells. The lysed red cells in turn cause compacted or scattered green dots on the pad. Without the lysing agents, it is theoretically possible for red cells to remain intact, preventing a positive hemoglobin reaction from occurring on the dipstick pad. Whereas most cases of hematuria are accompanied by some degree of spontaneous red cell lysis and hemoglobinuria, this phenomenon is still occasionally observed.

Hemoglobin should be reported as: negative, trace, small, moderate, or large rather than using the (+) designation.

Reaction interference

Excessive urinary nitrate levels associated with severe urinary tract infections may induce false-negative results. Vitamin C-induced false-negative reactions for both hemoglobin and glucose may represent a significant problem of unsuspected magnitude. For instance, a recent study of routine urinalysis results in a West Coast population documented a 23% incidence of significant urinary ascorbate levels. Since few published studies of individual dipstick performance have included checks for urinary ascorbate, unsuspected urinary contamination with vitamin C may have been responsible for many of the false-negative reactions for hemoglobin and glucose noted in published dipstick evaluations.

Manufacturers have devised two potential solutions to this problem. One has been the development of a vitamin C-resistant dipstick that utilizes an iodate-impregnated mesh layer to resist ascorbic acid interference. Another manufacturer has actually incorporated a vitamin C test pad onto its dipstick. Any urine testing positive for vitamin C with this pad can be followed up with a careful microscopic exam and consideration can be given to alternative testing for glucose and hemoglobin.

False-positive reactions owing to menstrual contamination may be seen and will also occur if strong oxidizing detergents are present in the specimen container. False-positive reactions may also be caused by bacterial or vegetable peroxidases, including *Escherichia coli* peroxidase. For this reason, sediments containing bacteria should be followed up closely on microscopic exam for the presence of red blood cells.

Myoglobin

Clinical significance

As myoglobin will be detected by the dipstick hemo
bin pad, it is important to be aware of potential cause
myoglobinuria. Myoglobin is a muscle protein respons
for oxygen transport. It is very similar to hemoglobi
nature. Myoglobinuria may occur following crush injur
heavy exercise (particularly in the untrained), grand
seizures, comas (particularly those resulting in decre
oxygen supply to the periphery of the body), and a var
of myopathies. The clinical picture of myoglobinuria usu
includes one of the aforementioned clinical scenarios
sociated with a positive dipstick hemoglobin test, redd
gold urinary pigment, granular casts on urinary microsc
exam, and an increased creatinine kinase in the serum.

Measurement

Whereas the existence of myoglobinuria may be
pected following the detection of the triad of findings n
earlier, its presence can be verified by a variety of diffe
techniques. Electrophoresis has been used as an anal
technique. Spectrophotometry is also possible as myogl
demonstrates characteristic absorption wavelengths. H
ever, spectrophotometry is not a definitive test. Filtra
techniques have been devised, capitalizing on the sma
size of the myoglobin molecule, which allows it to be filte
through an ultrafiltration membrane and thereby dis
guished from hemoglobin. This filtrate will continue to
positive on the hemoglobin pad of the dipstick. Hemogl
and myoglobin can also be distinguished by differential
ubility. However, none of the above techniques is eas
perform or completely reliable. For routine use, an imi
nological technique using immunodiffusion is probably b

Reaction interference

Contamination of urine with povidone iodine (Betad
used as a skin decontaminant for surgical procedures
produce a positive chemical test for blood secondary to
strong oxidizing properties of iodine. Following mictura
postsurgery, if iodine contamination is not considered, th
may be a concern that the surgery has caused muscle dam
and myoglobinuria.

Bilirubin

Clinical significance

After 120 days, as red blood cells reach the end of th
natural life span, they undergo lysis by the body and th
components are broken down either to be removed from
body as waste or recycled for the formation of new red ce
It is from this breakdown of the heme group in hemoglo
that bilirubin is formed (see Chapter 11). Bilirubin is
soluble in plasma and must be transported bound to albun
When it arrives at the hepatocyte, it is removed from
bumin and, conjugated through a series of enzymatic ste
with glucuronic acid. In its conjugated form bilirubin
soluble and is secreted into the biliary tract for removal fr
the body. Bilirubin and urobilinogen metabolism is su
marized in Box 29-2. Normally, almost none of the c
jugated bilirubin enters the circulation. With biliary tr
obstruction (stone or tumor) or obstruction involving

