

**CDC 4T051N**

**Medical Laboratory  
Journeyman—  
Administration and  
Chemistry**

**Volume 3. Renal Function and  
Procedures**



**Air Force Institute for Advanced Distributed Learning  
The Air University  
Air Education and Training Command**

**Author:** MSgt Karla L. Puterbaugh  
382d Training Squadron  
882d Training Group (AETC)  
382TRS/XYAC  
917 Missile Road, Suite 3  
Sheppard Air Force Base, Texas 736-2263  
DSN: 736-4089  
E-mail address: karla.puterbaugh@sheppard.af.mil

**Instructional Systems  
Specialist:** Pamela G. Brown

**Editor:** Pamela G. Brown

Air Force Institute for Advanced Distributed Learning  
The Air University (AETC)  
Maxwell Air Force Base, Gunter Annex, Alabama 36118-5643

THIS final volume of CDC 4T051N discusses urinalysis concepts. Unit 1 introduces you to microscopy and the human body. The anatomy and function of the urinary system, and urinalysis specimen collection is reviewed in unit 2. Unit 3, Renal Function and Urinalysis Procedures, provides practical information on performing blood tests that reflect renal function and performing macroscopic and microscopic urine examinations.

A glossary of terms, abbreviations, and acronyms used in this course is included at the end of this volume.

A bibliography is also included at the end of this volume.

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## Acknowledgments

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**Source: How to Improve Photography Through the Microscope**

Page 16	Illustration of S Plan APO 10 Objective
Page 17	Illustration of NC S Plan APO 40 Objective
Page 18	Illustration of S Plan APO 100 Objective

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In this volume, the subject matter is divided into self-contained units. A unit menu begins each unit, identifying the lesson headings and numbers. After reading the unit menu page and unit introduction, study the section, answer the self-test questions, and compare your answers with those given at the end of the unit. Then do the unit review exercises.

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# Unit 1. Microscopy and The Human Body

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We are, and will be, constantly reminded that our purpose as a medical laboratory technician is to meet the mission of the medical treatment facility (MTF). The laboratory accomplishes this by providing accurate, reliable, and timely services that aid in the diagnosis, treatment, and prevention of diseases affecting the health and welfare of all patients; whether they are active duty military personnel, retirees, dependents, or civilians. The microscope is one of the most important tools utilized for accomplishing this purpose or mission. For example, it's used throughout the laboratory for identifying urinary cells, casts, and crystals; microorganisms found in biological specimens and cultures; and formed elements in blood, body fluids, and tissues. The microscope is single-handily the most significant instrument used by the cytologist and pathologist. Therefore, it is essential that we devote a section to this valuable tool and how it helps us with our study of the human body.

## 1–1. Microscopy Introduction and Methods

Around 1600 the first compound microscope was made by Zacharias Janssen, a Dutch spectacle-maker. Between 1674 and 1723, Anton van Leeuwenhoek used a simple microscope to describe and draw “animalcules” he found in rain water and other fluids. The first microscopes were of poor quality and it wasn't until about 1830 that a considerably better microscope was contrived by Joseph Jackson Lister, the father of Joseph Lister, who is known as the “Father of Aseptic Surgery.” The modern compound microscope is a result of various improvements made to Lister's microscope. Let's begin our study of modern microscopes with a review of basic microscopic terms and components.

### 401. Review of the basic microscope

Microscopes are used in many different professions (e.g., industry, science, medicine, etc.). There are approximately 35 different types of microscopes listed in *Dorland's Illustrated Medical Dictionary*, 28<sup>th</sup> ed. Nonetheless, each microscope has the same purpose ... to enlarge images of small objects and/or to reveal minuscule details that cannot be seen with the naked eye. Today, within the medical arena, microscopes are used to diagnose disease, perform surgery, and determine cause of death. Of course, we are going to limit our study to the microscopes we use in the clinical laboratory. As stated earlier, the microscope is indeed an invaluable instrument. As with other instrumentation, you should know the terms associated with microscopy and be able to identify the components of a microscope.

#### Microscopic terms

Before we look at microscopic terms let's look at the measurements used in microscopy and the different types of microscopes.

#### Measurements

Magnifying glasses and microscopes are used to see microorganisms, cellular elements, and other small objects. These small objects are measured in units that aren't normally used in everyday life. If you recall from volume 1, unit 5, the standard or primary unit for length is meter (m). Common measurements used in microscopy are micrometer (μm) and nanometer (nm). A micrometer is equal to 10<sup>-6</sup>m and a nanometer is equal to 10<sup>-9</sup>m. The prefix “micro” indicates that the following unit

should be divided by one million and “nano” indicates that following unit should be divided by one billion. The table below should help you get a perspective of the measurements mentioned and how they relate to microscopy.

Object	Average Measurement(s)	Resolution by
Human	1 to 2 m (5 to 6 ½ ft)	Unaided eye
Some human muscles	0.6 m (2 ft)	Unaided eye
Chicken eggs	≈ 5 cm (2 inches)	Unaided eye
Frog egg	≈ 2 mm (0.07 to 0.08 inches)	Unaided eye or light microscope
Eukaryotic cell	10 to 100 $\mu\text{m}$	Light microscope or electron microscope
Nucleus	5 to 10 $\mu\text{m}$	Light microscope or electron microscope
Most Bacteria	1 to 10 $\mu\text{m}$	Light microscope or electron microscope
Mitochondria	1 to 3 $\mu\text{m}$	Light microscope or electron microscope
Mycoplasma	100 to 120 nm	Electron microscope
Viruses	50 to 90 nm	Electron microscope
Ribosomes	10 to 30 nm	Electron microscope
Proteins	3 to 10 nm	Electron microscope
Lipids	1 to 3 nm	Electron microscope
Small molecules	0.4 to 1 nm	Electron microscope
Atoms	0.1 nm	Electron microscope

### Types

The size of the object indicates the type of microscope required. The first type of microscope uses the optical principles of light rays and lenses. The second type uses electronic principles with electron beams and magnetic fields, which will be discussed in the next lesson. Optical microscopes are further divided into *simple* or *compound*.

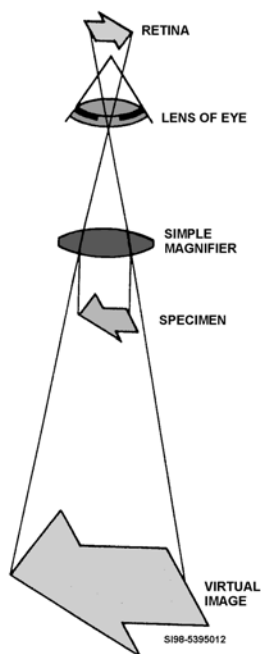


Figure 1–1. Illustration of simple magnification.

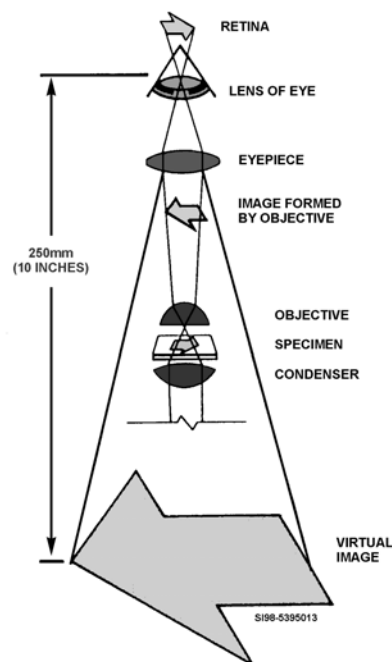


Figure 1–2. Illustration of compound magnification.



Simple microscopes are composed of a single lens (or a system of lenses that act as a single lens) that enlarges an object in 1 step as shown in figure 1–1. A magnifying glass is an example of a simple microscope. A compound microscope is a group of diverse lenses (aligned in a specific series) that enlarges the object in 2 steps; in step 1 the object is magnified by the objective and in step 2 it is further magnified by the eyepieces, as shown in figure 1–2. Because of the way light rays travel through the compound microscope, the image that is seen is upside down and reversed. The right side appears as the left, the top as the bottom, and vice versa. This is important to remember when moving the object or slide under examination. **NOTE:** When you look into a microscope, you are looking at an *image* of the object, and not the object itself. In order to grasp how a microscope works, it's important to understand the following terms.

### *Light principles*

Unit 6 of volume 1 stated that light is a form of electromagnetic energy that travels in waves. It is composed of both visible and invisible waves. Visible light frequencies represent the familiar spectrum, that is, the rainbow spectrum from violet to red. The violet-blue end of the spectrum is made up of shorter wavelengths and the red end of the spectrum is made up of longer wavelengths. Invisible light includes the ultra-violet (shorter than violet) and infra-red (longer than red) parts of the light spectrum. Both visible and invisible light can be used in microscopes. Light travels at a velocity of about  $3 \times 10^{10}$  cm (186,284 miles) per second and when it hits a surface, it can be absorbed, transmitted, deflected, reflected, refracted, or diffused. Because absorbance and transmission were discussed in volume 1, unit 6, we'll just look at the latter terms. *Dorland's* defines deflection as "deviation or movement from a straight line or given course" and reflected light as "light whose rays have been turned back from an illuminated surface." *Dorland's* defines refracted light as "light whose rays have been bent out of their original course by passing through a transparent membrane," and diffused light as "that which has been scattered by reflection and refraction."

### *Refractive index*

Light rays passing from an optically light (i.e., air) to an optically dense medium (e.g., glass, water, or oil) change speed and direction. The refractive index (RI) is the ratio of the speed of light in air to its velocity in another medium. The refractive index of air is assumed to be 1, the RI of water 1.334, heavy mineral oil has a 1.483 RI, and cedar oil has a 1.515 RI. Because the various wavelengths or colors of white light are slowed at different rates, when passing through a medium, aberrations are seen. The two main types of aberrations are *chromatic* aberration and *spherical* aberration.

#### *Chromatic aberration*

Because the colors of white light are slowed at different rates when passing through a particular medium or lens, the various wavelengths are brought into different foci instead of a common focus. Thus, the image exhibits color fringes and poor definition. Lens manufacturers strive to bring the main colors of red, blue, and green into a common focus by using different glass and lens elements to correct for chromatic aberrations.

#### *Spherical aberration*

Light rays passing through the medium or lens will also come into different foci depending upon whether they pass closer to the center (near-center) or periphery of the lens. This type of aberration produces images that are more or less blurred with poor definition and contrast. To correct for spherical aberrations, lens manufacturers try to bring light rays from the near-center and periphery to a common focus.

### *Lens characteristics*

"A piece of glass or other transparent substance so shaped as to converge or scatter the rays of light, especially the glass used in appropriate frames or other instruments to increase the visual acuity of the human eye," is *Dorland's* definition for a lens. *Dorland's* dictionary lists over 60 types of lenses that are, in general, man-made. However, a few are located in the human eye. Lenses can basically, be *flat*

(lying on 1 plane, even surface), *concave* (round, depressed surface), *convex* (round, elevated surface), or *combinations* of these primary shapes. Concave or negative lenses cause parallel light rays to diverge or separate. On the other hand, convex or positive lenses cause light rays passing through them to converge or collect to form a real image.

### **Resolution**

According to *Dorland's*, resolution is “the perception as separate of 2 adjacent objects or points. In microscopy, it’s the minimal distance at which 2 adjacent objects can be distinguished as separate. The resolving power of an instrument depends on the wavelength of the radiation used and numerical aperture of the system; it is expressed in micrometers per distance or lines per millimeter.”

### **Resolving power**

Resolving power is the ability of the lenses to distinguish fine details and structures. For example, if the resolving power is 1.0 nm, then the microscope is capable of distinguishing two points as separate objects if they are at least 1.0 nm apart. In general, the shorter the wavelength of the light used in the microscope, the greater the resolution. The white light used in a compound light microscope has a relative long wavelength and, hence, cannot resolve structures smaller than 0.2  $\mu\text{m}$  apart.

### **Numerical aperture**

The concept of numerical aperture (NA) was introduced by Abbe. The NA value can be derived mathematically using the refractive index of the medium (between the object and the lower lens of the objective) and one-half the angle of the cone of light admitted by the lens of the condenser or the light entering the objective. This is a complicated concept, so let’s just say “The NA can be thought of as an index of the light-gathering power of a lens.” I hope this explanation is a little better.

### **Illumination**

*Dorland's* defines illumination as “the lighting up of a part, cavity, organ, or object for inspection.” Because an object rarely generates its own light, illumination is usually furnished by some type of light source (illuminator). The light source, field diaphragm, iris diaphragm, and condenser make up the illumination system of a microscope.

### **Magnification**

“Apparent increase in size as under the microscope, the process of making something appear larger, as by use of lenses, or the ratio of apparent (image) size to real size” are *Dorland's* definitions for magnification. The magnification system of a microscope includes the objectives and eyepieces (oculars).

### **Microscope components**

Now it’s time to break down the microscope and examine each component of the different systems. Keep in mind we are describing a generic compound light microscope; each manufacturer utilizes the basic components a little differently. We’ll start with its foundation—the base—and then work our way up to the top. Use figure 1–3 to locate the components.

### **The base**

The base of the microscope provides the stability and rigidity for the microscope arm or frame. It also houses the light source or illuminator, a right angle mirror (if needed), and the field diaphragm. The frame supports the illumination, magnification, and adjusting systems, along with the stage. The adjusting system contains the coarse and fine adjustment knobs; condenser adjustment knob; and stage controls. In the more unique and expensive microscopes, the base is designed with special microscopist-arm rests.

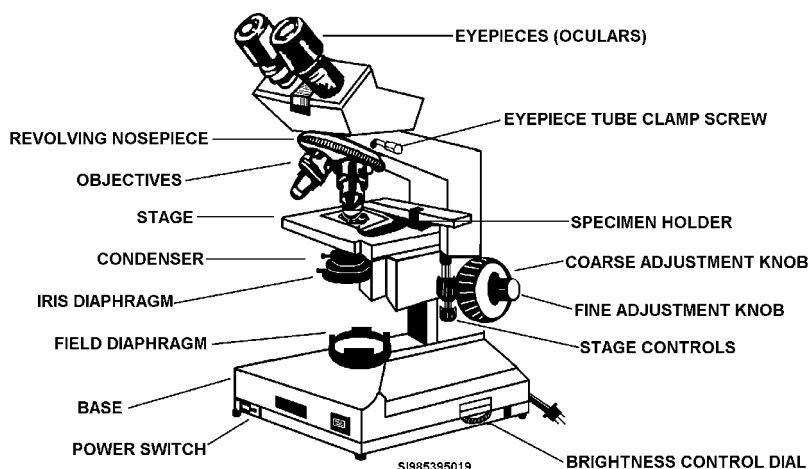


Figure 1-3. An illustration of a compound light microscope.

### ***Light source***

The light source is part of the microscope's illumination system and can be internal or external.

#### ***Internal***

Internal light sources are usually in the form of a lamp or bulb. The type of bulb employed depends on the type of microscope in use. The light path may be directed straight-up through the condenser or directed towards a right-angle mirror that deflects it to the condenser. If the bulb is of high intensity, the brightness control dial can regulate the intensity to ensure adequate illumination of the object and provide comfort for the microscopist. Sometimes sophisticated and well-equipped microscopes fail to yield sharp images because of incorrect use of the light source: incorrect bulb installation and/or alignment or using the wrong type of bulb. Excellent illumination of the object includes a bright, glare-free, evenly illuminated field of view.

#### ***External***

Some of the older microscopes use an external rather than an internal light source. External light sources are usually a separate bulb or illumination system that directs the light path to a mirror on the base of the microscope. The mirror deflects the light upward through the condenser to the object under examination. It is critical that the mirror is at the correct angle. This can be checked by removing an eyepiece and looking to see if the light is centered within the body tube. The body tube is the part of the microscope through which the light passes from the objective to the eyepiece.

### ***Field diaphragm***

A basic definition for a diaphragm is "a disk with an adjustable opening." Therefore, we can summarize that the field diaphragm adjusts the light path and restricts the area of illumination or field of view. You may also see the term *aperture* used with the diaphragm. Aperture essentially means "an opening, as a hole, slit, or gap." The field diaphragm can be easily opened or closed by using the control ring or dial.

### ***Iris diaphragm***

As we move up the microscope, the next component we'll discuss is the iris diaphragm. The iris diaphragm is located under the condenser but within the condenser body and is shown in figure 1-4. It also controls the amount of light passing through the object. It consists of a series of horizontally arranged interlocking plates with a central aperture. The iris diaphragm can be opened or closed by a simple lever. It may include a calibrated scale that increases adjustment accuracy and reproducibility. Because the amount of light passing through the diaphragm affects the NA, the calibration number

can refer directly to the NA value of the condenser and/or objective. **NOTE:** The field diaphragm controls the circular area of light illuminating the object and the iris diaphragm controls the angular aperture of the cone of light from the condenser.

### Condenser

The condenser is located directly under the stage. It concentrates, directs, and focuses the path of light onto the object under examination. Condensers also have an NA assigned to match or be slightly less than the NA of the objective in use. The NA of the condenser can be varied by changing its position. Therefore, the condenser should be adjusted every time you change objectives to guarantee optimal performance of the objective. Likewise, appropriate adjustments of the iris diaphragm are vital in obtaining proper illumination and contrast. Because of the aberrations mentioned earlier, condensers are manufactured according to different levels of correction required. The engraving on the condenser includes its NA and designation or type. Let's look at the three different types of condensers as shown in figure 1-4.

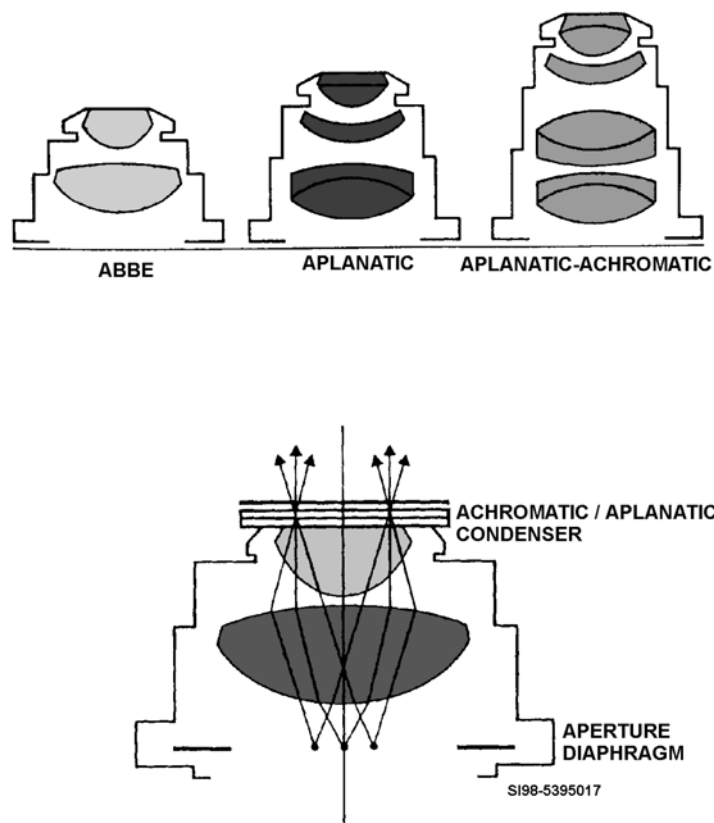


Figure 1-4. An example of the different types of condensers.

### Abbe

The Abbe condenser is for routine clinical laboratory observations and procedures. It is a conical lens system with two lenses as shown in figure 1-4. The Abbe condenser is uncorrected, simple, and the least expensive of the three types.