Box 29-2 An outline of bilirubin and urobilinogen metabolism

1. Synthesis from heme
   a. From hemoglobin
      - Senescent red cells
      - Ineffective erythropoiesis
   b. From other heme containing compounds
   ↓
2. Transport in plasma bound to albumin
   ↓
3. Uptake by liver
   Dissociation from albumin—entry into hepatocytes
   ↓
4. Conjugation with glucuronic acid
   ↓
5. Excretion of conjugated bilirubin into bile
   ↓
6. Degradation in gut—formation of urobilinogen—fecal excretion
   ↓
7. Enterohepatic circulation of some urobilinogen
   a. Mostly recycled by liver
   b. Small amount excreted into urine

passage from the hepatocyte into the biliary system, conjugated bilirubin will reflux back into the circulation and appear in the bloodstream. Since conjugated bilirubin is water soluble, it easily passes through the glomerulus and into the urine. Thus, the appearance of conjugated bilirubin in the urine is strong evidence for some type of obstruction lesion in the liver or biliary system.

Measurement

Bilirubin degrades quickly in the urine and must be measured within a few hours. An ancient assessment of bilirubin involved the shake test. Following a vigorous agitation of urine, the visual detection of yellow foam was taken as strong evidence for the presence of bilirubin.

Today, dipstick detection of bilirubin relies on the diazotizing of bilirubin to a complex organic molecule, which changes color in the process. For example, diazotized 2,4-dichloroaniline in an acid buffer has been employed as a stable diazonium salt, 2,6-dichlorobenzene-diazonium fluoroborate, used to detect bilirubin.

There are a variety of additional tests for measuring bilirubin in the urine. However, even if carried out with accuracy and precision, the urinary measurement of bilirubin has limited diagnostic utility. It is far better to proceed with specific serum tests of liver function, such as serum bilirubin, aspartate transaminase (AST), alkaline phosphatase, and γ-glutamyl transpeptidase than to waste time with the further quantifying of urinary bilirubin. Urine bilirubin should be reported as positive or negative.

Reaction interference

The most frequent problem area associated with bilirubin testing is false-negative results caused by the testing of specimens that are not fresh because bilirubin is an unstable compound rapidly destroyed following light exposure. Exposure to air causes oxidation of bilirubin to biliverdin, which does not react in standard test systems. False-negative results will also occur following the hydrolysis of bilirubin diglucuronide to free bilirubin because this substance is less reactive in reagent strip tests. High concentrations of ascorbic acid and nitrate have also been noted to lower the sensitivity of this test pad reaction.

Urobilinogen

Clinical significance

Once bilirubin enters the biliary tract it ultimately passes into the intestinal lumen. After traversing the small intestine and reaching the large intestine, the action of various bacteria break bilirubin down through a series of steps to a number of metabolites, including urobilinogen. Highly converted forms of bilirubin can be absorbed in the large bowel to a minimal degree. Ordinarily, minute amounts of urobilinogen find their way back into the bloodstream and are then re-excreted through the liver and into the biliary system. Various disorders of the liver or biliary system have the potential to impair this enterohepatic circulation. Significant amounts of urobilinogen will then be excreted in the urine. For this reason, increased urobilinogen can be found in the urine of those suffering from either obstructive liver disease or hepatocellular liver disease. In addition, persons with a hemolytic process will have an increased enterohepatic circulation of bilirubin by-products such that increased amounts of urobilinogen will appear in the urine.

Urobilinogen disappears very quickly from urine following degredation by oxidiation. Initially, the molecule is colorless, but after standing for a few hours the urine may be rendered dark.

Measurement

Dipstick measurement of urobilinogen depends upon linkage with complex organic molecules. One manufacturer's product uses a buffered p-dimethylaminobenzaldehyde. When this material combines with urobilinogen it produces a pink color. There are other methods for the measurement of urobilinogen such as the Wallace-Diamond test or Watson's quantitative test. As in the earlier discussion of bilirubin, further specific measurement of urobilinogen is usually not worth the effort. Following its initial detection, investigation should proceed using well-established serum tests of liver function. An approach to the simultaneous use of bilirubin, urobilinogen, and liver enzymes in defining hemolytic, hepatic, and biliary causes of jaundice is outlined in Table 29-4. Urinary urobilinogen should be reported as normal (3 to 17 μmol/L) or positive (34, 68, 135 μmol/L).

Reaction interference

The dipstick pads that test for urobilinogen using p-dimethylaminobenzaldehyde are susceptible to false-positive results from compounds such as p-aminosalicyclic acid, antipyrine, apronalide, chlorpromazine, phenazopyridine, phenothiazine, sulfadiazine, and sulfonamide. The utilization of p-methoxybenzene-diazonium-fluoroborate as a reagent on the Chemstrip dipstick produces a more specific test. However, false-negative results may occur when large

Table 29-4 Helpful tests in the evaluation of jaundice

	Hemolysis	Acquired hepatocyte disorder	Biliary tract obstruction
Urine test			
Bilirubin	−	+	+
Urobilinogen	↑	↑	− or ↓
Serum enzyme activity			
Alkaline phosphatase	±	Variable	↑
Alanine and/or asparate aminotransferase	±	↑	+ or ↑
γ-Glutamyl transpeptidase	±	Variable	↑

quantities of nitrite are present and highly pigmented urine samples may also interfere with the detection of this color reaction.

Leukocyte esterase

Clinical significance

Many white cells (particularly granulocytes) contain an enzyme known as leukocyte esterase. Detecting the presence of this esterase provides strong evidence that white cells are present in the urine. The inclusion of the esterase test on the dipstick allows the detection of the presence of white cells without necessarily requiring a visual search. It should be recognized that not all white cells produce leukocyte esterase and therefore it is possible to have white cells in the urine without a positive dipstick leukocyte esterase. In general, this pad is 90% to 95% sensitive and over 95% specific.

Measurement

The leukocyte esterase test requires up to 2 minutes (or longer) for the resulting color change. Manufacturers are currently working on developing faster reagent combinations. Test timing is critical, otherwise reduced sensitivity is obtained. The sensitivity of this test also varies with the number of leukocytes present. While positive reactions may be noted at 5 to 15 WBC/HPF, many dipsticks only give 80% to 90% positive results with greater than 15 WBC/HPF. However, beyond a general indication of leukocyturia, the esterase test has only a limited ability to predict the number of leukocytes that will ultimately be found on microscopic examination of the urinary sediment. Urine containing large amounts of yellow pigment may generate a green rather than purple color with the esterase reaction. The observation of this color also constitutes a positive test. Dipstick leukocyte esterase should be reported as positive or negative.

Reaction interference

Taking too early a reading is the most common cause of a false-negative leukocyte esterase test. False-negative results may also be noted with high specific gravity urine because crenation of leukocytes can prevent release of their esterases. Other conditions that have been reported to cause reduced leukocyte esterase reactions include elevated glucose (>3 g/dL), cephalexin, cephalothin, high concentrations of oxalic acid, tetracycline, and boric acid. For can cause a false-positive reaction.

Nitrite

Clinical significance

Occult urinary tract infections can be devastating ders that ultimately result in chronic renal failure. Tl tection of urinary tract infection has traditionally inv either performing a culture or at least identifying whit and bacteria in the formed elements of the urine. Rec a dipstick test has been introduced that attempts to id the presence of bacteria without the need for a visual s

Normal human beings excrete a certain amount of r in their urine. This nitrate is converted to nitrite by c species of bacteria. A positive dipstick pad to dete presence of nitrite will therefore, by inference, pr strong evidence for the presence of certain types of bac

For the test to be positive, the patient must first nitrate in the urine. The production of urinary nitra largely associated with the consumption of vegetabl therefore, individuals must be consuming adequate an of these foodstuffs to start off with.

In addition, only certain bacteria have the ability t duce the nitrate-nitrite conversion. *Escherichia coli*, *teus*, *Klebsiella*, *Enterobacter*, *Citrobacter*, and *Salm* are very aggressive nitrate reducers. *Enterococcus*, *S ylococcus*, and *Pseudomonas* are only partial conv Other bacterial species do not promote this conversic

Furthermore, the production of nitrite will increase urine is held in the bladder for a period of hours. Sinc is often not feasible, the reliability of the test is only 50% positive in random samples. For all the aforement reasons, a first-morning specimen is certainly the best ple for nitrite testing.