### Aplanatic

Aplanatic refers to aplanatism which means “freedom from spherical aberration.” Aplanatic condensers correct for spherical aberrations but not chromatic. This type of condenser is used for black and white photomicrography. The use of photomicrography is growing because it produces a permanent record of the object under examination. Remember it is the enlarged image of the object and not the object itself that is seen or photographed.

### *Aplanatic-achromatic*

Aplanatic-achromatic condensers, as you have probably concluded, correct for both spherical and chromatic aberrations. These condensers are used for critical microscopy and color photomicrography.

### ***Stage***

The microscope stage is a horizontal platform or shelf that may be rectangular or circular. The object under examination is placed on the stage. The stage has an opening in the center that allows light to pass from the light source through the diaphragms and onto the object. Most microscopes have a mechanical device that holds the object or slide in place and is known as a slide or specimen holder. The stage smoothly moves back-and-forth and side-to-side in order to bring the object into the field of view.

### ***Objectives***

Up next in our lesson are the objectives. The objectives are the major part of the magnification system because they form the primary image. Light rays, directed from the light source by the condenser, pass through the object under examination into the objective. Each objective contains a set series of different lenses. An objective must be able to gather light coming from different points of the object and reconstitute that light into the various corresponding points of the image. The quality and cost of an objective are based on its aberration correction performance and magnifying power. First we'll look at the different correction objectives and then their magnifying power.

### *Achromatic*

Achromatic objectives chromatically correct for 2 colors and spherically correct for 1 color. They are corrected chromatically to bring red and blue light into the same focus. Further, they are corrected for spherical aberration for the color green. Achromatic objectives are the least expensive.

### *Fluorite objectives or semi-apochromats*

Fluorite objectives (their lens elements contain some natural or synthetic fluorite) are chromatically corrected for 2 colors and spherically for 2 colors. These objectives are also corrected chromatically for red and blue light.

### *Apochromatic*

The highest level of correction is found in apochromatic objectives shown in figure 1-5. These objectives are corrected chromatically for 3 colors (red, blue, and green) and spherically for 2 colors. Because of their high level of correction, such objectives usually have, for a given magnification, higher numerical apertures than do achromatic or fluorite objectives.

### *Plano or flat-field objectives*

All 3 types of objectives project images that are curved rather than flat. To overcome this inherent condition, lens designers have produced flat-field objectives that yield flat images. Such lenses are called plan-achromats, or plan-fluorites, or plan-apochromats. Such correction, although expensive, is invaluable in photomicrography.

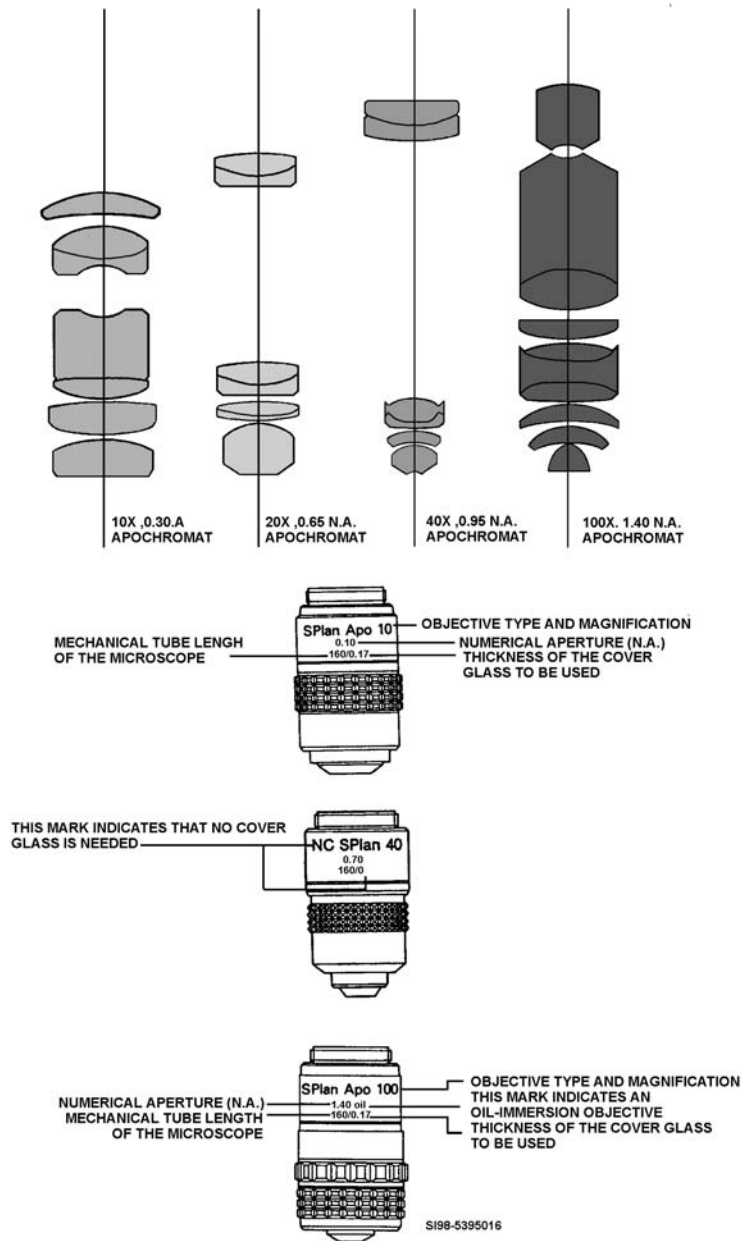


Figure 1-5. An example of different objectives.

### *Magnifying power*

Magnification is expressed in terms of diameters, power, times, or X. Total magnification is calculated by multiplying the objective lens magnification by the eyepiece lens magnification. For example, if the objective power is 40 X and the eyepiece power is 10 X, the total magnification of the object is 400 X. If relevant, axillary optics and tube factors must also be taken into account when calculating total magnification. Most microscopes come with 3 objectives with the following magnification powers: 10 X (low power), 40 X (high power), and 100 X (oil immersion). Some manufacturers use a color-coded band to also identify the magnification. Keep in mind that an objective's working distance decreases with increasing magnification. The working distance is the depth of space, in millimeters, between the top surface of the object and the front surface of the objective lens. This is important to remember when changing objectives in order to prevent damage to an objective lens.

### *Objective inscriptions*

Each objective is inscribed with critical information that is used for achieving optimal performance and image production. As shown in figure 1-5, this information includes the type of objective, its magnification, NA, tube length, thickness of cover glass to be used, and if immersion oil is required—OIL, OEL, or HI. If these letters aren't present the objective is to be used "dry." Dry objectives are designed to work with air in between the object under examination and the objective. Immersion objectives require oil in between the object and objective in order to increase their NA and resolving power. **NOTE:** Oil will not increase the NA or resolving power of dry objectives, it actually ruins them.

### *Revolving nosepiece*

The revolving nosepiece secures and allows easy rotation of the objectives. Similar manufacturer objectives (e.g., achromatic objectives of various magnifications), are designed to project the image of an object to approximately the same plane within the body tube. For that reason, only minimal adjustment is required when changing objectives. When this occurs, the objectives are described as being parfocal. Similar objectives are also designed to parcentric. This means, if any object is in the center of the field of view for one objective, it remains in the center when the objective is changed.

### *Body tube*

The body tube is connected to the revolving nosepiece and transmits the image from the objective lens to the eyepiece. It's usually 160 mm in length, but some manufacturers may use a 170 mm body tube. Generally, the body tube contains a prism to deflect the path of light from the objective to the eyepiece.

### *Oculars or eyepieces*

The eyepieces are part of the magnification system and work in conjunction with the objectives. They are located at the top of the microscope and fit into the body tube. The eyepiece further magnifies the real image projected by the objective. Each eyepiece is inscribed with its magnification power—10 X is the most common. When you view the image of the object, it appears to be 10 inches (250 mm) away or near the base of the microscope. For the best results, the eyepieces should be used in combination with the appropriate correction and type of objective. Basic eyepieces are either negative or positive, as shown in figure 1-6.

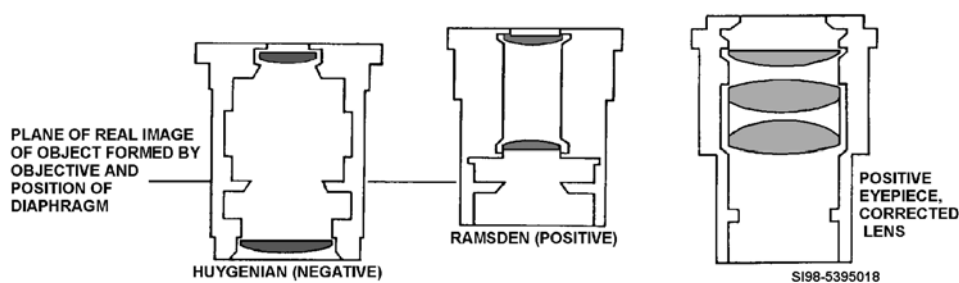


Figure 1-6. Examples of eyepieces.

### *Negative*

Negative or Huygenian eyepieces contain 2 lenses: an upper or eye lens and a lower or field lens. Both are plano-convex (flat on one side and round on the other) with the convex side towards the object under examination. Between these lenses is a fixed diaphragm which, by its size, defines the circular field of view. Negative eyepieces with only 2 lenses are routinely used with achromatic objectives and have less aberration correction than positive eyepieces.

### *Positive*

Positive or Ramsden eyepieces also contain 2 lenses: an eye-lens and a field lens. However, the flat side of the field lens is towards the object and the fixed diaphragm is below the field lens (not in between the lenses).

### *Compensating eyepieces*

Compensating eyepieces can be negative or positive and are used with fluorite and apochromatic objectives. They play a vital role in compensating for residual chromatic aberrations inherent in the design of highly corrected objectives. Therefore, it is important to use eyepieces and objectives designed for each other by the same manufacturer. Compensating eyepieces are inscribed with K, C, or comp, along with the magnification power. Eyepieces used with flat-field objectives are inscribed with *plan-comp*, and those designed to give a wide-diameter field of view are inscribed with W.F. or S.W.

### *Markers*

Negative or positive eyepieces can be designed with grids, scales, crosshairs, or micrometer discs for measuring, centering, or identifying parts of the object in the field of view. The markers are placed on the eyepiece diaphragm. Because the objective projects the image at the plane of the diaphragm, these markers appear as if they were superimposed onto the object in focus.

### ***Focus knobs***

Coarse and fine adjustment knobs are located on both sides of the microscope. The adjustment knobs serve to bring the objective and object under examination closer together or farther apart. Depending on the manufacturer, the adjustment knobs can either raise and lower the stage or raise and lower the objectives. The coarse adjustment knob allows for large-increment focusing movements and the fine adjustment knobs for small-increment focusing movements. The sensitivity or size of the interval of the fine adjustment knob is dependent on the precision required and manufacturer of the microscope.

Now that you know all about the various microscope components, its time to discuss the different types of microscopic methods.

## **402. Microscopic methods**

By this time in your career I am sure you realize that one method, technique, or instrument cannot perform all the procedures done in the laboratory. This is true of microscopes: the compound light microscope has its limitations. In this age of technical advances, even the microscope has evolved to perform many specialized functions. Let's look at some of the different microscopes and the illumination systems they employ.

### **Stereoscopes**

Stereoscopes are modified light, binocular microscopes, with 1 or 2 objectives, to give a 3-dimensional view of an object. They are occasionally used in the clinical laboratory to see very small organisms on culture plates. They are used most often in histology for viewing dissections.

### **Brightfield**

A compound light or brightfield microscope uses transmitted light to reveal structural details of an object. These details appear darker than the illuminated field of view, hence the name "brightfield." The object or specimen under examination must be stained to enhance light absorption and contrast. Remember, staining kills living cells and microorganisms. Brightfield microscopy is the primary type of microscope used in the clinical laboratory and is considered a cornerstone in diagnostic hematology. Figure 1-7 illustrates the basic principle of brightfield microscopy.



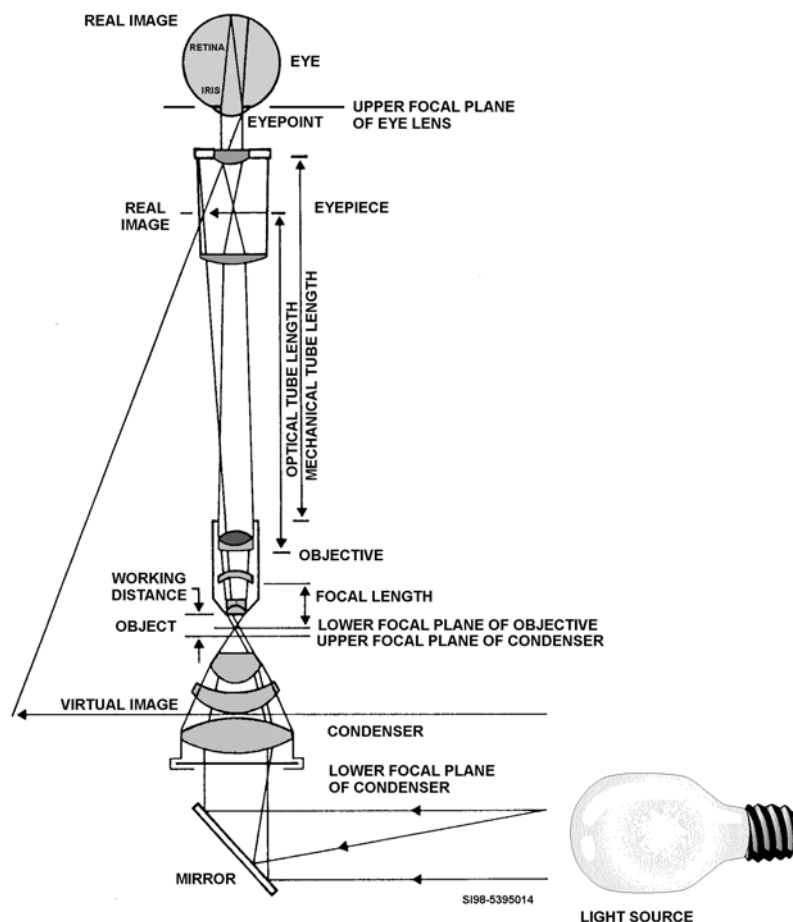


Figure 1-7. An illustration of how an object image is produced in brightfield microscopy.

### ***Köhler illumination***

Köhler illumination is a method that ensures optimum contrast and resolution for the maximum definition of object details by precisely focusing and centering the light path. It also spreads the light uniformly over the field of view. It is accomplished by adjusting the substage Abbe condenser, iris diaphragm, and field diaphragm, then removing the eyepiece to see a circular beam of light inside the body tube. The Köhler illumination procedure should be performed daily before using the microscope and when changing objectives because each magnification has a different field of view and requires different levels of illumination. After practice the procedure will take less than a minute to perform.

### ***Oil immersion***

A few comments should be made about using oil immersion in brightfield microscopy. Oil immersion objectives cannot be focused just by switching to these lenses, you should first focus and select the viewing area using the 10 X objective. Move the 10 X objective and then place a drop of immersion oil in the center of the object above the beam of light. Remember, don't drag other dry objectives across the oil. Move the oil immersion objective into place and only use the fine adjustment knob for focusing.

### ***Darkfield***

Darkfield microscopy is used for examining live microorganisms that are either invisible in brightfield microscopy, cannot be stained by standard methods, or their identifying characteristics are distorted by staining. In darkfield microscopy a special substage condenser with an opaque disc is

used. The disc blocks light that would enter the objective directly. Only the light that is deflected by the object enters the objective. Because there is no direct background illumination, due to no light rays passing from the condenser to the objective, the object appears light against a black background. Any brightfield or compound microscope can be converted to a darkfield microscope by replacing the usual condenser with a darkfield condenser. The darkfield microscope is useful in examining unstained microorganisms suspended in fluid (e.g., spirochetes found in leptospiral or syphilitic infections).

### **Phase-contrast**

The phase-contrast illumination system allows the detailed examination of internal structures in living cells and microorganisms as they move and change shape. As the name suggests, the object shows more contrast than with brightfield microscopy. The principle of phase-contrast microscopy is based on slight variations in thickness and refractive index in the object, into intensity or brightness differences that are detectable by the human eye. In other words, as light rays pass through the object their velocity is altered by differences in the thickness and physical properties of the various structures within the object or specimen. The light rays are refracted (bent) differently by the various structures and travel in different pathways (out of phase with one another). These phase differences are seen as varying degrees of brightness. Internal structures also become more sharply defined in phase-contrast microscopy. The internal details of a cell or microorganism appear as degrees of brightness against a dark or very dull background. A phase-contrast microscope is basically a brightfield microscope with a special condenser and objectives. An annular diaphragm (a black ring with a black center) is placed below or into the condenser. This produces a hollow cone or “doughnut” of light passing through the object. A corresponding absorption ring or phase-changing plate is built into the objective. The phase-shifting element creates a uniform phase change that increases the differences between the background light and the refracted light from the object, thereby enhancing image contrast. The annulus and absorption ring must be perfectly aligned or adjusted so they are concentric and appear superimposed. A phase-contrast microscope is used for counting platelets using the direct method and for observing microscopic elements in wet preparations of urinary sediments and vaginal smears.

### **Interference-contrast**

This type of microscopy provides a “three-dimensional” or “shadow-cast” image of the object under examination. This is accomplished by inserting a pair of beam-splitting prisms in the light path (Normarski-interference). One prism actually divides the light and reorients it into phased and perpendicular waves of light (a reference beam and an object beam). The second recombines the light after it has passed through the object under examination. When the two beams are recombined through the second Normarski prism, the object appears to be three-dimensional when viewed through the eyepiece of the microscope. Interference-contrast microscopy (ICM) is of particular value in teaching because of the ease in demonstrating fine details and points of structure. The cost of ICM equipment is somewhat high, and therefore, it’s difficult to justify in most routine laboratories.

### **Fluorescence**

Fluorescence microscopy is discussed in-depth in 4T051P, volume 2, *Immunology*, because of its application of antigen-antibody techniques. However, a brief introduction is presented in this lesson. Fluorescence microscopy takes advantage of the phenomena that certain objects or substances have the natural ability to fluoresce. Fluorescent substances absorb short wavelengths of light and give off light at a longer wavelength that can be seen by the use of special light filters. Fluorescent microscopes use special components to separate excitation wavelengths from emission wavelengths. The exciting light, usually blue-green to ultraviolet, is provided by a high pressure arc (mercury, xenon, or halogen) lamp. The color of emitted light depends on the nature of the substance. Some objects, substances, or microorganisms naturally fluoresce under special lighting. But, if the object doesn’t naturally fluoresce, it can be stained with one of a group of fluorescent dyes called *fluorochromes*. Some examples are fluorescein isothiocyanate (FITC), auramine O, rhodamine,

acridine orange, and calcofluor white. Each fluorochrome has a special attraction for a certain substance (e.g., bacteria, fungi, protein, etc.). The color observed in the fluorescent microscope depends on the secondary or barrier filter used in the eyepiece. A yellow filter absorbs the green fluorescence of fluorescein and transmits only the color yellow. In practice, the actual brightness of fluorescence observed by the eye depends on these factors: (1) the efficiency with which the dye converts excited light into fluorescent light, (2) the concentration of the dye in the object, and (3) the intensity of the exciting (absorbed) radiation. Fluorescence techniques are used for immunological studies, primary and final identification of microorganisms, and in hematological studies of various cell lines.