Obviously, a negative nitrite test does not exclu presence of a urinary tract infection. However, a po test provides strong evidence for a urinary tract inf and should be followed up via appropriate culture.

Measurement

The presence of urinary nitrite is detected by linki aromatic amine with nitrite to produce a diazonium pound with a characteristic color. The pink color re is not directly quantitative in relation to the number o teria present. However, any degree of color should terpreted as a positive nitrite test and is suggestive or more organisms/mL. A negative test result is not that significant bacteriuria does not exist. The sensitiv the nitrite test is also reduced in urine with a high sp gravity. Urinary nitrites should be reported as posit negative.

Reaction interference

Ascorbic acid concentrations of 25 mg/dL or greate cause false-negative results when specimens contain amounts of nitrite ion (0.06 mg/dL or less). Other c of false-negative results include the inhibition of ba metabolism by antibiotics and ongoing reduction of r to nondetectable nitrogen, which may occur if large nu of bacteria are present in the bladder. False-positive r

may be obtained if nitrite testing is not performed on fresh samples as a multiplication of contaminant bacteria can rapidly generate measurable amounts of nitrite.

MICROSCOPIC EXAMINATION

General

A traditional part of urinalysis is the performance of a microscopic examination. This is generally carried out by taking a standard amount of urine, centrifuging the specimen for a fixed period of time, removing the supernatant fluid, resuspending the sediment in a standard volume (usually 1 mL), and finally examining this sediment under the microscope. For the microscopic exam, commercially available slides with incorporated counting chambers of a constant volume have been available since the early 1970s. Their use is highly desirable because they offer a standardized sediment analysis that eliminates many of the variables inherent in "drop on slide" techniques.

Standardized sediment analysis enables the observer to observe and quantitate cells (red cells, white cells, bacteria, yeast, epithelial cells, and tumor cells), crystals, which are formed from the precipitation of salts; and casts, which are formed in the kidney tubules. Each of these substances may be a valuable indicator of pathology. Whether or not to routinely stain urine sediment is a question of some debate as is the necessity for phase or interference contrast microscopy. Given a proper level of professional expertise and good equipment, bright field microscopy of unstained urine should identify all clinically important and relevant sediment. However, polarization of the sediment may be helpful in some circumstances, especially in identifying crystals and lipids. For instance, six-sided cystine crystals that are nonbirefringent can be differentiated from six-sided urate crystals that are birefringent. Doubly refractile lipids show a typical "Maltese cross" pattern under polarized light. Many foreign contaminants such as talc or starch granules are birefringent, whereas most noncrystalline human sediment elements are not.

When to perform the microscopic examination

It has been argued that, under routine circumstances, microscopic examination of the urinary sediment is not necessary. This argument is based upon the rationale that the only features of the urinary sediment that are not detected by the dipstick in asymptomatic individuals are certain bacteria and/or some white cells. The presence of other urinary constituents such as red cells or casts will be heralded by positive dipstick reactions for hemoglobin, esterase, or protein. Whereas some investigators advocating a "dipstick only" screening approach have claimed a false-negative detection rate for significant urinary constituents of only a few percent, other studies have demonstrated false-negative rates as high as 30%.

Most clinicians would generally agree that an important advantage of the microscopic examination performed on an asymptomatic patient is the possible detection of occult urinary tract infection. However, with the recent development of the nitrite and leukocyte esterase pads, the ability to detect occult infections has increased. Some studies have found that the diagnostic efficiency of the esterase-nitrite combination is similar to that of sediment microscopy in detecting or excluding urinary tract infections. However, the impact of the pretest probability of infection on the overall test reliability and infection detection rates has also been emphasized. Proponents of the "dipstick only" approach would still argue that the chances are very small of finding an occult infection in an asymptomatic individual with an otherwise normal urinalysis. In addition, the high labor costs associated with performing additional microscopic procedures further detract from cost effectiveness.

These arguments will continue to be debated in the literature. In the interim, each laboratory should develop an individual approach that ideally would be based on a survey of the false-negative dipstick results in their own urinalysis patient population. However, at the present time, whenever a positive pad is detected on the dipstick it is necessary to complete the urinalysis by performing a microscopic examination.