### **Electron microscopy (EM)**

Electron microscopes are used for the study of fine details and ultra structures of cells, microorganisms, tissues, and other objects. Electron microscopes are very expensive and complex because they use a beam of electrons instead of a beam of light. Free electrons also travel in waves. Their wavelength is equal to 0.005 nm, as compared to visible light wavelengths of 397–723 nm. The equation for EM resolution is the same as for light microscopy, giving an electron microscope much greater resolving power and magnification capabilities because of the shorter wavelengths they incorporate. A light microscope has a magnification power of 1,000 X in comparison to an electron microscope that has a magnification power of 250,000 X. Electron microscopes use electromagnetic lens to focus a beam of electrons through an evacuated tube onto an object. Its major components are an electromagnetic condenser, electromagnetic objective, electromagnetic projector(s), and a fluorescent screen. The condenser deflects and focuses a cone of the electron beam onto the object. The object is usually stained with an electron-absorbing or electron-scattering substance to provide contrast. The objective deflects the portion of the electron beam that has passed through the object, to form and magnify the image. The projector(s) are equivalent to the light microscope's eyepieces and further enlarge the image and project it onto a fluorescent screen or photographic emulsion. The fluorescent screen is a plate coated with material that fluoresces as electrons strike it. Electrons deflected or absorbed by the object do not reach the screen, whereas those that pass through the object do. The result is a transmission image formed by a shadow of the electron-dense components of the object. There are two types of electron microscopes, the transmission electron microscope (TEM) and the scanning electron microscope (SEM).

#### *Transmission electron microscopy*

In TEM, electrons pass through the object and are scattered. Magnetic lenses focus the image onto a fluorescent screen or photographic plate. The object under examination appears 2-dimensional with weak contrast unless various stains (e.g., salts of heavy metals such as lead, osmium, tungsten, and uranium) are used. These metals can be used for positive staining (fixed on the object) or negative staining (background). Negative staining is used for very small objects (i.e., viruses, bacterial flagella, and protein molecules). TEM permits the visualization of internal ultrastructures of cells and tissues.

#### *Scanning electron microscopy*

In SEM, electrons sweep across the object and knock off other electrons from the object surface. The electrons are picked up by a collector then amplified and transmitted onto a viewing screen or photographic plate. An SEM provides striking 3-dimensional views of the object and is especially useful in studying the surface ultrastructures of intact cells, viruses, and microorganisms.

### **Uses of microscopy**

Now that you have knowledge about different types of microscopy, and especially brightfield microscopy, let's get to work and use this valuable tool in urinalysis, microbiology, and hematology for studying the human body.

## Self-Test Questions

After you complete these questions, you may check your answers at the end of the unit.

### 401. Review of the basic microscope

1. Read the statements at the top of the next page and determine the correct term for each statement. Locate and circle the term in the block of letters below. Terms may be listed forwards, backwards, horizontally, vertically, or diagonally.

M	I	C	R	O	M	E	T	E	R	Z	B	A	S	E
X	M	Z	A	B	L	G	L	Q	E	W	R	O	Q	C
V	A	N	M	A	B	B	E	T	T	Y	U	B	O	O
W	G	P	K	L	M	R	N	V	E	X	Z	J	S	N
Q	E	A	B	G	H	D	S	I	M	P	L	E	T	D
E	Y	E	P	I	E	C	E	S	O	Z	I	C	X	E
Z	L	O	H	J	B	C	S	Q	N	K	G	T	G	N
N	V	E	Y	U	Q	J	G	W	A	L	H	I	J	S
U	A	R	C	O	M	P	O	U	N	D	T	V	Z	E
M	M	P	H	T	D	F	K	L	Q	P	S	E	X	R
E	A	H	R	Z	R	G	O	D	W	N	O	C	B	X
R	G	F	O	N	V	O	B	E	D	O	U	A	R	E
I	N	O	M	O	G	H	M	X	O	I	R	P	E	D
C	I	C	A	S	Z	O	G	A	O	T	C	L	W	N
A	F	U	T	E	Y	P	O	L	G	A	E	A	O	I
L	I	S	I	P	N	E	K	O	F	N	D	N	P	E
A	C	K	C	I	N	O	S	V	A	I	E	A	G	V
P	A	N	J	E	F	S	I	E	I	M	F	T	N	I
E	T	O	E	C	I	A	R	T	T	U	Q	I	I	T
R	I	B	S	E	E	V	I	B	U	L	S	C	V	C
T	O	S	U	W	L	E	S	C	H	L	M	U	L	A
U	N	Z	S	X	D	S	X	Y	Z	I	O	N	O	R
R	P	A	Q	Y	Z	S	T	A	G	E	P	S	S	F
E	M	B	O	D	Y	T	U	B	E	L	Q	J	E	E
S	P	H	E	R	I	C	A	L	Q	S	A	A	R	R

Statements	
1. A measurement equal to $10^{-6}$ .	15. The process of making something appear larger.
2. A measurement equal to $10^{-9}$ .	16. Provides the stability and rigidity for the microscope arm or frame.
3. Microscope composed of a single lens.	17. Can be internal or external.
4. Microscope composed of a group of diverse lenses.	18. A diaphragm that restricts the area of illumination or field of view.
5. What you see when looking into a microscope.	19. A diaphragm that controls the amount of light passing through the object under examination.
6. Light is in this form of energy.	20. Concentrates, directs, and focuses the path light onto the object under examination.
7. The ratio of the speed of light in air to its velocity in another medium.	21. An uncorrected and simple condenser.
8. Aberration due to wavelengths of white light being slowed at different rates.	22. A condenser that corrects for spherical aberrations.
9. Aberration due to light rays passing through the periphery or near-center.	23. Horizontal platform or shelf that may be rectangular or circular.
10. Flat, concave, convex, and combination, are descriptions of these.	24. The major part of the magnification system.
11. The perception as separate of 2 adjacent objects or points.	25. Secures and allows easy rotation of objectives.
12. The ability of the lenses to distinguish fine details.	26. Usually 160 mm in length and may contain a prism.
13. The index of the light-gathering power of a lens.	27. Further magnifies the real image projected by the objectives.
14. This system contains the light source, condenser, and diaphragms.	28. Serve to bring the objective and object under examination closer or farther apart.

#### 402. Microscopic methods

- Match the terms in column B with the statement in column A by placing the letter of the column B item beside the number of the column A item that most nearly describes it. Each element in column B may be used once, more than once, or not at all.

Column A	Column B
____ (1) An illumination system that allows detailed examination of internal structures in living objects as they change shape.	a. Stereoscope.
____ (2) Uses transmitted light to reveal structural details of an object; primary type of microscope used in the clinical laboratory.	b. Brightfield.
____ (3) Uses special components to separate excitation wavelengths from emission wavelengths.	c. Köhler illumination.
____ (4) Examines live microorganisms and uses a condenser with an opaque disc.	d. Oil immersion.
____ (5) Objects appear as 2-dimensional and use salts of heavy metals as stains.	e. Darkfield.
____ (6) Produces a 3-dimensional view and used in histology for viewing dissections.	f. Phase-contrast.
____ (7) Object details appear darker than the illuminated field of view.	g. Fluorescence.
____ (8) Is used to study fine details and ultrastructures of cells, microorganisms, tissue, and other objects.	h. Electron microscopy.
____ (9) Light rays are refracted by various structures within the object showing varying degrees of brightness.	i. Transmission electron microscopy.
____ (10) A method that ensures optimum contrast and resolution.	j. Scanning electron microscopy.
____ (11) Provides a striking 3-dimensional view by knocking off electrons from the object.	
____ (12) Use only the fine adjustment for focusing when using this type of objective.	

## 1-2. The Human Body

You may be asking yourself “What is the relationship between the microscope and the human body?” I’m sure you realize that as a laboratory technician you use the microscope to identify normal and abnormal cellular elements and microorganisms found in body fluids and tissue. By doing this, you

help physicians diagnose, treat, and prevent disease that, in the end, accomplishes the Air Force mission. However, just like our mission is a part of the Air Force “big picture,” I want you to recognize that the macroscopic or microscopic specimen you hold in your hand is also a part of another big picture—the human body.

### 403. Introduction to the human body

In the first 2 volumes of this course you have learned about laboratory management, collecting venous blood specimens, clinical chemistry theory, laboratory instrumentation, and all the various chemical elements we test for in the laboratory. Although we still have a long road to travel through the rest of these CDCs, I feel it is important to take a “pit stop” and look at the big picture. Sometimes we are so close to a painting that we can see the direction of the artist’s brush strokes and each individual color, but we miss the beautiful sunset. After a brief review of the brush strokes, let’s take a few steps back and look at the whole picture.

#### In order to live

For life to exist and thrive the human body must be able to obtain or maintain the requirements shown in the table below.

Requirement	Purpose
Water	Water is not only fundamental to life, but is a part of life; the body is made up of about 60% water by weight. Every day we lose water through urine, feces, sweat, and exhaled air. We must replace this fluid loss because most of the chemical activities critical to human life take place in a water medium.
Oxygen	In the human body, oxygen is required to oxidize or break down fuel molecules in order to obtain their energy. Most cells die within a few minutes if deprived of this vital gas.
Food	The food we consume is broken down into different chemical elements or compounds needed by each cell of the body. These elements or compounds are used as raw materials to make new chemical substances and body parts or as fuel molecules.
Appropriate temperature	The human body could not survive on a dark, frozen planet or on a hot, burning planet. Although the Earth’s temperatures are appropriate for life, they fluctuate greatly and the body must, at times, work hard to maintain its own narrow internal temperature limits.
Suitable environment	Atmospheric pressure is important for normal breathing and proper gas exchange in the lungs. An increase in pressure can drive excess nitrogen gas into the blood and tissues causing nitrogen narcosis, also called “rapture of the deep.” Decreased pressure prevents adequate oxygenation of the body and cells. The absence of pressure, as in space, causes the blood to boil and the body to explode. A note of caution for all you would-be astronauts, “Don’t step outside the shuttle without your pressurized suit.” Also, a suitable environment for life must include protection from harmful radiation.

#### Living things verses nonliving things

With the above requirements being met to support life, let’s look at the difference between living and nonliving things. Living things move, support self-regulated metabolism, respond to internal and external changes, grow, and reproduce; nonliving things are not capable of these on their own. Most of these differences are self-explanatory, nonetheless, I think a review of metabolism is important.

#### Metabolism

Metabolism is derived from the Greek word *metaballein* that means to change, alter, or to turn about. *Dorland’s* defines metabolism as “the sum of all the physical and chemical processes by which living organized substance is produced and maintained (anabolism), and also the transformation by which energy is made available for the uses of the organism (catabolism).” In other words, *anabolism* is the

phase of metabolism in which the body uses energy to produce the chemical substances required for growth, repair, and maintenance of all body systems. *Catabolism* is the breaking-down phase of metabolism. Catabolic reactions convert the energy of food into forms that can be used by the body to provide energy needed to carry on the processes of life. Metabolism includes 3 interdependent activities: nutrition, synthesis, and cellular respiration.

1. Nutrition—Nourishing the body through the breakdown of nutrients.
2. Synthesis—Using some of these nutrients to manufacture or synthesis new substances for the building of new body parts or as fuel for cellular respiration.
3. Cellular respiration—Providing a process of gas exchange within the cells as nutrients are slowly broken down for fuel. The energy released is packaged within a special energy-holding molecule called *adenosine triphosphate* or *ATP*.

Metabolic activities are continuously occurring in every living cell. To maintain a constant internal environment or steady state, metabolism must be carefully regulated. The organism must *know* when nutrients are needed, when to manufacture what, and when to breakdown substances for fuel or energy. It must also know when not to produce too much of any substance. The automatic tendency to maintain a relative constant internal environment is call homeostasis or homeodynamics.

### ***Homeostasis or homeodynamics***

Homeostasis is acquired from the Greek terms *home(o)* which means sameness or similarity and *stasis* which means standing. It is thought that an adult human body contains about 75 trillion cells that are organized to form tissues, organs, and body systems. The human body is such a complex organism that it seems strange so little actually goes wrong with it. It appears a 19<sup>th</sup> century physician, Sir William Osler, understood this concept when he stated, “Strange that a harp of a thousand strings should remain in tune so long.” So what keeps our bodies in tune? The majority of the credit goes to its control mechanisms for maintaining a constant, appropriate internal environment—homeostasis. How does homeostasis work or how is this dynamic equilibrium (homeodynamics) maintained? All body systems participate in the regulatory mechanisms of homeostasis by doing what they were designed to do. If they don’t, negative feedback is sent to activate control mechanisms (i.e., nervous or endocrine system that activates another system or systems to compensate for the disruption). Homeostasis is the fundamental concept of physiology.

## **404. Body systems**

You should recall from your earlier biology studies that anatomy is the study of body structure and relationships among its parts. Gross anatomy includes the structures that can be studied by dissection with an unaided eye or stereoscope. The study of microscopic tissue anatomy is known as histology and the study of individual cellular anatomy is called cytology. Physiology is the study of body functions—physical and chemical. Let’s take a deeper look at the anatomy and physiology of the human body by reviewing the body systems.

### **Building blocks**

The human body is not an unorganized, shapeless heap. It is exquisitely organized into several different levels: chemical, cellular, tissue, organ, system, and organism. Did you notice how the levels build upon each other or move from the simple to the complex? The *chemical* level is made of atoms and molecules. The atoms of oxygen and hydrogen combine to form a molecule of water. (Remember, the human body consists of 60% water by weight.) Water, along with other molecules, makes up the most complex level—the *cellular* level. There are over 100 distinct types of cells within the human body. Cells are considered the basic building blocks of the body and are the smallest units of living material, capable of carrying on all the activities necessary for life. (**NOTE:** You’ll study different cell lines in greater detail in the 4T051C CDCs.) Cells come together to form *tissues*. Tissue is a group of closely associated cells specialized to perform a specific function or a group of functions. Different types of tissue may be organized into functional structures known as *organs*. A

coordinated group of tissues and organs proceed to make an *organ* or *body system*. These body systems work together, with far greater precision and complexity than most complicated machines, to form a complex, living *organism—the human body*.

### The body systems and homeostasis

Just as the microscope's illumination system, magnifying system, and adjustment system work together to produce an image of an object, the body is composed of systems that work together to keep the body functioning within the narrow limits that define human life. **NOTE:** The regulatory functions of the body systems are ultimately controlled by the nervous and endocrine systems.

System	Components	Basic Function	Homeostasis Function
Circulatory	Cardiovascular and lymphatic vascular systems.	Transports materials from one area of the body to another; defends the body against disease.	Distribution of oxygen, nutrients, wastes, body fluids and solutes, and body heat.
Dermal	Skin, nails, hair, sebaceous glands, and sweat glands.	Protects and covers the body, acts as a sensory receptor.	Controls body temperature through sweat glands, a barrier from harmful substances and a carrier for helpful substances.
Digestive	Oral cavity, oral pharynx, esophagus, stomach, small intestines (duodenum, jejunum, ileum), large intestines (cecum and colon), rectum, and anal canal; and associated glands—salivary, pancreas, liver, and gallbladder.	Absorbs nutrients (small intestines) and water (large intestines) through absorptive cells (enterocytes) and blood vessels, excretes wastes, and toxins.	Maintains adequate supplies of fuel molecules and building materials, eliminates wastes and toxins.
Endocrine	Hypothalamus, pituitary, thyroid, parathyroid, and adrenal glands; gonads, pancreas, paraganglia, and pineal body.	Regulates body chemistry and many body functions by releasing hormones into the bloodstream.	Regulates metabolic activities and blood levels of various substances.
Muscular	Striated muscles (skeletal and cardiac) and smooth muscles.	Provides movement for parts of the skeleton, pumps blood, and aids in movement of internal structures.	Cardiac muscle circulates blood, smooth muscles maintain blood pressure, help move nutrients through the body.
Nervous	Central nervous system (brain and spinal cord) and peripheral nervous system (peripheral nerves, ganglia, and nerve plexuses).	Autonomic nervous system (controls involuntary visceral functions) and somatic nervous system (controls sensory and voluntary functions).	Primary regulatory system, controls all other systems.
Reproductive	Male—testes, scrotum, penis, accessory glands and ducts; female—ovaries, uterine tubes, uterus, and vagina.	Reproduction, provides for continuation of species.	Passes on genetic codes for all cells.
Respiratory	Lungs, pharynx, larynx,	Exchanges gases between	Maintains oxygen content in



System	Components	Basic Function	Homeostasis Function
	trachea, bronchi, and associated structures.	blood and air.	tissue and cells, eliminates carbon dioxide and other waste.
Skeletal	Bones, cartilage, and ligaments.	Provides the body's supportive framework; protects internal organs; provides blood cell formation.	Maintains calcium level and cellular elements in blood
Urinary	Kidneys, ureters, urinary bladder, and urethra.	Filters blood, removes wastes and foreign substances; regulates ion, salt, and water concentrations of fluids; produces renin and erythropoietin.	Regulates blood chemistry in conjunction with endocrine system.

### Stressors

The human body is a magnificent machine with many diverse parts or systems. In order for the best possible image to be magnified and projected by a microscope, each system must work in the way it was intended to ensure adequate illumination and production of an aberration-free, sharp, clear image. Likewise, each body system and its components must properly perform its functions to maintain the body's steady state. There are external and internal changes that can disturb homeostasis; these changes are called *stressors*. External stressors include heat, cold, noise, abnormal pressure, harmful radiation exposure, and lack of oxygen. Internal stressors may be changes in blood pressure, pH, or salt concentration; and high or low blood-sugar levels. The homeostatic mechanisms of the body can handle the many stressors that occur routinely. However, other stressors are more severe and may cause serious disruptions or stress on the body. If the homeostatic mechanisms are unable to restore the delicate balance or steady state of the body, the stress can lead to body system malfunctions and failures, disease, and death. For the preservation of the human body, our job is to help the physician identify problems with the homeostatic mechanisms through laboratory procedures. As the previous volumes presented the importance of the analytes of the different systems, the next unit will discuss the importance of the urinary system.

## Self-Test Questions

After you complete these questions, you may check your answers at the end of the unit.

### 403. Introduction to the human body

1. What are the requirements for life?
2. What are the differences between living and nonliving things?
3. What is anabolism?
4. What is catabolism?

5. What are the 3 interdependent activities of metabolism? Briefly describe each.
6. What must an organism do to carefully regulate metabolism?
7. What is homeostasis?
8. How does homeostasis work?

#### 404. Body systems

1. What are the different levels of the human body? List them in building order.
2. Which is the most complex level?
3. Of what does a body system consist?
4. What controls the regulatory functions of the body systems?
5. Match the terms in column B with the statement in column A by placing the letter of the column B item beside the number of the column A item that most nearly describes it. Each element in column B may be used once, more than once, or not at all.

<i>Column A</i>		<i>Column B</i>
___ (1) Maintains adequate supplies of fuel molecules and building materials, eliminates wastes and toxins.		b. Circulatory.
___ (2) Provides movement for parts of the skeleton, pumps blood, aids in movement of internal structures.		c. Dermal.
___ (3) Maintains oxygen content in tissue and cells, eliminates carbon dioxide and other waste.		d. Digestive.
___ (4) Controls body temperature through sweat glands, a barrier from harmful substances and carrier for helpful substances.		e. Endocrine.
___ (5) Filters blood, removes wastes and foreign substances; regulates ion, salt, and water concentrations of fluids; produces renin and erythropoietin.		f. Muscular.
___ (6) Primary regulatory system, controls all other systems.		g. Nervous.
___ (7) Provides the body's supportive framework; protects internal organs; provides blood cell formation.		h. Reproductive.
___ (8) Distribution of oxygen, nutrients, wastes, body fluids and solutes, and body heat.		Respiratory.
___ (9) Passes on genetic codes for all cells.		i. Skeletal.
___ (10) Regulates metabolic activities and blood levels of various substances.		j. Urinary.
___ (11) Transports materials from one area of the body to another, defends the body against disease.		
___ (12) Protects and covers the body, acts a sensory receptor.		

6. List examples of external stressors.

7. List examples of internal stressors.

## Answers to Self-Test Questions

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<sup>1</sup> M	<sup>5</sup> I	C	R	O	M	E	T	E	R		<sup>16</sup> B	A	S	E
	M						<sup>10</sup> L		E			<sup>24</sup> O		<sup>20</sup> C
	A			<sup>21</sup> A	B	B	E		T			B		O
	G						N		E			J		N
	E						<sup>3</sup> S	I	M	P	<sup>17</sup> L	E		D
<sup>6</sup> E <sup>27</sup>	Y	E	P	I	E	C	E	S	O		I	C		E
	L						S		N		G	T		N
<sup>13</sup> N		E							A		H	I		S
U			<sup>4</sup> C <sup>8</sup>	O	M	P	O	U	<sup>2</sup> N	D	T	V		E
M	<sup>15</sup> M		H	T							S	E		R
E	A		R		R					N	O			X
R	G	<sup>28</sup> F	O	<sup>25</sup> N		O				O	U	<sup>22</sup> A	R	E
I	N	O	M	O			M			I	R	P	E	D
C	I	C	A	S				A		T	C	L	W	N
A	F	U	T	E					G	A	E	A	O	I
L	I	S	I	P	N					N		N	P	E
A	C	K	C	I		O				I	E	A	G	V
P	A	N		E	<sup>18</sup> F		<sup>19</sup> I			M		T	N	I
E	T	O		C	I		R	T		U		I	I	T
R	I	B		E	E		I		U	L		C	V	C
T	O	S			L		S			L			L	A
U	N				D					<sup>14</sup> I	O		O	R
R						<sup>23</sup> S	T	A	G	E		S	S	F
E		<sup>26</sup> B	O	D	Y	T	U	B	E				E	E
<sup>9</sup> S	P	H	E	R	I	C	A	L					<sup>12</sup> R	<sup>7</sup> R <sup>11</sup>

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1. (1) f.
- (2) b.
- (3) g.
- (4) e.
- (5) i.
- (6) a.
- (7) b.
- (8) h.

- (9) f.
- (10) c.
- (11) j.
- (12) d.

**403**

1. Water, oxygen, food, appropriate temperature, and suitable environment.
2. Living things move, support self-regulated metabolism, respond to internal and external changes, grow, and reproduce; nonliving things are not capable of these on their own.
3. The phase of metabolism in which the body uses energy to produce the chemical substances required for growth, repair, and maintenance of all body systems.
4. The breaking-down phase of metabolism; converts the energy of food into forms that can be used by the body to provide energy needed to carry on the processes of life.
5. Nutrition, synthesis, and cellular respiration; nourishing the body through the breakdown of nutrients; using some of these nutrients to manufacture or synthesis new substances for the building of new body parts or as fuel for cellular respiration; providing a process of gas exchange within the cells as nutrients are slowly broken down for fuel.
6. *Know* when nutrients are needed, when to manufacture what, and when to breakdown substances for fuel or energy; and when not to produce too much of any substance.
7. A control mechanism for maintaining a constant, appropriate internal environment.
8. All body systems participate in the regulatory mechanisms of homeostasis by doing what they were designed to do; if they don't, negative feedback is sent to activate control mechanisms (nervous or endocrine system that activate another system or systems to compensate for the disruption).

**404**

1. Chemical, cellular, tissue, organ, system, and organism.
2. Cellular.
3. A coordinated group of tissues and organs.
4. Nervous and endocrine systems.
5. (1) c. (2) e. (3) h. (4) b. (5) j. (6) f. (7) i. (8) a. (9) g. (10) d. (11) a. (12) b.
6. Heat, cold, noise, abnormal pressure, harmful radiation exposure, and lack of oxygen.
7. Changes in blood pressure, pH, or salt concentration; and high or low blood-sugar levels.

**Do the unit review exercises before going to the next unit.**

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## Unit Review Exercises

**Note to Student:** Consider all choices carefully, select the *best* answer to each question, and *circle* the corresponding letter. When you have completed all unit review exercises, transfer your answers to ECI (AFIADL) Form 34, Field Scoring Answer Sheet.

**Do not return your answer sheet to AFIADL.**

1. (401) The *common* measurements used in microscopy are
  - a. millimeter and nanometer.
  - b. millimeter and centimeter.
  - c. micrometer and nanometer.
  - d. micrometer and centimeter.
2. (401) Which statement *best* describes simple and compound microscopes?
  - a. Simple microscopes use light principles; compound microscopes use electronic principles.
  - b. Simple microscopes use electronic principles; compound microscopes use light principles.
  - c. Simple microscopes contain a group of diverse lenses; compound microscopes contain a single lens.
  - d. Simple microscopes contain a single lens; compound microscopes contain a group of diverse lenses.
3. (401) Which term is described as “light whose rays have been bent out of their original course by passing through a transparent membrane”?
  - a. Diffused.
  - b. Reflected.
  - c. Refracted.
  - d. Deflected.
4. (401) All of the following are examples of *basic* lens shapes *except*
  - a. flat.
  - b. convex.
  - c. concave.
  - d. compound.
5. (401) The resolving power of microscope lenses is its ability to
  - a. gather light.
  - b. prevent aberrations.
  - c. provide illumination.
  - d. distinguish fine details and structures.
6. (401) The magnification system of a microscope includes
  - a. condenser and eyepieces.
  - b. objectives and eyepieces.
  - c. condenser and illuminator.
  - d. objectives and illuminator.
7. (401) What component concentrates, directs, and focuses the path of light onto the object under examination?
  - a. Condenser.
  - b. Internal light.
  - c. Iris diaphragm.
  - d. Field diaphragm.

8. (401) What is the total magnification of an object when the objective power is 20 X and the eyepiece power is 10 X?
  - a. 10 X.
  - b. 20 X.
  - c. 200 X.
  - d. 2,000 X.
9. (401) Markers (grids, scales, etc.) used for measuring objects are placed on the
  - a. objective.
  - b. condenser.
  - c. iris diaphragm.
  - d. eyepiece diaphragm.
10. (402) To enhance light absorption and contrast of objects in brightfield microscopy,
  - a. use oil.
  - b. stain the objects.
  - c. adjust the condenser.
  - d. increase the light intensity.
11. (402) Which step is *incorrect* when using an oil objective?
  - a. Pass a dry objective through the oil.
  - b. Focus initially using the 10 X objective.
  - c. Place an oil drop on the object above the light beam.
  - d. Move the oil objective into place and focus using the fine adjustment knob.
12. (402) Which microscopic method is useful in examining unstained microorganisms suspended in fluid?
  - a. Darkfield.
  - b. Brightfield.
  - c. Fluorescence.
  - d. Interference-contrast.
13. (402) The detailed examination of internal structures in living cells and microorganisms as they move and change shape can be accomplished by using
  - a. darkfield microscopy.
  - b. phase-contrast microscopy.
  - c. scanning electron microscopy.
  - d. transmission electron microscopy.
14. (402) Which microscopic method allows the study of fine details and ultra structures and what is its principle?
  - a. Phase-contrast; use of electron beams.
  - b. Electron microscopy; use of electron beams.
  - c. Phase-contrast; use of variations in refractive index and light intensity.
  - d. Electron microscopy; use of variations in refractive index and light intensity.
15. (403) Which human body requirement is critical because *most* of the chemical activities take place in this medium?
  - a. Food.
  - b. Water.
  - c. Environment.
  - d. Air (oxygen).

16. (403) All of the following are activities of metabolism *except*
- a. nutrition.
  - b. synthesis.
  - c. cellular respiration.
  - d. internal temperature.
17. (403) The automatic tendency of the human body to maintain a relative constant internal environment is known as
- a. hemostasis.
  - b. homeostasis.
  - c. physiological dynamics.
  - d. psychological dynamics.
18. (404) When discussing the various levels of organization of the human body, atoms of hydrogen and oxygen combining to form water *best* illustrates the
- a. organ level.
  - b. cellular level.
  - c. chemical level.
  - d. organism level.
19. (404) A coordinated group of tissues and organs proceed to make
- a. a cell.
  - b. an organism.
  - c. a body system.
  - d. a functional structure.
20. (404) Which body system transports materials from one area of the body to another and helps defend the body against disease?
- a. Skeletal.
  - b. Nervous.
  - c. Circulatory.
  - d. Respiratory.
21. (404) Which statement *best* describes the homeostasis function of the endocrine system?
- a. Controls body temperature.
  - b. Passes on all genetic codes for cells.
  - c. Maintains calcium levels and cellular elements in blood.
  - d. Regulates metabolic activities and blood levels of various substances.

## **Student Notes**



## Unit 2. The Urinary System

<b>2–1. Anatomy and Function of the Urinary System .....</b>	<b>2–1</b>
405. Anatomy .....	2–1
406. Renal function and urinary system disorders.....	2–7
<b>2–2. Urine Specimen Collection, Transportation, and Preservation .....</b>	<b>2–15</b>
407. Specimen collection for urinalysis procedures .....	2–16
408. Transportation and preservation of urine samples .....	2–19

**W**hen any body system is mentioned it is easy to say, “This is the most important one.” However, as shown in unit 1, the body is designed to run smoothly only if all systems are working in unison. If most of the systems are working together and just one fails to function properly, then the body’s internal balance is disrupted. The urinary system is particularly important in eliminating the waste byproducts of metabolism. If the kidneys malfunction, toxic chemicals build up in the blood stream, and literally poison the cells of other body systems. Death eventually occurs if steps are not taken to correct the problem. In addition, the urinary system plays a vital role in regulating and maintaining fluid and electrolyte balance within the body. Another body system that shares many of the same structures with the urinary system is the reproductive system. Hence, you may see the terms *urogenital system* or *genitourinary system* if the text is discussing the anatomy and physiology of both systems as one system. In this unit we’ll take a closer look at the anatomy and function of the urinary system, and the collection of urinalysis specimens.

### 2–1. Anatomy and Function of the Urinary System

The urinary system is one of the most remarkable systems in the body. Most of us think that it simply forms urine. Although urine production and excretion is one of its primary functions, it also plays a major role in regulating blood pressure and volume, fluid balance and homeostasis, and red blood cell production. The basic function of urine production and elimination is relatively simple, but the mechanism through which the urinary system maintains homeostasis is quite detailed. This section explores the structures of the urinary system and their functions, with special emphasis on the kidneys. Note that the term *renal* pertains to the kidney. Therefore, renal function can be used in place of kidney function, but renal system shouldn’t be used in place of the urinary system.

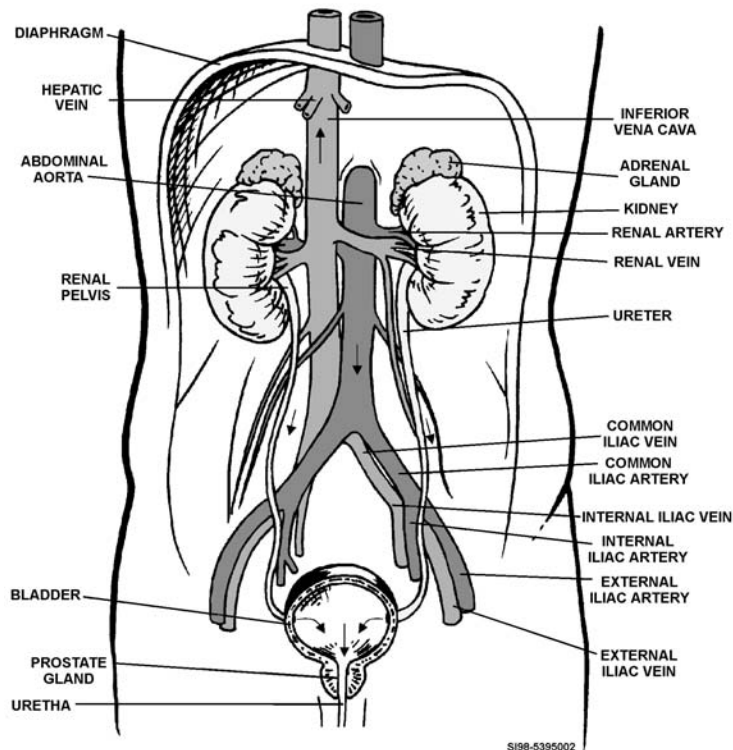
#### 405. Anatomy

The urinary system is made up of two kidneys, two ureters, a bladder, and a urethra. The kidneys are the main functional organs of the system—the remainder of the system is basically plumbing. In this lesson, you’ll learn about the gross anatomy of the urinary system and the microscopic anatomy or internal structures of the kidneys.

#### Kidneys

As shown in figure 2–1, the kidneys are a pair of unique organs located under the dome of the diaphragm, behind the peritoneum (known as retroperitoneal space) in the posterior wall of the abdominal cavity, on either side of the vertebral column. The kidneys are protected by the lower ribs and deep muscles of the back. They are approximately 11 cm (4.5 inches) long, 6 cm (2.5 inches) wide, and 2.5 cm (1 inch) thick. They are reddish-brown in color and are covered by three tissue layers. The innermost layer is called the renal capsule, the middle layer is the adipose capsule, and outermost layer is the renal fascia. The lateral surface of each kidney is convex while the medial side of each is concave, giving the entire organ a bean-shaped appearance. The concavity gives rise to a hollow chamber called the renal sinus; the entrance to the sinus is called the hilum. The renal artery, which rises from the abdominal aorta; the renal vein, which empties into the inferior vena cava;

lymph vessels; nerves; and the renal pelvis, which becomes a ureter, all pass through the hilum into each kidney.



**Figure 2-1. Gross anatomy of the urinary system and connecting blood vessels.**

### Ureters

The ureters are long, slender, muscular tubes that vary in length depending on the size of the individual. They are about 6 mm (0.25 inch) in diameter and may be anywhere from 25 cm to 32 cm (10 to 12 inches) long. They are located in the retroperitoneal space and run parallel with the vertebral column. Towards the bottom of the pelvic cavity, they start running forward, enter the peritoneal cavity, and converge to meet behind and at the bottom of the bladder. Nearly 2.5 cm (1 inch) of the ureter enters the bladder by passing diagonally through the bladder wall. The ureter transports the urine, produced in the kidney, to the urinary bladder for storage. The ureters are made up of three layers.

Layer	Composition	Function
Internal	A mucosal epithelium layer that is continuous with the epithelium of the bladder and pelvis of the kidney.	Helps protect the ureter from the chemical actions of the urine; allows stretching without tearing.
Middle	A muscularis layer made up of outer circular and internal longitudinal smooth muscle fibers.	These smooth muscle fibers contract in waves (peristalsis) and help propel urine.
Outer	Made up of fibrous and areolar connective tissue and adipose tissue.	Like most organs, the connective tissue secures organs to surrounding structures and adipose tissue provides protection.

The muscles of the ureters are capable of the same rhythmic contraction (peristalsis) found in the digestive system. Urine is moved along the ureter from the kidneys to the bladder by peristalsis at frequent intervals. The ureters connect to the bladder on the bottom and at the point where they join small folds of mucous membrane cover the openings. These folds act as flap valves to prevent urine from reentering the ureters. When a peristaltic wave of urine reaches the bladder and spurts into it, a

valve is needed to prevent gravity and the constant tone of the bladder's muscle layer from forcing urine back into the ureter.

### Urinary bladder

The urinary bladder is a hollow organ that acts as a reservoir for urine just as the gallbladder is a storage bag for bile. It is located within the pelvic cavity, behind the pubic symphysis (joint); anterior to the rectum in a male and anterior to the uterus in a female. It is held in place by folds of peritoneum. Its size ranges from the size of a walnut when empty to the size of a small melon when fully distended. The ability to distend so dramatically is a result of its unique composition. The bladder is made up of four layers.

Layer	Composition
First (internal)	The innermost layer or mucosa layer, like the ureters, is made up of several layers of mucous epithelium for protection against the urine. Unlike the epithelium of the ureters, the bladder's epithelium has rounded cells that look like the cortex of the cerebrum. As the bladder distends, these cells flatten out to increase the surface area and hence the volume capacity of the bladder.
Second	The submucosa layer is composed mainly of connective tissue.
Third	The detrusor muscle layer is made up of two longitudinal muscle layers separated by a circular muscle layer.
Fourth (outer)	The outermost layer is called the serous coat and is formed by the peritoneum on the top part of the bladder and connective tissue on the bottom.

The internal floor of the bladder is triangular in shape. The posterior part of the floor forms the base of the triangle with the ureters entering approximately at the corners. The apex of the triangle is roughly where the urethra connects to the bladder. The floor of the bladder does not change much when the bladder is full. Rather, the sides and top project outward and upward as the bladder fills with urine.

### *Changes within the bladder*

When the bladder is empty, the muscular wall becomes thick and the entire organ feels firm. As the organ fills, the muscular wall becomes thinner. The organ may increase from a length of 5 cm (2 inches) up to as much as 12.5 cm (5 inches) or even more. This can be compared to the changes a balloon goes through as it is inflated. A deflated balloon has its wrinkles and folds, and the wall of the balloon is relatively thick. As the balloon inflates, the rubber in the wall stretches and becomes thinner, just like the wall of the bladder as it inflates with urine. As the bladder fills with urine, stretch receptors send impulses to the sacral spinal cord to trigger the micturition (urination) reflex center.

### *Urination*

This reflex center, located in the sacral segments of the spinal cord, sends out parasympathetic impulses to the detrusor muscle of the bladder and rhythmic contractions begin. The sensation of "I've gotta go" accompanies the contractions. This usually occurs when the bladder contains about 150 ml of urine. As the bladder fills to about 300 ml, the urge to void becomes very strong. Fortunately, conscious nervous control from the mid-brain and cerebral cortex can override the micturition reflex. Near the outlet of the bladder, circular muscle fibers contract to prevent emptying, and form what is known as the internal sphincter. In addition, conscious control keeps the external urethral sphincter contracted to prevent the flow of urine from the bladder. With flap valves at the urine inflow sites (ureters), and a sphincter at the output site (urethra), you can see how the bladder can become distended with urine as peristalsis continues. In the infant, a center in the lower part of the spinal cord receives impulses from the bladder and sends motor impulses out to the bladder musculature; the organ is emptied in an action that is automatic (i.e., a reflex action). However, with training, the child learns to control this reflex.

## Urethra

The urethra is a tube-like structure that drains urine from the bladder and conveys it to the outside of the body. Like the other structures of the urinary plumbing, its innermost wall is made up of a mucous membrane. The outer part of the urethra is a relatively thick layer of smooth muscle with its fibers generally running longitudinally. The urethra has two sphincters—the internal urethral and the external urethral sphincters. The external urethral sphincter is made up of involuntary muscle tissue subject to reflex activity. Both are stimulated by reflex impulses, but the reflex impulses of the external urethral sphincter can be inhibited. Voluntary control of this sphincter is possible only with a mature nervous system, hence the need for diapers for small children. Voluntary control usually develops at about two years of age as the nervous system matures.