Clinical significance of the formed elements of urine

Red blood cells

Whereas more than 0 to 3 RBC/HPF in the urine is considered abnormal, some authors have suggested that 8 RBC/HPF is an appropriate level dividing normal and abnormal hematuria. RBC are found in the urine after passing through the glomerulus in glomerulonephritis. In addition, they may enter the urine following direct bleeding into the lower urinary tract as may occur with urinary infections, tumors, or renal calculi. Hematuria may also follow trauma or exposure to toxic chemicals or drugs. In female patients, the possibility of menstrual contamination must also be considered. On microscopic examination red blood cells are noted as uniform, colorless disks. However, in concentrated urine red cells may shrink and appear crenated. In dilute alkaline urine RBC can lyse rapidly, releasing hemoglobin so that only the red cell membrane remains as a "ghost cell." Ghost cells may be missed unless specimens are reviewed under subdued light.

Recent attention has been directed to urinary red blood cell morphology in the form of "dysmorphic red blood cells" as an indicator of glomerular bleeding. Dysmorphic red blood cells vary in size and are fragmented or have cellular protrusions. Although the exact number of dysmorphic RBC necessary to represent significant renal pathology has been disputed, one study has suggested that an incidence of 14% or greater of dysmorphic RBC is highly suggestive of an intrarenal source. The presence of less than 14% dysmorphic red cells suggests an extrarenal bleeding site. Urine samples stored up to 5 hours at 4°C to 20°C did not show any apparent change in the percentages of dysmorphic RBC present. It should be noted however, that other studies have been unable to demonstrate a clear correlation between the presence of dysmorphic red cells and the site of bleeding. Intraobserver discrepancy in the intrepretation of red cell morphology has also been a problem.

As explained earlier, the presence or absence of red blood cells noted on microscopic examination does not always correlate with the results of the dipstick pad test for hemoglobin. Urinary tract hemoglobin loss secondary to in-

travascular hemolysis can produce a positive dipstick pad result for hemoglobin with no RBCs noted on microscopic examination. Similarly, occasional specimens will be found with significant numbers of RBCs on microscopic examination but a negative dipstick pad result for hemoglobin. In this instance, possibilities include the failure of red cells to lyse, releasing hemoglobin, or the presence of significant amounts of urinary ascorbic acid or another oxidant, providing false-negative dipstick pad results.

White blood cells

White cells present in the urine at more than 3 to 5 HPF point to an infectious or inflammatory process somewhere within the urinary tract. White blood cells are larger than red blood cells and are easier to identify because of the presence of cytoplasmic granules and nuclei. Although neutrophils are the most commonly seen white cells in the urine, on occasion, other leukocytes such as eosinophils, lymphocytes, or monocytes may be noted. Supravital staining may be used to enhance white cell nuclear detail and Hansel's or Wright's stain should be used if eosinophiluria is suspected. Degenerated neutrophils called "glitter cells" are sometimes found in the urinary sediment. The nuclear lobes of these cells are no longer clearly delineated and in dilute urine their cytoplasmic granules display Brownian movement, resulting in a glittering phenomenon. Although glitter cells are most commonly associated with pyelonephritis, they may also be noted when the urinary specific gravity is low.

Epithelial cells

The three major types of epithelial cells that may be found in the urine are squamous, transitional, and renal tubular cells. There may be no medical significance to the presence of moderate numbers of squamous or transitional cells in the urine as they are derived from the normal lining of the genitourinary system. When large numbers of epithelial cells are present, this may point to poor specimen collection, signifying that the first part of the voided sample was taken rather than a midstream portion.

Microorganisms

Bacteria are not normally present if the urine has been collected under sterile conditions. However, urine allowed to stand at room temperature may contain noticeable numbers of bacteria following the multiplication of contaminants. Bacteria will appear either as rods or cocci in the urine. Providing contamination or overgrowth has not occurred, the presence of bacteria correlates strongly with the presence of urinary tract infection, especially when seen in association with white blood cells. Yeast in the form of *Candida albicans* may also be present in the sample as a contaminant. Yeast may be confused with red cells but may be distinguished by the observation of budding forms and an ovoid rather than a round shape.