### *In females*

The urethra differs in females and males, since in males it is also a part of the reproductive system. The female urethra is a thin-walled tube about 3.75 cm long. It is behind the pubic joint embedded in the muscle of the front wall of the vagina. The external opening is called the urethral meatus and is located just in front of the vaginal opening or within the lower part of the front wall of the vagina.

### *In males*

The male urethra is about 20 cm long. Early in its course it passes through the prostate gland, where the two ducts carrying the male sex cells within the sperm join it. From here it leads through the penis, the male organ of copulation, to the outside. The male urethra serves the dual purpose of conveying the spermatozoa and draining the bladder, while the female urethra performs only the latter function.

## Internal structures of the kidney

The kidney is a glandular organ; that is, most of the tissue is epithelium with just enough connective tissue to serve as a framework. As is the case with most organs, the most fascinating aspect of the kidney is too small to be seen with the naked eye. When the kidney is cut in half lengthwise, specific anatomical areas and structures can be readily identified. There are two main regions within the kidney covering. The inner region is called the medulla. The outermost region, which lies under the covering, is called the cortex. See figure 2-2 for an illustration.

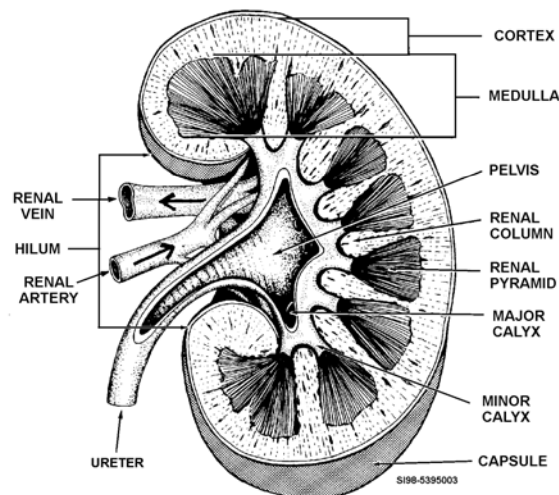


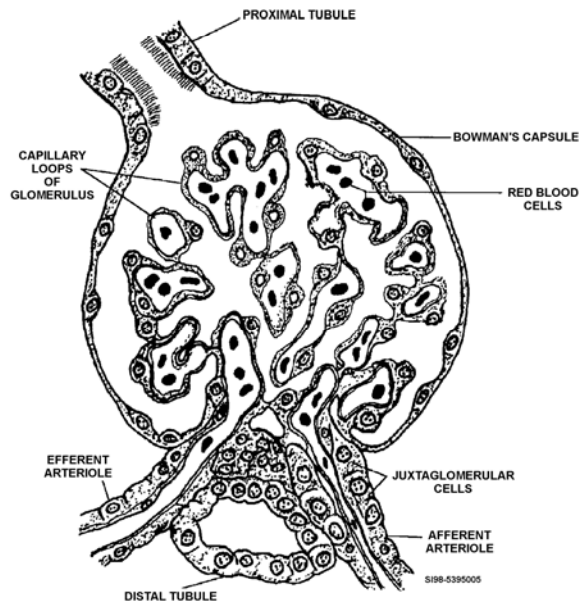
Figure 2-2. Longitudinal view of the internal structures.

### *Medulla*

The medulla is composed of tissue lighter in color than that of the cortex and contains the renal pyramids. These are funnel or pyramid-shaped, with a striated appearance and they contain collection tubules. Tissue from the cortex projects between adjacent renal pyramids to form the renal columns. The apices of the pyramids project toward the center or hilum of the kidney, while the bases face the cortex. The narrow apex of each pyramid forms a renal papilla, which projects into a small, tube-shaped collecting chamber called a minor calyx. The minor calyces (the plural of calyx) join together to form larger collecting areas called major calyces. Each papilla contains several small openings through which urine passes from the collecting tubules of the renal pyramids into the minor and major calyces. The major calyces empty urine into a large central cavity called the renal pelvis. From the renal pelvis urine then passes into the expanded proximal end of the ureter, which joins with the kidney at the hilum.

The nephron is primarily a tiny coiled tube with a bulb at one end called the Bowman's capsule. This bulb surrounds a cluster of capillaries called the glomerulus. The glomerulus of the nephron is a collection of capillaries that invaginates into Bowman's capsule. The combination of these two structures is often called a renal corpuscle. Bowman's capsule can be compared to a bean bag chair. The chair would represent the capsule, and your body sitting in the chair would be like the glomerulus, as shown in figure 2–3. As you sit in the chair, the sides of the chair wrap around your backside, legs, and trunk. The capsule folds around the glomerulus in much the same manner. With this type of arrangement there are two membranes between the blood in the capillaries and the inside of the capsule—the capillary membrane and the capsule membrane (the one created by the

invagination of the capillaries). Of course, the membranes are separated by a small space as shown in figure 2-4. Bowman's capsule extends completely around the glomerulus to seal and entirely surround the capillary bed. A small blood vessel, called the afferent arteriole, supplies the glomerulus with blood; another small vessel, called the efferent arteriole, carries blood from the glomerulus to the capillaries surrounding the coiled tube of the nephron. Since these capillaries surround the tube, they are called the peritubular capillaries as illustrated in figure 2-3. The only area where Bowman's capsule is not sealed thoroughly is where the afferent and efferent arterioles enter the capsule.



**Figure 2-4. Magnified view of a Bowman's capsule and glomeruli.**

### *Tubules*

Before we discuss the flow of blood through the nephron unit, let's move back to Bowman's capsule. The structure that connects to the capsule is called a tubule (i.e., small tube). Portions of the tubule are twisted and, therefore, referred to as being convoluted. The tubule is divided into three sections—the proximal and distal convoluted tubules, and the loop of Henle. The loop of Henle separates the proximal and distal convoluted tubules as you can see in figure 2-3. As the name implies, the loop of Henle is not twisted, but is simply a U-shaped loop of the tubule. The point of reference for determining whether the tubule is proximal or distal is the capsule. Keeping this in mind, the proximal convoluted tubule attaches to the capsule, the loop of Henle separates the proximal and distal tubule, and the distal tubule connects to the collecting tubule. The collecting tubule runs inside a renal pyramid and empties into a renal papilla.

### *Renal blood supply*

The kidney receives its blood supply from the renal artery. This artery branches into several small arteries, usually one to a pyramid. From there, the arteries further divide, forming the afferent arterioles. The afferent arteriole supplies the renal corpuscle with blood and the efferent arteriole receives the blood from the corpuscle. In this way, the efferent arteriole acts much like a venule in systemic circulation in as much as it receives the blood from the capillaries. So why isn't it called a venule? Because the efferent arteriole establishes another capillary bed after it leaves Bowman's capsule. This capillary bed is called the peritubular capillary network. Likewise, the renal vein divides and subdivides to complete the blood circuit through the kidney. Like most blood vessels, the arteries and veins share the same pathway, running parallel with each other. Within the kidney, the blood vessels divide and travel through the renal columns, then further divide and run between the cortex and medulla.

### *Nephron fluid circuits*

The nephron can, generally, be subdivided into two fluid circuits—the blood circuit and the filtrate (urine) circuit. It is the interaction between these two circuits that performs the absorption function of the kidney.

#### *Blood circuit*

As we just mentioned, the kidney receives its blood supply from the renal artery entering the kidney through the hilum. The artery subdivides into smaller arteries and, finally, into arterioles to provide blood to the nephron unit. The blood enters the unit by way of the afferent arteriole, circulates

through the glomerulus invaginated in Bowman's capsule, and leaves the capsule through the efferent arteriole. The efferent arteriole then gives rise to the peritubular capillary network and vasa recta. The blood leaves the peritubular capillary network through a venule, which joins with other venules to form the renal vein. The renal vein passes through the hilum of the kidney to empty the blood into the inferior vena cava.

#### *Filtrate circuit*

The filtrate circuit begins in Bowman's capsule as the filtrate passes through the capsule's membrane. From this point it passes through the proximal convoluted tubule, the loop of Henle, the distal convoluted tubule, and into the collecting duct. The filtrate from several nephrons empty into a single collecting duct. From the collecting duct, the fluid empties into the minor and major calyx, then into the pelvis of the kidney, and finally into the ureter. The ureter passes through the hilum and on to the bladder and urethra.

### **406. Renal function and urinary system disorders**

As stated in the last unit, homeostasis is critical to maintaining life and the urinary system plays an important role in homeostasis. In this lesson we'll discuss renal function and some of the disorders of the urinary system.

#### **Excretory mechanisms**

The urinary system can also be considered an excretory system, because one of its main functions is to remove certain waste products from the blood and eliminate them from the body. The terms *excretion* and *elimination* are often used interchangeably. Excretion is the function of removing useless substances or waste products and elimination indicates the actual expulsion or emptying of the hollow organs in which these waste products have been temporarily stored. Thus, the kidney is said to excrete, while the urinary bladder eliminates. Although the focus of this lesson is the urinary system, it is important to remember that body systems work interdependently to maintain homeostasis. One way to do this is through eliminating waste products. See the table below for a brief review of the excretory systems.

System	Eliminated Substances
Urinary system	Constituents of the urine are water soluble waste products containing nitrogen and salts.
Digestive system	Water, some salts, bile, and the residue of digestion are all contained in the feces.
Respiratory system	Carbon dioxide and water are excreted. Water appears as vapor, as breathing on a windowpane will demonstrate.
Dermal system	Water, salts, and very small quantities of nitrogenous wastes appear in perspiration, though evaporation of water from the skin may go on most of the time without our being conscious of it.

It's time to look at the specific renal functions that keep us healthy and alive. And then we'll move into the subject of urinary system disorders.

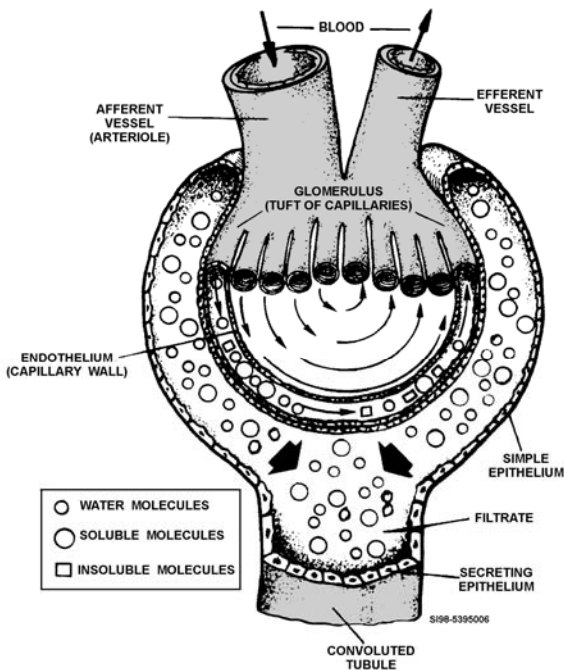
#### **Urine production**

The removal of metabolic waste from the blood and excretion of the waste products from the body is the major function of the kidneys and urinary system. The kidney uses two processes for urine production—filtration and reabsorption. Let's discuss the process of filtration first.

#### ***Filtration***

Filtration occurs as water and dissolved substances are forced out of the glomerulus and into Bowman's capsule, where urine production begins. The membranes that form the walls of the glomerular capillaries are sieve-like and permit a free flow of water and soluble materials through them. These capillary walls, as is the case with other capillaries, are impermeable to blood cells and large protein molecules, so these substances remain in the blood. The process of filtration is random.

That is, any blood, fluid, or dissolved substance small enough to pass through the pores of the glomerular capillaries does! (It's not quite that simple, but not too difficult either.)



**Figure 2-5. An illustration of the process of filtration through Bowman's capsule and the glomerulus.**

### *Glomerular filtration*

The afferent arteriole entering the glomerulus allows blood to enter more rapidly than it can leave through the efferent arteriole. Thus, the pressure of the blood in the glomerulus is about 3 to 4 times as high as it is in other body capillaries. Consequently, the fluid in the blood is constantly being squeezed into Bowman's capsule, as shown in figure 2-5. This process is known as glomerular filtration, and the fluid that enters Bowman's capsule is called the glomerular filtrate. As blood passes through the glomerulus, about one-fifth of the blood plasma volume is filtered out of the capillaries and into Bowman's capsule. It is composed of amino acids, urea, uric acid, creatine, creatinine, water, and a host of ions including sodium, potassium, chlorine, phosphate, sulfate, calcium, and bicarbonate. The concentrations of these substances in the filtrate are much the same as the concentrations found in blood plasma. So, the glomerulus essentially filters out blood

plasma from the blood. In addition to water and the normal soluble substances in the blood, other substances, such as drugs, may also be filtered and become part of the glomerular filtrate. About the only difference between filtrate and plasma or interstitial fluid is the very small amount of protein in the filtrate. As you can see, the process of filtration does very little to produce urine; the composition of the filtrate is very similar to that of interstitial fluid. It is critical, however, to the initial processes of urine production.

### *Filtration and blood pressure*

Changes in the systemic blood pressure have little effect on the rate of filtration in the glomerulus. You would think that an increase in blood pressure would increase the filtration rate as capillary hydrostatic pressure is the major force pushing fluid through the capillary membranes. Fortunately, other pressures oppose the loss of fluid from the glomerulus. For example, the osmotic (inward) pressure of the protein that remains in the glomerulus capillaries attracts water and counteracts some of the hydrostatic (outward) pressure of the blood.

### *Juxtaglomerular apparatus and filtration*

Another mechanism involves the juxtaglomerular apparatus. This apparatus is made up of the distal tubule passing between the afferent and efferent arterioles. Basically, special cells of the tubule at their contact with the arterioles sense a decrease in the concentration of chloride ions as a result of decreased filtration rate (pressure). The cells then signal the afferent arteriole to relax (dilate) in order to increase blood flow through the glomerulus. At the same time, the enzyme renin (pronounced ree-nin) is released to stimulate the production of a vasoconstrictor. The vasoconstrictor acts on the efferent arteriole causing a decreased flow through the glomerulus. This backup of blood also causes an increase in blood pressure and filtration rate. With these regulating mechanisms, the glomerular filtration rate remains relatively constant despite changes in systemic blood pressure. Now that Bowman's capsule has received the filtrate, let's discuss the next process of urine formation, reabsorption. As aforementioned, about one-fifth of the blood's fluid is filtered into Bowman's



capsule. If some mechanism did not exist to reabsorb this fluid, you would become severely dehydrated.

### ***Reabsorption***

Glomerular filtrate begins its journey along the tubular system of the nephron. The process of reabsorption takes place in the tubules. The structures of the nephron lend themselves very well to this process. For example, the diameter of the efferent arteriole is smaller than that of the peritubular capillaries. So, as the fluid flows from the efferent arteriole into the peritubular capillaries, the pressure drops remarkably. This allows the filtrate in the tubules to diffuse easily into the interstitial space and be reabsorbed by the capillaries. Also, the peritubular capillaries have very large pores to ease the reabsorption. As you can see, the tubules alone cannot perform the function of reabsorption; it is the interaction of the tubules and peritubular capillaries that accounts for the reabsorption.

### ***Removing fluid***

Reabsorption involves the use of several different mechanisms to remove fluid, ions, and other dissolved substances from the tubules. Since a large percent of the filtrate is water, osmosis occurs between the tubules and capillaries. Osmosis is possible because the concentration of water in the tubules is greater than that of the peritubular capillaries. Stated differently, the water moves from an area of lesser solute concentration (hypotonic) to an area of greater solute concentration (hypertonic). If the last sentence seems contradictory to the one before it, remember a greater solute concentration has less water than a lesser solute concentration.

### ***Active transport and passive transport***

Active transport is used to remove sodium from the tubules. Since sodium is a positively charged ion, some negatively charged ions like chloride, phosphate, and bicarbonate are attracted to the sodium and move with it. This is called passive transport. Other substances are diffused out of the tubules into the capillaries. The small amount of protein in the filtrate is reabsorbed by the epithelial cells of the tubules by pinocytosis. The protein is then converted to amino acid and released to the interstitial fluid for capillary absorption. As the filtrate continues through the tubules, more water is reabsorbed by the capillaries until approximately 99% is recovered. Ions, dissolved substances, and waste products in the filtrate are either reabsorbed or left to remain in the tubule according to the body's needs. By the time the filtrate enters the collecting duct, all that remains is excess to the needs of the body. It is the waste product called urine.

### ***Body fluid homeostasis***

The kidneys wouldn't have much to do if our fluid intake was always in equilibrium with our fluid loss! When our fluid intake is low and output high, the body calls on the kidneys to further concentrate urine to help conserve fluids. Likewise, if the input is high, the kidneys must increase output to keep from flooding the internal environment. This keeps the body in balance and helps it maintain a constant appropriate internal environment—homeostasis.

### ***Regulating urine concentration or dilution***

To help regulate urine concentration or dilution, the kidney uses a counter-current mechanism, which increases the concentration of solutes. Basically, a counter-current mechanism is made up of the loop of Henle, which is a long, hairpin-U-shaped tube with the limbs close to each other. Fluid travels down the descending limb, through the U bend, then up the ascending limb. Because the fluid travels in a downward direction in one limb, but an upward direction in the other, the flow or current in one limb is counter to the flow in the other. This allows the contents in one limb to differ from the contents of the other.

### Loop of Henle

It may help you to refer to figure 2-6 as we discuss this process. In the kidney, the nephron's loop of Henle and the vasa recta extend deep into the medulla. Fluid containing NaCl enters the descending limb of the U. The walls of the descending limb, and bottom of the U are much thinner than those of the ascending limb, and they are also more permeable; they freely allow water and solutes to pass through. This means as urine flows down the loop of Henle, it becomes approximately equal in concentration to the interstitial fluid around the descending limb. However, the walls of the ascending limb are much thicker, and tissue forming the walls is highly impermeable to water, but not as impermeable to NaCl. As the urine travels up the ascending limb, the NaCl passes through the walls, while the water remains trapped. Thus, the fluid emerging from the ascending limb has a lesser concentration than the urine within the limb. This also means that the interstitial fluid in the medulla is highly concentrated, and increases in concentration as more NaCl is absorbed. Keep this counter-current mechanism in mind as you read the next two paragraphs.

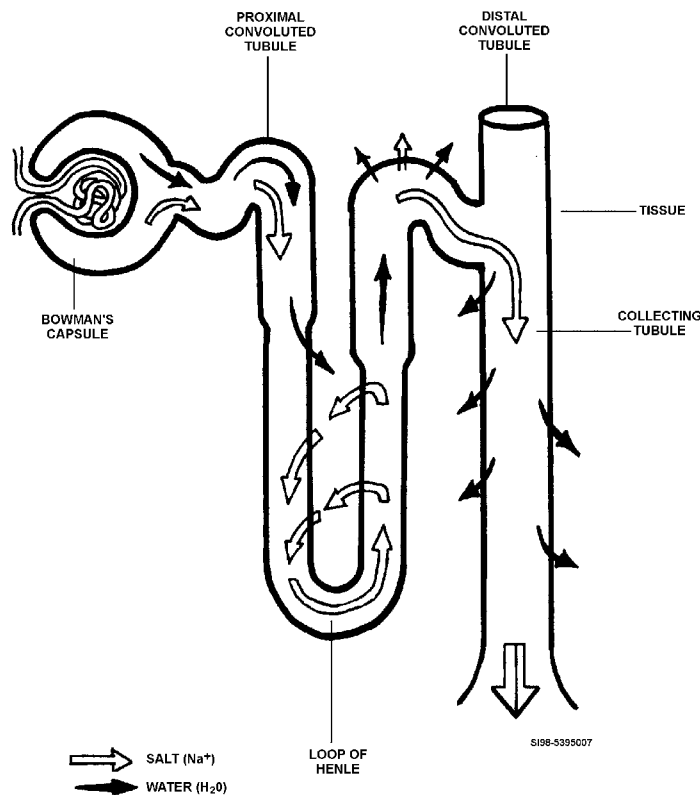


Figure 2-6. An illustration of the loop of Henle's counter-current mechanism.

### Concentration

Concentrating urine uses the high concentrations of interstitial fluid in the medulla established by the vasa recta and loop of Henle by the counter-current process. When ADH is secreted into the body tissue, the target cells in the walls of the nephron's distal tubule and collecting duct respond by dramatically increasing their permeability to water. As the fluid passes through the distal tubule into the cortical portion of the collecting duct, water freely osmoses into the interstitial fluid and is absorbed. The water also osmoses from the medullary portion of the collecting duct into the interstitial fluid of the medulla because of the concentration differences. Therefore, a very concentrated filtrate (urine) remains in the collecting duct as water is removed and absorbed by the capillaries—including the vasa recta. Concentrating urine, then, is one of the main functions of the juxtamedullary nephron. Again, it is

accomplished by high concentrations of NaCl in the medulla's interstitial fluid and the increased permeability of the distal tubule and collecting duct from anti-diuretic hormone (ADH) secretion.

### Dilution

The dilution of urine is fairly simple; the kidney simply allows water to remain in the tubules of the nephrons and enter the renal pelvis. In the absence of the ADH, produced by the posterior pituitary, the walls of the distal tubule and collecting duct are nearly impermeable to water. So, water remains in the tubules and enters the collecting duct and pelvis of the kidney; from the renal pelvis, the urine travels through the ureters to the bladder.

### Blood pressure regulation

Blood pressure is determined by several factors. For the purpose of our discussion, we only talk about how the kidneys' response to changes in blood volume influences blood pressure. When blood volume in the body is increased, blood pressure throughout the arterial vascular network increases. The converse also is true; decreased volume leads to decreased pressure. The kidneys play an important role in regulating blood volume, so indirectly they help regulate blood pressure. Here's how the kidneys accomplish this feat. When blood volume increases, cardiac output and arteriole pressure increases. As a result, afferent arteriole pressure in the renal corpuscle increases and the rate of glomerular filtration increases. Urine output continues to rise until a normal blood volume is established. The urine produced in this instance is very diluted (i.e., mostly water). If blood volume is low and blood pressure drops, filtration is decreased. ADH is secreted by the posterior lobe of the pituitary, increasing the permeability of the tubules. More water is reabsorbed by the capillaries, which helps elevate the fluid volume in the blood. The urine produced when this occurs is more concentrated because it contains a higher ratio of solutes to water.

### Red blood cell production

Another important function of the kidney is helping to regulate the production of red blood cells. They accomplish this by secreting an enzyme called erythropoietic factor. This enzyme acts on a protein in the plasma and forms erythropoietin. This substance stimulates the cells of the bone marrow that eventually produce red blood cells.

### Disorders of the kidneys

Kidney disorders may be acute or chronic. Acute conditions usually arise suddenly and, most frequently, are the result of infection with inflammation of the nephrons. These diseases commonly run a course of a few weeks followed by complete recovery. Chronic conditions arise slowly and often are progressive with gradual loss of kidney function.

#### *Acute renal failure*

Acute renal failure may result from a medical or surgical emergency or from toxins that damage the tubules. This condition is characterized by a sudden, serious decrease in kidney function that may be fatal without immediate medical treatment.

#### *Chronic renal failure*

Chronic renal failure results from a gradual loss of nephrons. As more and more nephrons are destroyed, the kidneys gradually lose the ability to perform their normal functions. As the disease progresses, nitrogen waste products accumulate to high levels, and this condition is known as uremia. A few of the characteristic signs and symptoms of chronic renal failure are shown in the table below.

Symptom	Characteristics
Dehydration	Excessive loss of body fluid may occur early in renal failure when the kidneys cannot concentrate the urine and large amounts of water are eliminated.
Edema	Accumulation of fluid in the tissue spaces may occur late in chronic renal disease when the kidneys cannot eliminate water in adequate amounts.
Hypertension	May occur as the result of fluid overload and the increased production of renin.
Anemia	Occurs when the kidneys cannot produce the hormone to activate red bone marrow cell production.
Uremia	If these levels are very high, urea can be changed into ammonia in the stomach and intestine and cause ulcerations and bleeding.

Even without kidney disease, the kidneys lose some of their ability to concentrate urine due to the aging process. More water is needed to excrete the normal amount of waste products. Older persons find it necessary to drink more water, and they eliminate larger amounts of urine (polyuria) even at night (nocturia).

***Acute glomerulonephritis***

Acute glomerulonephritis is the most common disease of the kidneys. This condition usually occurs in children about 1 to 4 weeks after a streptococcal infection of the throat. Antibodies formed in response to the streptococci attach to the glomerular membrane and injure it. These damaged glomeruli allow protein, especially albumin, to filter into Bowman's capsule and ultimately appear in the urine (albuminuria). They also allow red blood cells to filter into the urine (hematuria). Usually, the patient recovers without permanent kidney damage. Sometimes, in an adult patient, the disease becomes chronic with a gradual decrease in the number of functioning nephrons, leading to chronic renal failure.

***Pyelonephritis***

Pyelonephritis refers to an inflammation of the kidney pelvis and the tissue of the kidney itself. It may be either acute or chronic. In acute pyelonephritis, the inflammation results from a bacterial infection. Bacteria most commonly reach the kidney by ascending along the lining membrane from an infection in the lower part of the urinary tract. More rarely, bacteria can be carried to the kidney by the blood. Acute pyelonephritis is often seen in persons with partial obstruction of urine flow with stagnation (urinary stasis). This may occur during pregnancy in the female or it may be due to an enlarged prostate in the male. Usually, the disease responds to the administration of antibiotics, fluid replacement, rest, and fever control. Chronic pyelonephritis is a more serious disease and is frequently seen in patients with urinary tract blockage. It may be caused by persistent or repeated bacterial infections. There is progressive damage of kidney tissue, eventually leading to chronic renal failure.

***Hydronephrosis***

Hydronephrosis refers to the distention of the renal pelvis and calyces caused by an accumulation of fluid due to an obstruction to normal urine flow. The obstruction may occur at any level in the urinary tract. The most common causes of obstruction are, in addition to pregnancy or an enlarged prostate, a kidney stone that formed in the pelvis and dropped into the ureter, a tumor that presses on a ureter, or scars due to inflammation. Removal of the obstruction within a few weeks, before the kidney is damaged, may result in complete recovery. If the obstruction is not removed, the kidney will be permanently damaged.

***Kidney tumors and cysts***

Tumors of the kidneys usually grow rather slowly, but occasionally rapidly invading types are found. Blood in the urine and dull pain in the kidney region are warnings that should be heeded at once. Immediate surgery may be lifesaving. A polycystic kidney is one in which many fluid-containing sacs develop in the active tissue and gradually, by pressure, destroy the functioning parts. This disorder runs in families, and until now treatment has not proved very satisfactory, except for the use of dialysis machines or kidney transplants.

***Dialysis***

Dialysis means the diffusion of dissolved molecules through a semi-permeable membrane. These molecules tend to pass from the area of greater concentration to one of less concentration. In patients who have defective kidney function, the accumulation of urea and other nitrogen waste products can be reduced by passing the patient's blood through a dialysis machine. This is where the principle of molecules leaving the area of greater concentration operates to remove the excess products from the blood. There are two methods of dialysis in use: hemo-dialysis and peritoneal dialysis. Both are based on the principle of diffusion of dissolved molecules through a semi-permeable membrane. In hemo-dialysis, the membrane is made of cellophane; in peritoneal dialysis, the surface area of the peritoneum acts as the membrane.

### *Kidney transplants*

Many hundreds of kidney transplants have been performed successfully during the last several years. Kidneys have so much extra functioning tissue that in the normal individual no problem is posed by losing one kidney. Records show that the percentage of transplant successes is greatest when living, closely related donors are used. However, organs from a deceased donor have also proved satisfactory in many cases.

### ***Kidney stones***

Kidney stones, or calculi, are made of certain substances, such as uric acid and calcium salts, which precipitate out of the urine instead of remaining in solution. They usually form in the renal pelvis, although the bladder can be another site of formation. The causes of this precipitation of stone-building materials include infection of the urinary tract and stagnation of the urine. These stones may vary in size from tiny grains resembling bits of gravel up to large masses that fill the kidney pelvis and extend into the calyces. These are described as staghorn calculi. There is no way of dissolving these stones, since substances that would be able to do so would also destroy the kidney tissue. Sometimes, instruments can be used to crush smaller stones and thus allow them to be expelled with the urine, but most often surgical removal is required.

### **Disorders of the ureters**

Abnormalities in the structure of the ureter include double portions at the kidney pelvis and constricted or abnormally narrow parts, called strictures. Narrowing of the ureter may also be caused by abnormal pressures from tumors or other masses outside the tube. Obstruction of the ureters may be the result of stones from the kidneys or a “kinking” of the tube due to a dropping of the kidney, a condition known as *ptosis*. The passage of a small stone along the ureter causes one of the most excruciating pains known. This intense pain is called renal colic and usually requires morphine or an equally powerful drug for relief. The first barber surgeons operating without benefit of anesthesia were permitted by their patients to cut through the skin and the muscles of the back to remove stones from the ureters. Cutting for stones in this way was relatively successful, in spite of lack of sterile technique. Because this approach went through the back it avoided the peritoneal cavity which decreased the possibility of deadly peritonitis. Modern surgery for a kidney stone, including the use of special instruments threaded through the urinary tract from the outside, may cause temporary disability and a short convalescence.

### **Disorders involving the bladder**

A distended bladder lies in an unprotected position in the lower abdomen, and a blow may rupture it, necessitating immediate surgical repair. Infection and tumors may involve the bladder, and blood in the urine is a rather common symptom of these. Inflammation of the bladder is called cystitis and is 10 times as frequent in women as in men. This may be due, at least in part, to the very short urethra in the female compared with that of the male. Usually, bacteria ascend from the outside through the urethra into the bladder. Pain, urgency, and frequency are common symptoms. Obstruction, by an enlarged prostate gland or from pregnancy, may lead to stagnation and cystitis. Reduction of the general resistance to infection, as in diabetes, may lead to cystitis. The danger of cystitis is that the infection may ascend to other parts of the urinary tract.

### **Disorders of the urethra**

Congenital anomalies (defects) present at birth involve the urethra as well as other parts of the urinary system. The opening of the urethra to the outside may be too small, or the urethra itself narrowed. Occasionally, it happens that there is an abnormal valve-like structure located at the point where the urethra enters the bladder. These valve-like folds of tissue can cause a back pressure of the urine, with serious consequences if they are not removed surgically. There is also a condition in the male in which the urethra opens on the under surface of the penis instead of at the end. This is called hypospadias. Urethritis, in which inflammation of the mucous membrane and the glands of the urethra are involved, is much more common in the male than in the female and is often due to

gonorrhea, although many other bacteria also may be responsible for the infection. Straddle injuries to the urethra are common in men. This type of injury occurs when, for example, a man walking along a raised beam slips and lands with the beam between his legs. Such an accident may catch the urethra between the hard surfaces of the beam and the pubic arch, and rupture the urethra. In accidents in which the bones of the pelvis are fractured, rupture of the urethra is fairly common.

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### Self-Test Questions

**After you complete these questions, you may check your answers at the end of the unit.**

#### **405. Anatomy**

1. Where are the kidneys located?
2. How many tissue layers does the kidney have and how are they designated?
3. Where are the ureters located and what is their function?
4. How is urine moved along the ureter?
5. Where is the bladder located and what is its function?
6. What keeps the bladder from automatically emptying?
7. What are the differences between the female and male urethra?
8. What are the two main regions within the kidney covering?
9. Where is the medulla located and what does it contain?
10. Where is the cortex located and what does it contain?
11. What is the basic functional unit of the kidney and about how many are there?

12. Of what is the renal corpuscle composed?

13. What are the three sections of the tubule that is connected to Bowman's capsule?

14. What are the two fluid circuits of the nephron?

#### 406. Renal function and urinary system disorders

1. Match the terms in column B with the statement in column A by placing the letter of the column B item beside the number of the column A item that most nearly describes it. Each element in column B may be used once, more than once, or not at all.

Column A	Column B
____ (1) The fluid in the blood that is constantly being squeezed into Bowman's capsule.	a. Excretion.
____ (2) If the input is high, the kidneys must increase output to keep from flooding the internal environment.	b. Elimination.
____ (3) Kidneys secrete an enzyme called erythropoietic factor.	c. Urinary system.
____ (4) Occurs as water and dissolved substances are forced out of the glomerulus and into Bowman's capsule	d. Digestive system.
____ (5) Takes place in the tubules, includes the interaction of the tubules and peritubular capillaries.	e. Respiratory system.
____ (6) Most common disease of the kidneys.	f. Dermal system.
____ (7) Indicates the actual expulsion or emptying of the hollow organs.	g. Filtration.
____ (8) When our fluid intake is low and output high, the body calls on the kidneys to further concentrate urine to help conserve fluids.	h. Glomerular filtrate.
____ (9) Inflammation of the kidney pelvis and the tissue of the kidney itself.	i. Reabsorption.
____ (10) Usually grow rather slowly, but occasionally rapidly invading types are found.	j. Body fluid homeostasis.
____ (11) Made of certain substances, such as uric acid and calcium salts, which precipitate out of the urine instead of remaining in solution.	k. Blood pressure regulation.
____ (12) Removal of metabolic waste from the blood and excretion of the waste products from the body is the major function.	l. Red blood cell production.
____ (13) It is composed of amino acids, urea, uric acid, creatine, water, and a host of ions including sodium, potassium, chlorine, phosphate, sulfate, calcium, and bicarbonate.	m. Acute glomerulonephritis.
____ (14) Is frequently seen in patients with urinary tract blockage or obstruction.	n. Pyelonephritis.
____ (15) The function of removing useless substances or waste products.	o. Hydronephrosis.
____ (16) The kidneys play an important role in regulating blood volume.	p. Kidney tumors and cysts.
____ (17) Involves the use of several different mechanisms to remove fluid, ions, and other dissolved substances from the tubules.	q. Kidney stones.
____ (18) Carbon dioxide and water are excreted.	r. Disorders of the ureters.
____ (19) Usually occurs in children about 1 to 4 weeks after a streptococcal infection of the throat.	s. Disorders involving the bladder.
____ (20) Many fluid-containing sacs develop in the active tissue and gradually, by pressure, destroy the functioning parts.	t. Disorders of the urethra.
____ (21) Cystitis is 10 times more frequent in women as in men.	
____ (22) Include double portions at the kidney pelvis and constricted or abnormally narrow parts, called strictures.	
____ (23) Inflammation of the mucous membrane and the glands; much more common in the male than in the female and is often due to gonorrhea.	

## 2-2. Urine Specimen Collection, Transportation, and Preservation

Now that you know urine is produced by the kidneys and how it is eliminated by the urinary system, it's time to learn about collecting this important body fluid. Variables such as collection container, collection method, transportation, and preservation can affect the outcome of the analysis, therefore it is important to know how to overcome these variables through standardization. Your laboratory

should have an OI addressing procedures for collecting, transporting, and preserving urine specimens. An excellent reference for these instructions is the National Committee for Clinical Laboratory Standards (NCCLS), *Urinalysis and Collection, Transportation, and Preservation of Urine Specimens*, Approved Guideline, GP16-A2, November 2001. Some of the information in this guideline was used to prepare this text with permission from NCCLS.

#### **407. Specimen collection for urinalysis procedures**

Proper specimen collection requires knowledge of the routine and special methods utilized in urinalysis. At times, the urine specimen is referred to as a liquid tissue biopsy of the urinary system. It is usually painlessly obtained and provides quick, valuable information. As with all other patient samples, it must be correctly collected to generate accurate and reliable results.

##### **Services request**

As stated in unit 4 of volume 1 of this course, the very first step in specimen collection is the order or request for the laboratory to perform a procedure. This is initiated by the physician or another qualified individual. The lab slip or requisition form can be a computer printout or any style of a manually filled out form. You should familiarize yourself with the various types of forms seen in your MTF. Nonetheless, whatever type of form or system used, each request should encompass the same type of information. This information includes patient's name, SSN or hospital identification number, and date of birth; provider's name; test ordered; date received; requesting location, and room and bed number if an inpatient. For urinalysis request, the reason for ordering the test (chief complaint, symptoms, surgery, etc.), medications patient is taking, how the specimen was obtained (mid-stream, catheter, aspiration, etc.), and the time and date of collection should also be annotated on the request.

##### **Patient identification**

Whether the specimen is acquired in the lab, clinic, or ward; correct patient identification is essential. Before giving collection containers and instructions, check the patient's ID card, ask him or her to state his or her name and SSN, or have him or her visually verify the information on the request form. If the patient is too young, mentally incompetent, or doesn't speak the language, ask a nurse, relative, or friend to identify the patient. **NOTE:** If the patient inquires about the test being done, don't tell or inform him or her about the procedure(s). This should be left to the physician. Remind the patient to speak with his or her physician about the test being ordered.

##### **Safety precautions**

Remember that blood and body fluid precautions should be consistent for all patient specimens or samples. Treat all specimens and patients as though they could transmit an infectious agent. Wear PPE when handling urine specimens.

##### **Urine specimen containers**

The primary collection container and transport container, if necessary, should be clean, leak-proof, and preferably made of a clear, disposable material that will not react with urinary constituents. The typical container, used in most Air Force MTFs, has a 120 ml capacity with a 6.0 cm diameter opening. The container should also have a wide base to help prevent accidental spillage. The container and lid should be free of interfering substances. If a urine culture is requested, a sterile container with a tight-fitting lid is required. If both a urinalysis and culture are ordered together, the culture is done before the urinalysis unless a sterile technique is used to make aliquots from a part of the urine specimen. Sterile containers are also suggested if more than a 2-hour delay is expected between specimen collection and analysis. Some laboratories prefer to use sterile containers for all urine collection. The container is labeled with a label that will stay attached during refrigeration. The label should include the full name, SSN or hospital identification number, date and time of specimen collection, identity of the preservative, if used, and a barcode label if required. To guarantee proper specimen identification, place labels on the container, not on the lid. Now that our containers are ready and labeled, let's look at the collection of the different types of urine specimens.



**Type of specimens**

The type of urine specimen to be collected is dictated by the test to be performed and/or the time interval requested by the physician. Although it would seem that the attending physician should give collection instructions, the task is usually handed down to the laboratory. Some urinalysis procedures may require the patient to eliminate certain foods from his or her diet before the specimen is collected. If you aren't sure about patient instructions for a procedure, check the laboratory guide and OI. Whether verbal or written, instructions must be accurate, concise, and clear. Remember, collecting a proper specimen is the first step toward reporting accurate and reliable test results.

***Random***

The random specimen may be collected at unspecified times. Several hours of urinary continence (self-restraining from urination) before collection may be necessary to provide a specimen suitable for analysis. Because random or un-timed specimens are suitable for only a few chemical tests, generally, urine specimens must be collected over a predetermined interval of time, such as 2, 8, or 24 hours.