Casts

Casts represent a collection of protein and cellular debris that have gathered together in the kidney tubule, particularly where there has been low fluid flow. The coalescence of these materials forms a structure molded by the tubule itself, which subsequently appears in the urine as a tapered tube. The ends of the tubule may be pointed rather than r in which case it traditionally has been called a cylir rather than a cast. Cylindroids have the same clinic nificance as casts. The size of the cast depends on i of origin and the length of time it remains in the k tubule. Casts formed in the earliest parts of the ne tend to be the thinnest. The material that makes up th can cause it to differ in physical appearance. Where actual chemical constituents of casts have not been pletely analyzed, their major component is a glycop called Tamm-Horsfall protein, derived from the ren bular cells. Tamm-Horsfall protein is not detected t urinary protein dipstick pad and, consequently, is n sponsible for any increased urinary protein noted in containing casts.

Casts are best seen and examined utilizing low-po microscope fields (LPFs). On occasion, phase micro may be helpful.

1. Hyaline casts: These are pale and transparent. presence can be nonspecific and may be due increased concentration of solid materials in the tubules.
2. Red blood cell casts: Red blood cell casts vary g in appearance. The presence of true red bloo casts indicates bleeding at the level of the nep Whereas red blood cell casts are primarily asso with glomerulonephritis they may also be seer any condition that damages the glomerulus, tul or renal capillaries. RBC casts can usually b ognized because they are refractile and colored color of RBC casts ranges from a brown-yell red.
3. White blood cell (WBC) casts: WBC casts are true casts. They are, instead, conglomeratio white cells that have been packed closely toget a tubelike formation. White blood cell casts e granules and nuclei unless disintegration has b Staining to show nuclear detail or phase micro may be necessary to distinguish white cell casts epithelial casts. The finding of white cell casts u suggests the presence of a urinary tract infecti
4. Epithelial casts: The finding of significant nu of epithelial casts suggests the presence of an inflammatory process such as glomerulonephr pyelonephritis in the kidney. Epithelial casts c distinguished from white blood cell casts by th that epithelial cells have a round, centrally l nucleus.
5. Granular casts: The mechanism responsible f formation of granular casts is not well unders They may represent a further evolution of epit cell casts in which the cells themselves have d erated, leaving only a rough, granular appeai On occasion, white WBC cells may also be emb in granular casts, forming part of the component ture. The presence of granular casts generally cates a chronic disorder as they are rarely se acute inflammation.
6. Waxy casts: Waxy casts are refractile, lack dif tiated structures, may have serrated borders, and display cracked or broken ends. Waxy casts a made of wax. Scanning electron micrograph s

have shown they represent an advanced stage of hyaline casts. Waxy casts are most commonly associated with severe chronic renal disease.

7. Fatty casts: Fatty casts represent a breakdown product of epithelial casts that contain fat bodies. These casts may contain a few fat droplets within a hyaline matrix or may be completely filled with fat globules of different sizes. Fat is best recognized via polarized microscopy, which gives lipid globules a distinctive Maltese cross configuration and a doubly refractile appearance. Alternatively, fatty casts may be positively identified with the use of a Sudan III stain. The presence of fatty casts is usually associated with severe renal disease and nephrotic syndrome.
8. Broad casts: Broad casts are formed in the collecting ducts when urine flow is low. As such, broad casts have been called "renal failure casts." All types of casts may be present in broad forms.
9. Pseudo casts: Fibrin, epithelial cells, mucous strands, white blood cells, red blood cells, and bacteria from time to time may coalesce together, giving the appearance of a cast. Mucous strands usually have a much less structured morphology vs. true casts and frequently exhibit longitudinal striations.

A summary of various casts with their clinical correlations is provided in Table 29-5.

Crystals

Crystals form as a result of the precipitation of inorganic salts contained in the urine. Any fresh urine sample may contain crystals, especially if concentrated. Unfortunately, refrigerated specimens or those allowed to stand for any length of time frequently contain large numbers of crystals that may interfere with the microscopic detection of other urine elements. Whereas some crystals will dissolve on warming, others may require acidification of the urine.