***Timed***

The timed specimen is collected at a specified time within a 24-hour period, (e.g., at 10 am) or at a specified time in relation to another activity (e.g., 2 hours after eating a meal or immediately after prostatic massage). The collection period for timed specimens should be long enough to minimize the influences of short-term biological variations of constituents excreted during the day. In other words, since urinary substances are excreted in varying concentrations throughout the day, it is imperative to collect timed specimens in order to accurately quantitate certain substances. Before beginning a timed collection, the patient should be given written instructions with regard to diet or drug ingestion to avoid interference of ingested compounds with analytical procedures. When specimens are to be collected over a specified period of time the patient's close adherence to instructions is important. If a patient has a bowel movement during the collection period, precautions should be taken to prevent fecal contamination. Collection of a timed specimen from a baby is difficult, and requires coordination with the physician, nursing personnel, parents, and the laboratory. Fortunately such specimens are rarely required.

***First morning or 8-hour***

Although a first morning specimen may not seem to be a timed specimen, in actuality it is. The first morning or 8-hour specimen is normally collected immediately on the patient's arising from a night's sleep. This is also known as an overnight or early-morning specimen. Other 8-hour periods may also be used to accommodate insomniacs, night-shift workers, and in certain pediatric situations. A first-morning fasting specimen is generally the most concentrated specimen and thus is preferred for microscopic examinations and for the detection of abnormal amounts of constituents such as proteins, or of unusual compounds such as beta-chorionic gonadotropin ( $\beta$ -hCG). Specimens to verify orthostatic proteinuria are collected after an 8-hour period of lying down. The bladder is emptied immediately before lying down and the specimen is collected on arising so that the urine collected is that which accumulated while the patient was in the recumbent position. Any urine voided during the night should be collected and pooled with the first morning voided specimen.

***Double-voided specimens***

The double-voided specimen is the urine excreted during a timed period following a complete emptying of the bladder; it is used, for example, to assess glucose excretion during a glucose tolerance test. Its collection must be timed in relation to the ingestion of glucose.

### 24-hour urine

If it is necessary to measure the total amount of solutes excreted in a 24-hour period, a strictly timed 24-hour specimen is required because many solutes exhibit diurnal variations. The lowest concentrations of catecholamines, 17-hydroxysteroids, and electrolytes occur in the early morning, whereas highest concentrations occur at noon or shortly thereafter. Collect the specimen in one or more disposable, wide-mouthed, clean, plastic container(s), large enough to hold about 3 l (liters), with a plastic lid. See the illustration in figure 2-7. Keep the collection container in the refrigerator or on ice during the 24-hour period. Provide amber-colored containers for light-sensitive analytes. For

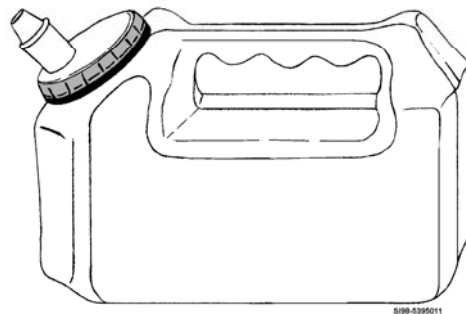


Figure 2-7. An illustration of a 24-hour urine container.

nonambulatory patients with a catheter, store the bag on ice; if the patient is ambulatory, empty the bag periodically and refrigerate the contents. The bladder should be emptied at the time the collection is to begin, and this urine is discarded. Thereafter, all urine should be collected until the end of the scheduled time. If a collection has to be made over several hours, urine should be passed into a separate container at each voiding and then emptied into a larger container for the complete specimen. This two-step procedure prevents the danger of a patient's splashing himself with a preservative such as acid. The large container should be stored in a refrigerator (2 to 8°C) during the entire collection period.

### Catheter and suprapubic tap specimens

Catheter specimens are used for microbiologic examination in critically ill patients or in those with urinary tract obstruction. Catheterized specimens may also be necessary if contamination by vaginal contents in female patients may contaminate the specimen or alter results. However, catheter specimens should not normally be obtained just for examination of chemical constituents because of the risk of introducing bacteria into an otherwise sterile bladder. In infants and small children, a suprapubic tap specimen is a useful alternative. Unlike a catheter, it rarely causes infection. This procedure is explained in more detail in volume 1 of the 4T051B, *Microbiology*, CDCs.

### Clean-catch method

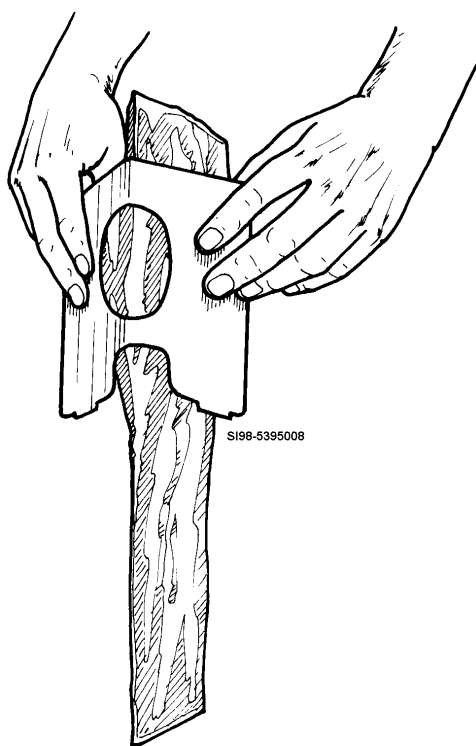
Even though tests in the clinical chemistry laboratory are not generally affected by lack of sterile collection procedures, the patient's genitalia should be cleansed before each voiding to minimize the transfer of surface bacteria to the urine for microbiology studies. Cleansing is essential if the true concentration of white cells is to be obtained. **NOTE:** The clean catch method is required for urine culture specimens and can also be used for any timed specimen. A clean-catch specimen is not a type of specimen but a method or procedure used for collecting a specimen. Hence, you can have a random (unclean) specimen or random clean-catch specimen. Clean-catch just means that the area around the urethra was cleaned before the specimen was collected.

### In females

Before collection of a clean catch urine, the patient should wash her hands with soap or a towelette. Instruct the female patient to squat over the toilet and with a sterile cleansing towelette or equivalent, cleanse the urethral meatus and surrounding area. She should begin urination, passing the first portion into the toilet and collecting the mid-portion without contaminating the container. Any excess urine can pass into the toilet.

***In males***

Before collection of a clean catch urine, the patient should wash his hands with soap or a towelette. Instruct the uncircumcised male patient to withdraw the foreskin to expose the urethral meatus. Using a sterile cleansing towelette or the equivalent, cleanse the penis, beginning at the urethra and working away from it. He should urinate the first portion into the toilet and collect just the mid-portion; without contaminating the container. Excess urine can pass into the toilet. **NOTE:** Bacterial examination of the first 10 ml of urine voided is most appropriate to detect urethritis while the midstream specimen is best for investigating bladder disorders.



**Figure 2-8. Example of a pediatric urine collection bag.**

***In infants and small children***

Before collection of a clean catch urine, the individual applying the urine bag should wash his or her hands with soap or a towelette. In males, the scrotal or perineal area is first cleaned and dried, and any natural or applied skin oils are removed. In females, the vaginal area is cleaned and dried. A urine bag, as illustrated in figure 2-8, is securely attached to the infant or small child. For an un-timed specimen a plastic bag is placed around the infant's or child's genitalia and left in place until urine has been voided. After urination, the bag is carefully removed to avoid contamination; using a sterile technique transfer the urine into a sterile container for culturing, if ordered.

**408. Transportation and preservation of urine samples**

Once the urine specimen is collected, it must be transported to the laboratory in a manner that will not interfere with the quality and integrity of the specimen. The same applies to the method of preservation, it must not interfere with the constituents being analyzed.

**Transportation**

The container used during transportation may or may not be the collection container, yet it must have the same characteristics as the collection container. It should have a tight-fitting lid or secure closure to prevent leakage of the specimen during transportation. The lid or closure should be easily applied and removed.

**Specimen acceptability**

When the specimen is delivered to the laboratory, you should ensure specimen integrity and suitability for analysis by inspecting the specimen for the following items: (1) the patient's information on the request slip must match the information on the container label; (2) the specimen must be <2 hours old if not preserved; (3) the specimen must be in the proper container and the lid must be tightly in place; (4) the outside of container must not be contaminated with the specimen; (5) the specimen volume must be adequate and be free of contaminating materials; and (6) the presence or absence of chemical preservative must be consistent with the procedure requested. If the specimen doesn't meet the criteria for acceptability, contact the attending physician or ward or clinic nursing personnel immediately for a decision on further action. Never discard an unacceptable specimen until the appropriate health care providers have been consulted and a mutually agreeable decision has been reached.

## Preservation

There is no substitute for a fresh urine specimen, and in all cases analysis should be accomplished as soon as possible. Occasionally, it will not be possible to analyze the specimen immediately. Because most urine constituents are unstable, specimens not analyzed within 2 hours of collection require preservation. Also, bacteria can destroy glucose, and pH changes can occur if urine is allowed to stand. Casts and red and white blood cells are especially susceptible to lysis in urine specimens with a low specific gravity ( $<1.010$ ) and in urine specimens with an alkaline pH ( $>7.0$ ). Refrigeration or chemical preservatives are recommended if there is a delay in analysis ( $>2$  hours from collection); the specimen is being tested for an otherwise unstable analyte; or the specimen is being stabilized for culture or microbiological studies.

## Refrigeration

If the specimen cannot be transported and analyzed immediately, it should be refrigerated (2 to 8°C) after collection. Refrigeration, instead of chemical preservatives, is recommended for routine urinalysis. Refrigeration is adequate for most chemical components, except for bilirubin and urobilinogen. If the specimen is to be refrigerated, the laboratory should ensure that the refrigerator is properly maintained and that delays in specimen delivery don't compromise specimen quality or integrity. Preservation is even more successful when refrigeration is combined with a chemical preservative. **NOTE:** Refrigeration can precipitate amorphous urates and/or phosphates; these can obscure the microscopic examination and make it difficult to perform.

## Chemical preservatives

Chemical preservatives are most often used for 24-hour urine requests, special studies, and urine specimens sent to reference laboratories. Unfortunately, there is not a good overall chemical preservative. Each chemical preservative has its drawbacks in that it interferes with a different urine analyte or the analytical method in some way. Therefore, it is imperative that you review your laboratory's guide and/or OI, in addition to the reference laboratory's shipping guide, for each requested analyte and its analytical test method. Because the preservative is analyte, analytical method, and reference laboratory dependent, it is beyond these CDCs to discuss each one specifically. You can review the references listed in the bibliography for more information on urine

preservatives and their preservation characteristics and mechanisms. The table below is a list of the most common preservatives and the analyte they preserve.

Procedure	None	Freeze	Boric acid	Concentrated Hydrochloric acid	Glacial acetic acid	Toluene
Aldosterone		X	X		X	
Amino acids			X	X		X
5-Aminolevulinic acid				X	X	
Calcium				X		
Catecholamines				X	X	
Beta-hCG	X		X			
Citrate			X			X
Copper	X			X		
Cortisol (free)	X		X		X	
Cystine		X		X		X
Estrogen			X		X	
Homogentisic acid	X					
Homovanillic acid			X	X	X	
5-Hydroxyindole-			X	X		

Procedure	None	Freeze	Boric acid	Concentrated Hydrochloric acid	Glacial acetic acid	Toluene
acetic acid						
Hydroxyproline			X	X		X
17-Ketosteroids			X		X	
Magnesium	X			X		
Mercury	X			X		
Metanephrines				X	X	
Nitrogen	X			X		
Osmolality	X	X				
Oxalate	X			X		
Porphyrins						
Pregnanetriol			X		X	
Uric acid	X		X			X
Urobilinogen	X	X				
Vanillylmandelic acid			X	X		
Zinc	X			X		

After the urine is collected, transported, accepted, and preserved it's ready for macroscopic and microscopic examination. These examinations will be presented in the next unit.

### Self-Test Questions

After you complete these questions, you may check your answers at the end of the unit.

#### 407. Specimen collection for urinalysis procedures

1. What is the *first* step in specimen collection and who initiates this step?
2. Besides the identification information, what information should be added to the urinalysis request form?
3. Before giving a patient a collection container and instructions, what should you do?
4. What criteria should the primary collection container and transport container meet?
5. Where should the label be placed and what information should it include?
6. If you aren't sure about patient instructions for a procedure, where can you look for the information?

7. What characteristics should verbal or written instructions have?
8. At what time is a random specimen collected?
9. How do you describe a timed specimen?
10. Why are timed specimens important?
11. What is given to a patient before collecting a time specimen and what does it include?
12. Which specimen is preferred for microscopic examination and provide a description of this specimen?
13. What specimen is used to measure the total amount of solutes during a day and why?
14. How do you describe the collection process of a 24-hour specimen?
15. When should a catheter specimen be collected and on whom?
16. When should a clean-catch specimen be collected?
17. What does clean-catch mean?

**408. Transportation and preservation of urine samples**

1. What characteristics must the transport container have?
2. To ensure specimen integrity and suitability, for what should you inspect?

3. When can you discard an unacceptable specimen?
4. If there is a delay in analysis, what should you do?
5. When is refrigeration, instead of chemical preservatives, recommended and what is the exception?
6. What is the drawback of chemical preservatives?
7. Who or what should you review before using a urine chemical preservative?

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### Answers to Self-Test Questions

#### 405

1. Under the dome of the diaphragm, behind the peritoneum (known as retroperitoneal space) in the posterior wall of the abdominal cavity, on either side of the vertebral column.
2. 3; the innermost layer is called the renal capsule, the middle layer is adipose capsule, and outermost layer is the renal fascia.
3. They are located in the retroperitoneal space and run parallel with the vertebral column; transport the urine, produced in the kidney, to the urinary bladder for storage.
4. Peristalsis.
5. It is located within the pelvic cavity, behind the pubic symphysis (joint); anterior to the rectum in a male and anterior to the uterus in a female; reservoir for urine.
6. The *internal sphincter* and conscious control keep the *external urethral sphincter* contracted.
7. The female urethra is shorter than the male urethra and the male urethra is part of the reproductive system.
8. Medulla and cortex.
9. Inner region of the kidney; renal pyramids.
10. Outermost region of the kidney; renal corpuscles, proximal and distal convoluted tubules, peritubular capillaries, and medullary rays.
11. Nephron; about a million.
12. Bowman's capsule and glomerulus.
13. The proximal and distal convoluted tubules, and the loop of Henle.
14. The blood circuit and the filtrate (urine) circuit.

#### 406

1. (1) h.  
(2) j.  
(3) l.  
(4) g.  
(5) i.

- (6) m.
- (7) b.
- (8) j.
- (9) n.
- (10) p.
- (11) q.
- (12) c.
- (13) h.
- (14) n, o.
- (15) a.
- (16) k.
- (17) i.
- (18) e.
- (19) m.
- (20) p.
- (21) s.
- (22) r.
- (23) t.

**407**

1. The order or request for the laboratory to perform a procedure; physician or another qualified individual.
2. The reason for ordering the test (chief complaint, symptoms, surgery, etc.), medications patient is taking, how the specimen was obtained (mid-stream, catheter, aspiration, etc.), and the time and date of collection.
3. Check the patient's ID card, ask him or her to state his or her name and SSN, or have him or her visually verify the information on the request form.
4. Should be clean, leak-proof, and preferably made of a clear, disposable material that will not react with urinary constituents, have a wide base to help prevent accidental spillage, and have a lid free of interfering substances.
5. Place labels on the container, full name, SSN or hospital identification number, date and time of specimen collection, identity of the preservative, if used, and a barcode label if required.
6. Laboratory guide and OI.
7. Instructions must be accurate, concise, and clear.
8. Unspecified time.
9. The timed specimen is collected at a specified time within a 24-hour period, (e.g., at 10 am) *or* at a specified time in relation to another activity (i.e., 2 hours after eating a meal or immediately after prostatic massage).
10. Since urinary substances are excreted in varying concentrations throughout the day, it is imperative to collect timed specimens in order to accurately quantitate certain substances.
11. Before beginning a timed collection a patient should be given written instructions with regard to diet or drug ingestion to avoid interference of ingested compounds with analytical procedures.
12. The first morning, early-morning fasting, or 8-hour specimen; normally collected immediately on the patient's arising from a night's sleep.
13. A strictly timed 24-hour specimen is required because many solutes exhibit diurnal variations.
14. At the beginning of the collection, the bladder is emptied and urine is discarded; at each voiding during the collection period, urine is collected in a separate container and then emptied into a larger container which is stored at 2 to 8°C until delivery to the laboratory.
15. For microbiologic examination in critically ill patients or in those with urinary tract obstruction and/or if contamination by vaginal contents in female patients may contaminate the specimen or alter results.
16. Microbiology studies or urine cultures and for a true concentration of white cells.



17. Clean-catch just means that the area around the urethra was cleaned before the specimen was collected.

**408**

1. The same characteristics of the collection container; a tight-fitting lid that is easily applied and removed.
2. (1) The patient information on the request slip must match the information on the container label; (2) the specimen must be <2 hours old if not preserved; (3) the specimen must be in the proper container and the lid must be tightly in place; (4) the outside of container must not be contaminated with the specimen; (5) the specimen volume must be adequate and be free of contaminating materials; and (6) the presence or absence of chemical preservative must be consistent with the procedure requested.
3. Never discard an unacceptable specimen until the appropriate care providers have been consulted and a mutually agreeable decision has been reached.
4. Refrigerate or use chemical preservatives.
5. For routine urinalysis; bilirubin and urobilinogen measurements.
6. Unfortunately, there is not a good overall chemical preservative because each preservative interferes with a different urine analyte or the analytical method in some way.
7. Your laboratory's guide and/or OI, in addition to the references laboratory's shipping guide.

**Do the unit review exercises before going to the next unit.**

## Unit Review Exercises

**Note to Student:** Consider all choices carefully, select the *best* answer to each question, and *circle* the corresponding letter. When you have completed all unit review exercises, transfer your answers to ECI (AFIADL) Form 34, Field Scoring Answer Sheet.

**Do not return your answer sheet to AFIADL.**

22. (405) The purpose of the ureters is to
  - a. drain urine from the bladder.
  - b. prevent the flow of urine from the bladder.
  - c. reabsorb nutrients from metabolism for later use by the body.
  - d. transport urine produced in the kidneys to the bladder for storage.
23. (405) As the urinary bladder begins to fill, the muscular wall
  - a. becomes firm.
  - b. starts peristalsis.
  - c. becomes thinner.
  - d. starts urinary circulation.
24. (405) The tube-like structure that drains urine from the bladder and conveys it to the outside of the body is the
  - a. urethra.
  - b. ureter.
  - c. internal sphincter.
  - d. external sphincter.
25. (405) What are the two main regions within the kidney covering, and where are they positioned?
  - a. Medulla - inner region; cortex - outermost region.
  - b. Medulla - outermost region; cortex - inner region.
  - c. Proximal convoluted tube - inner region; loop of Henle - outermost region.
  - d. Proximal convoluted tube - outermost region; loop of Henle - inner region.
26. (405) The *basic* functional unit of the kidney is the
  - a. cortex.
  - b. medulla.
  - c. nephron.
  - d. renal column.
27. (405) The combination of the glomerulus and Bowman's capsule is often called a
  - a. major calyx.
  - b. minor calyx.
  - c. capillary bed.
  - d. renal corpuscle.
28. (405) Kidney tubules are divided into three sections: the proximal and distal convoluted tubules, and the
  - a. loop of Henle.
  - b. renal corpuscle.
  - c. efferent arteriole
  - d. afferent arteriole.