Although crystals vary in appearance and are sometimes entrancingly beautiful to look at, most do not have clinical significance. The main reason to attempt to classify urinary crystals is to identify the few types that may be associated with systemic illness, such as inborn errors of metabolism or liver disease. Abnormal crystals of primary concern include cystine, leucine, tyrosine, and some drug crystals. Hexagonal cystine crystals may be noted in patients with hereditary cystinosis and may predispose to renal calculi. Leucine crystals that appear as spheres with concentric striations and tyrosine crystals that appear as bundles of needles may occasionally be seen together in cases of serious hepatic damage. When either is seen in isolation, they may signify a rare hereditary metabolic disorder. A variety of drug crystals may be present in the urine. Sulfa crystals, ampicillin crystals, and some crystals associated with radiographic dyes may produce renal tubular damage in certain clinical conditions, especially if associated with dehydration.

To properly identify crystals it is important to be aware of urinary pH. For instance, uric acid and calcium oxalate crystals are frequently found in acidic urine, whereas phosphates and calcium carbonate constitute the most common crystals noted in alkaline urine. The appearance of crystals under polarized light is also helpful and there are various confirmatory chemical tests that may be performed for individual species of crystals. However, many problems associated with the identification of specific crystals may be simply resolved by inquiring about patient medications. A summary of the appearance and other identifying features of various crystals is provided in Table 29-6. In most circumstances, it is a mistake for the technologist to spend an inordinate amount of time trying to specifically identify the wide variety of crystals that may, on occasion, be present in a urine sample.

Method of performing the microscopic examination

A commercial system is recommended.

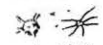
1. Mix the sample and pour off a standard amount into a disposable tube. (Always note volume if it is impossible to collect the full amount.)
2. Centrifuge for 5 minutes at 400 RCF (relative centrifugal force). This will generally be 1500 to 2000 rpm but must be determined for each centrifuge.
3. Remove the tubes from the centrifuge and place in an appropriate plastic rack. Remove lids. Turn the whole rack completely upside down to drain the supernatant liquid. Dry the lip of the tube by wiping

Table 29-5 Urinary cast summary

Type	Origin	Clinical significance
Hyaline	Tubular secretion of Tamm-Horsfall protein	Glomerulonephritis, pyelonephritis, chronic renal disease, congestive heart failure, stress and exercise, 0-2/HPF normal
Red blood cell	Red blood cells enmeshed in, or attached to, Tamm-Horsfall protein matrix	Glomerulonephritis, strenuous exercise
White blood cell	White blood cells enmeshed in, or attached to, Tamm-Horsfall protein matrix	Pyelonephritis
Epithelial cell	Tubular cells remaining attached to Tamm-Horsfall protein fibrils	Renal tubular damage
Granular	Disintegration of white cell casts, bacteria, urates, tubular cell lysosomes, protein aggregates	Stasis of urine flow, stress and exercise, urinary tract infection, 0-1/HPF normal
Waxy	Evolution of hyaline casts	Stasis of urine flow
Fatty	Renal tubular cells, oval fat bodies	Nephrotic syndrome
Broad casts	Formation in collecting ducts	Marked stasis of urine flow
Pseudo casts	Mucus, fibrin, or contaminants	May be mistaken for true casts

Modified with permission from Strasinger SK: *Urinalysis and body fluids*, ed 2, Philadelphia, 1989, FA Davis.

Table 29-6 Major characteristics of urinary crystals

Crystal	pH	Color-shape	Solubility	Appearance
Uric acid	Acid	Yellow-brown	Alkali	
Amorphous urates	Acid	Brick dust or yellow brown	Alkali and heat	
Calcium oxalate	Acid/neutral (alkaline)	Colorless (envelopes)	Dilute HCl	
Amorphous phosphates	Alkaline Neutral	White-colorless	Dilute acetic acid	
Calcium phosphate	Alkaline	Colorless	Dilute acetic acid	
Triple phosphate	Alkaline	Colorless (coffin lids)	Dilute acetic acid	
Ammonium biurate	Alkaline	Yellow-brown (thorny apples)	Acetic acid with heat	
Calcium carbonate	Alkaline	Colorless (dumbbells)	Gas from acetic acid	
Cholesterol	Acid	Colorless (notched plates)	Chloroform	
Cystine	Acid	Colorless (hexagonal shapes)	Ammonia, dilute HCl	
Leucine	Acid/neutral	Yellow (concentrically ribbed spheres)	Hot alkali or alcohol	
Tyrosine	Acid/neutral	Colorless-yellow (bundles of needles)	Alkali or heat	
Bilirubin	Acid	Yellow	Acetic acid, HCl, NaOH, ether, chloroform	
Sulfonamides	Acid/neutral	Green	Acetone	
Ampicillin	Acid/neutral	Colorless	Refrigeration produces bundles	
Radiographic dyes	Acid	Colorless	10% NaOH	

Modified with permission from Strasinger SK: *Urinalysis and body fluids*, ed 2. Philadelphia, 1989, FA Davis.

with absorbent tissue. This should leave a uniform volume of sediment in all tubes. (Flush sink with dilute bleach after dumping urine samples down the drain.)

4. Resuspend the sediment by agitating the tube contents with a Pasteur pipette.
5. Draw out the resuspended sediment. Deliver one drop onto the side chamber.
6. Examine preparation immediately before evaporation occurs.
7. Inspect the slide generally over a wide area using low power.
8. Reduce the light intensity to a minimum and scan several fields searching for casts. Alternatively, phase contrast microscopy can be used. Note: Casts are reported as number per low power field.
9. Turn to the high power lens and increase the light intensity. Scan several fields to evaluate cells. Discard slides into dilute bleach.

Examine and report microscopic findings

The following terminology should be valid if the high-powered field consists of a X10 eyepiece and a X40 objective lens. This results in a 0.375 mm diameter field, which is the standard field for reporting numbers per high-powered field. The standard low power field consists of a X10 eyepiece and a X10 objective lens, providing a field of 1.5 mm.

Using the average number of cells observed in four or five fields, the microscopic examination is reported using the following terminology (WBC is used as an example):

1. For small numbers of cells in which some high p fields do not contain any cells, report "0 to 2 W HPF."
2. When a countable number of cells per high p field are present, count four to five fields, aver and report "5 to 10 (or 5 to 8 or 10 to 15) W HPF."
3. When large numbers of cells are present, count quarter of a field and report this number times (rounded to the nearest ten) per HPF. Suppose 24 cells are counted, 24 × 4 = 96. Report "a 100 WBC/HPF."
4. If there are too many cells to count, report "fi packed."
5. Note the presence of clumping, in other words, "W clumps present."
6. Red blood cells are reported using the same sy as for WBCs.

Report epithelial cells as:

Occasional	0-2 per HPF
Few	3-4 per HPF
Moderate	5-10 per HPF
Many	10-15 per HPF
Gross	Packed field

Microorganisms. The discovery of bacteria in a prop handled specimen indicates that a second collection sh be made with a midstream portion collected and immedi examined. Report as: few, moderate, many, or field pac

Yeast must be distinguished from red blood cells, w they resemble. They tend to be ovoid, variable in size,

often demonstrate budding. If any question exists, a drop of 2% acetic acid added to the sediment will destroy red cells but will leave yeast intact.

Casts/cylindroids. Casts are best detected by using, on occasion, lower power fields and varying illumination; phase-contrast microscopy or staining may be helpful. Casts should be counted per low power field, but initial identification is sometimes easier using high power.

When reporting casts, it is permissible to use the terms "rare" or "occasional" when there is less than one cast in two or three low power fields. As is practical, report different types of casts separately. For example, "0 to 1 hyaline casts per low power field, 1 to 2 fine granular casts per low power field, and rare waxy cast."

Mucous strands or fibers. Mucous strands appear normally in small numbers, particularly in male patients. Increased numbers may be found in chronic inflammation of the urethra and bladder. Mucous strands may be difficult to distinguish from casts and phase-contrast microscopy allows for easier discrimination.

Crystals. When the type of crystals cannot be clearly identified but are not of a clinically significant variety, they should be reported as "nonpathologic unidentified crystals present."

Report crystals as few, moderate, or many.

Report amorphous sediment as light, moderate, or heavy.

Report mucus as light, moderate, or heavy.

Report spermatozoa when present.

Report yeast cells, trichomonas, parasites, or ova.

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