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29. (405) The kidney receives its blood supply from the
- renal vein.
  - renal artery.
  - abdominal vein.
  - abdominal artery.
30. (405) Nephrons can generally be subdivided into two fluid circuits that are known as the
- distal and proximal circuits.
  - blood and the filtrate (urine) circuits.
  - afferent and efferent arteriole circuits.
  - systemic and diastolic pressure circuits.
31. (406) The two processes the kidney uses to produce urine are
- filtration and excretion.
  - secretion and excretion.
  - filtration and reabsorption.
  - secretion and reabsorption.
32. (406) As glomerular filtrate begins to move along the tubular system of the nephron, the process of reabsorption takes place in the
- tubules.
  - renal corpuscle.
  - afferent arteriole.
  - efferent arteriole.
33. (406) What kidney disorder or disease is characterized by a sudden, serious decrease in kidney function that may be fatal?
- Pyelonephritis.
  - Hydronephrosis.
  - Acute renal failure.
  - Chronic renal failure.
34. (406) Which kidney disease is the most common and usually occurs after a streptococcal infection?
- Uremia.
  - Pyelonephritis.
  - Hydronephrosis.
  - Acute glomerulonephritis.
35. (406) Which disorder results from fluid-containing sacs destroying functional kidney parts and how is it treated?
- Ptosis; surgical removal.
  - Polycystic; surgical removal.
  - Ptosis; dialysis or kidney transplant.
  - Polycystic; dialysis or kidney transplant.
36. (406) Which one of the following is an inflammation of the bladder and in whom is it most frequent?
- Cystitis; men.
  - Urethritis; men.
  - Cystitis; women.
  - Urethritis; women.

37. (407) When performing urinalysis testing, all of the following are correct *except*
- a. ensuring correct patient identification.
  - b. informing the patient about the procedure(s).
  - c. treating the urine specimen as if it was infectious.
  - d. wearing personal protective equipment when handling urine specimens.
38. (407) The *first step* toward reporting accurate and reliable urinalysis results is
- a. collecting a proper specimen.
  - b. annotating time of collection.
  - c. performing confirmatory procedures on all positive test.
  - d. measuring total urine volume on 24-hour urine specimens.
39. (407) Which specimen is generally the *most* concentrated and preferred for microscopic examination?
- a. Catheter.
  - b. Random.
  - c. First-morning.
  - d. Double-voided.
40. (407) Which specimen is *required* for measuring the total amount of solutes excreted during a day?
- a. Random.
  - b. 24-hour urine.
  - c. First-morning.
  - d. Double-voided.
41. (407) Which one of the following is *not* a type of urine specimen?
- a. Timed.
  - b. Random.
  - c. Clean-catch.
  - d. First-morning.
42. (408) To guarantee specimen integrity and suitability, inspect the specimen for *all* the following *except*
- a. adequate volume.
  - b. contamination with bacteria.
  - c. age, <2 hours old if not preserved.
  - d. correct patient information on slip and container label.
43. (408) If the urine specimen for routine analysis cannot be analyzed within 2 hours,
- a. freeze the sample.
  - b. refrigerate the sample or use chemical preservatives.
  - c. have the patient come back in 2 hours and recollect the sample.
  - d. call the health care provider for permission to discard the sample.
44. (408) Refrigeration of urine samples is adequate for *most* chemical components *except*
- a. protein and glucose.
  - b. bilirubin and glucose.
  - c. protein and urobilinogen.
  - d. bilirubin and urobilinogen.

45. (408) Refrigeration of urine samples can precipitate out
- a. uric acid crystals that obscure microscopic examinations.
  - b. uric acid crystals that obscure macroscopic examinations.
  - c. amorphous urates and/or phosphates that obscure microscopic examinations.
  - d. amorphous urates and/or phosphates that obscure macroscopic examinations.

## **Student Notes**

## Unit 3. Renal Function and Urinalysis Procedures

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UNITS 1 and 2 laid the foundation for this unit. If you have a difficult time understanding some of the principles in this unit, please review those units. However, a review of metabolism is essential before we move on. Remember that anabolism is the phase of metabolism in which the body uses energy to produce the chemical substances required for growth, repair, and maintaining all body systems. Catabolism is the breaking-down phase of metabolism. Catabolic reactions convert the energy of food into forms that can be used by the body to provide energy needed to carry on the processes of life. Metabolism includes three interdependent activities: nutrition, synthesis, and cellular respiration. Metabolic products can be sequentially derived from the catabolism of either exogenous (dietary) or endogenous (tissue) proteins, complex lipids, complex carbohydrates, nucleic acids, etc. Because the kidneys excrete waste products of metabolism, it is important to comprehend the concept of metabolism. Many of the procedures within this unit measure the analytes derived from metabolism.

### 3–1. Renal Function Procedures on Blood Specimens

It has been stated numerous times that the obvious role of glomerular filtration and tubular secretion of the kidneys is to excrete waste products of metabolism. Another equally important role of the kidneys is to maintain a constant, optimal composition of the blood. This, in turn, maintains a constant, optimal chemical composition of the interstitial (in the spaces between the tissues) and intracellular (within the cells) fluids throughout the body. Due to the kidneys' role in homeostasis, they are not only considered excretory organs but, also, regulatory organs. And, because they produce erythropoietin, renin, and prostaglandin; are a location or target for hormones produced or activated elsewhere; and are a place of degradation for other hormones (e.g., insulin and aldosterone), they are also considered endocrine organs. As you can see the kidneys affect many different systems, so it is crucial that the kidneys function properly. As laboratory technicians we help physicians diagnose deficient kidney function through the procedures we perform on blood and urine.

#### 409. Urea, creatine, and creatinine procedures

First of all, we are going to take a brief look at the tests for renal function that involve blood specimens. Because volume 1, unit 4, of this course describes blood collection, the information will not be repeated in this lesson. We will assume that the blood was collected, transported, identified, and processed appropriately. Let's begin with a general explanation of renal function tests before we discuss each analyte.

#### Renal function tests

Renal function testing and liver function testing experience many of the same problems because of the various enzyme and transport systems that coexist within each system. Some are related while

others are physically and physiologically quite separate. Like the liver, the kidney has not one but a great many functions that may or may not be affected in a given pathologic process or disease. For example, pathological processes going on in one section of the nephron may or may not directly affect those in other segments of the nephron or parts of the kidney. Unfortunately, the tests available to the clinical laboratory are inadequate for measuring the performance of each delicate network within the urinary system. To adequately measure the urinary system's performance it often requires complicated research setups or in-depth procedures.

### ***Renal evaluations***

Basically, the laboratory can only measure what passes into and out of the kidney. What goes on inside the kidney is mostly speculation and can only be measured by indirect means. It is also difficult to differentiate between localized and generalized damage, between temporary and permanent malfunction, and between primary and secondary deformity. Most function tests cannot reveal the etiology (the cause or origin of the disease or disorder) of dysfunction, but only whether or not dysfunction is present and a rough estimate of its severity. Therefore, it is vital to understand the physiologic basis for each test and its correlation with other symptoms and laboratory results. Practical renal evaluations include the nephron functions of glomerular filtration, the tubules reabsorptive capacity for water and electrolytes as manifested by their urine concentration abilities, and the kidney's ability to excrete endogenous and exogenous compounds. Because glomerular filtration is the initiating phase of all nephron functions, measurement of the glomerular filtration rate (GFR), or some variable that bears a constant relationship to it, provides the most useful general index for physicians to assess the severity and progress of renal damage. Most clinical laboratory information used to assess renal function is derived from, or related to, the measurement of the clearance of some substances from the kidneys.

### ***Clearance test***

Clearance is a theoretical concept and is defined as the volume of plasma from which a measured amount of a substance can be completely cleared (eliminated or excreted) into the urine per unit of time. The renal clearance of basically any substance can be calculated by using the following formula:

$$\text{Clearance (ml/min)} = \frac{\text{Urine concentration}}{\text{Plasma concentration}} \times \text{Timed urine volume (ml/min)}$$

Renal clearance depends on the plasma concentration and excretory rate, which, in turn, involves the GFR and renal plasma flow (RPF). Clearance of substances that are filtered exclusively or predominantly by the glomeruli but neither reabsorbed nor excreted by other regions of the nephron can be used to measure the GFR. The clearance technique, with appropriate choice of substance for the measurement, allows for quantitative estimation of the GFR, RPF, and tubular reabsorption or excretion. The primary or reference substance for measuring GFR is inulin (a naturally occurring polysaccharide and not to be confused with the hormone insulin) and *p*-aminohippuric acid (PAH) is the reference substance for measurement of RPF. PAH is a glycine amide of *p*-aminobenzoic acid (PABA) that is filtered by the renal glomeruli and excreted into urine by proximal tubules. Because PAH is almost completely cleared from the blood by the kidney tubules, its clearance is a measure of tubular excretory capacity. An amount of aminohippuric acid—*USP* preparation is injected, according to the patient's weight; blood and urine specimens are collected at specified times, and the results are plugged into the above formula. This is the basic clearance procedure used for all injected compounds. Also, other nonbiological compounds, such as phenolsulfonphthalein (PSP) and iodopyracetate (Diodrast), are excreted by the proximal tubule and have been used for the evaluation of renal tubular excretory capacity.



### ***Creatinine clearance test***

Because the necessary blood and urine assays for inulin and PAH are too difficult and too time-consuming to be practical in clinical laboratories, creatinine clearance is almost universally used for the clinical assessment of GFR. The creatinine clearance procedure includes the measurement of serum or plasma and urine creatinine. Creatinine naturally occurs in the body as a result of metabolism. Each individual has a normal value or reference range for creatinine, but, if there is kidney damage or disease, creatinine values will be higher than normal. However, the creatinine clearance test can only estimate mild to moderate diffuse glomerular damage. We will take a more in-depth look at the creatinine analyte, as well as other metabolic analytes in the next area, starting with urea.

### **Urea**

Nitrogen (N) exists in the body in many forms, especially in components of complex substances. Substances that contain nitrogen are classified into two main groups: protein nitrogen (protein substances containing nitrogen) and nonprotein nitrogen (NPN) substances. There are more than 15 different NPN compounds in plasma. Urea, the major constituent, makes up about 45% of the total NPN compounds. Other NPN compounds are creatine, creatinine, uric acid, ammonia, and amino acids. The kidneys provide a specialized mechanism for the elimination of these nitrogenous waste products.

### ***Physiology***

The utilization of proteins (in the form of amino acids) by the body's cells produces, among other things, waste materials that contain the element nitrogen. Chief among these nitrogen-containing products is urea. The formation of urea occurs primarily in the liver through hepatic enzymes. Approximately 90% of urea is excreted through the kidneys with the rest through the gastrointestinal tract or skin. Urea is neither actively reabsorbed nor excreted by the tubules but is filtered freely by the glomeruli. In a normal kidney, 40 to 70% of highly diffusible urea moves passively out of the renal tubule and into the interstitial fluid, ultimately to re-enter the blood or plasma. This back-diffusion of urea is also dependent on urine flow rate, with more entering the interstitial fluid in slow-flow states. Consequently, urea clearance test under estimates GFR and, hence, renal damage.

### ***Clinical significance***

As stated earlier, if waste products (toxic chemicals) are allowed to build up in the blood stream, all of the body's cells and systems are literally poisoned. This poisoning can lead to the death of the cell, organ, system, and the body.

### ***Increased urea levels***

Elevated serum or plasma urea and other nitrogenous compound levels (e.g., creatinine) is known as azotemia. Uremia is the term used for elevated blood urea levels. Azotemia is classified into different groups based on etiology. Prerenal azotemia results from inadequate perfusion of the kidneys or increased synthesis of urea. Renal azotemia is due to reduced glomerular filtration resulting from acute or chronic renal disease. Postrenal azotemia is from an obstruction of the urinary tract. The table below presents some of the common causes of prerenal, renal, and postrenal urea azotemia.

<b>Urea Azotemia</b>	<b>Causes</b>
Prerenal	Hypovolemic shock, hypotension, congestive heart failure (CHF), renal vein thrombosis, cirrhosis and ascites, catabolic states (fever, stress, burns, etc.), high-protein diet, GI bleeding, starvation, hyperthyroidism, Cushing's syndrome, hemolysis, and certain drugs (e.g., cortisol and tetracycline).
Renal	Glomerular disease, tubular disease, interstitial disease, and vascular damage.
Postrenal	Prostatic carcinoma, benign prostatic hyperplasia, carcinoma of the bladder or ureters, retroperitoneal tumor, and kidney stones.

Because urea production can be due to several nonrenal sources, as shown in the table, it is basically useless as a measurement of GFR except in the case of chronic renal failure. Thus, a urea measurement by itself has no clinical value. However, in conjunction with a creatinine measurement a discrimination between prerenal and postrenal azotemia can be made, as will be shown in the creatinine area.

#### *Decreased urea measurements*

Urea may be abnormally low when there is a low rate of urea production; hemodilution (an increase in fluid content and a decrease concentration of erythrocytes in the blood); or increased rate of urea excretion.

Condition	Causes
Low rate of urea production	Low protein intake, administration of androgens or growth hormone, and severe liver dysfunction.
Hemodilution	Overhydration, psychogenic polydipsia, and diabetes insipidus.
Increased rate of urea excretion	Hemodilution, pregnancy, and postdialysis states.

#### *Laboratory procedures and limitations*

Although blood urea nitrogen (BUN) continues to be the terminology used for ordering a serum or plasma urea nitrogen procedure, analysis of whole blood for urea is obsolete. Therefore, plasma or serum is the specimen of choice for urea measurements. Urine and other biological samples can also be used. If plasma is required, the blood sample must be collected using the appropriate anticoagulant. Anticoagulants containing nitrogen (e.g., ammonium salts of heparin) must not be used, because they can falsely elevate urea values. If an enzymatic method is used for the measurement of urea, the blood cannot be collected in sodium fluoride tubes because fluoride inhibits enzymes. For all urea procedures, the serum or plasma should be separated from the clot or cells within 1 hour. Laboratory procedures for urea include indirect and direct methods.

#### *Indirect methods*

Indirect methods are based on preliminary hydrolysis of urea with urease followed by some process that quantitates the ammonium ion. Spectrophotometric methods include the Berthelot's reaction and an enzymatic assay using glutamate dehydrogenase. In the Berthelot's reaction, urea is hydrolyzed with urease to carbonic acid and ammonia. Sodium phenate and hypochlorite react with ammonia in the presence of sodium nitroprusside to produce a blue indophenol complex with a maximum absorbance at 630 nm. The limitations or disadvantages of this method include nonspecificity and long reaction times. In the enzymatic assay, the highly specific urease enzyme converts urea into ammonia, which is coupled with a reaction utilizing glutamate dehydrogenase with NADH (reduced form of nicotinamide adenine dinucleotide) and  $\alpha$ -ketoglutarate. This reaction is monitored by measuring the decrease in absorbance at 340 nm as NADH is converted to  $\text{NAD}^+$  (oxidized form of nicotinamide adenine dinucleotide). In this method, interferences may be seen with other enzymes that are elevated in serum and can also oxidize NADH. Electrochemical approaches that quantitate ammonium ions are conductimetry and potentiometry.

#### *Direct methods*

Direct methods are usually variations of the Fearon reaction: the condensation of diacetyl with urea to form the chromogen diazine. In this method, the diacetyl complexes with urea in an acid solution containing an oxidizing agent to form a yellow diazine compound (Fearon reaction), which can be measured at 550 nm, or fluorometrically at 415 nm. The advantage of this method is that it is easily automated. Because nonspecific substances may also produce chromogenes, it has a lower specificity than enzymatic assays.

## Creatine and creatinine

As mentioned above, creatinine can also be used to estimate renal function and should be measured in conjunction with urea. Let's look at its physiology.

### Physiology

Creatine is synthesized in the kidneys, liver, and pancreas by two enzymatically mediated reactions. The blood transports creatine throughout the body, especially to the muscles and brain. In the muscles, it combines with phosphorous as phosphate to form the high-energy compound phosphocreatine. Interconversion of phosphocreatine and creatine is a special feature of the metabolic processes of muscle contraction. Some of the free creatine in the muscles spontaneously converts to creatinine, an anhydride of creatine. Between 1 and 2% of muscle creatine is converted to creatinine on a daily basis. Because the amount of endogenous creatinine produced is proportional to muscle mass, normal creatinine levels vary with age and sex (male versus female). Dietary (exogenous) intake of creatinine causes only minor variations in daily creatinine excretion. Therefore, an individual's creatinine excretion rate is relatively constant and parallels endogenous production. Because its plasma levels are maintained within narrow limits, its clearance may be an indicator of GFR.

### Clinical significance

Like urea, creatinine is a result of metabolism and will provide an estimate of renal function. Unlike urea, creatinine levels are not affected by diet and remain constant. Both urea and creatinine can cause azotemia. The table below displays some of the common causes of prerenal, renal, and postrenal creatinine azotemia.

<b>Creatinine Azotemia</b>	<b>Causes</b>
Prerenal	Hypovolemic shock, hypotension, congestive heart failure (CHF), cirrhosis and ascites, muscle hypertrophy, muscle necrosis, anabolic steroid use, severe exercise, and phenacetide a drug.
Renal	Drugs (e.g., cimetidine, trimethoprim, probenecid), glomerular disease, tubular disease, interstitial disease, and vascular damage.
Postrenal	Benign prostatic hyperplasia, carcinoma of the prostate, bladder, or uterus, retroperitoneal tumor, and kidney stones.

As stated above, creatinine levels and urea levels should be determined together. A urea/creatinine ratio can be calculated, using the formula below, and can help physicians distinguish between prerenal and postrenal conditions.

$$\frac{\text{Serum urea concentration (mg/dl)}}{\text{Serum creatinine concentration (mg/dl)}}$$

For a normal individual the reference interval for the urea/creatinine ratio ranges between 12 and 20. Lower ratios usually indicate acute tubular necrosis, low protein intake, starvation, or severe liver disease (decreased urea synthesis). A high ratio with a normal creatinine level denotes prerenal azotemia or high protein intake. A high ratio with an elevated creatinine level is associated with postrenal obstruction or prerenal azotemia consequential to renal disease. The urea/creatinine ratio is only a tool or guide and its usefulness depends on the accuracy of the urea and creatinine determinations.

### Laboratory procedures and limitations

Serum, plasma, or diluted urine can be used for creatinine determinations. Serum or plasma must be separated from the clot or cells in order to prevent hemolysis. Hemolysis causes falsely elevated creatinine due to the release of noncreatinine chromogens from the cells. In serum and plasma, creatinine is stable for up to 1 week if stored at 2 to 8°C. As with urea procedures, if plasma is to be tested, do not use an ammonia heparin anticoagulant.

### *Spectrometry*

The methods most widely used today are based on the Jaffé reaction, first described in 1886. The reaction occurs between creatinine and the picrate ion formed in an alkaline medium. This reaction produces a red-orange complex, but despite extensive study, its structure remains uncertain. Concentration of alkali in the reagent system is critical. In most methods wavelengths of 505 to 520 nm are specified for spectrophotometry and a narrow-bandpass spectrophotometer is required to provide appropriate analytical sensitivity and specificity. The Jaffé reaction is nonspecific because of the noncreatinine, Jaffé-reacting chromogens found in the specimens: protein, glucose, ascorbic acid, guanidine, acetone, cephalosporins, and  $\alpha$ -keto acids. Depending on the method, these quasi-creatinine compounds cause a falsely elevated result and overestimates the true plasma creatinine level. Numerous modifications to the Jaffé method have improved the specificity for creatinine in plasma. One approach for eliminating interferences is to use an acid blank in the Jaffé procedure.

### *Enzymatic or partially enzymatic assays*

Enzymatic assays have also been around for awhile. The two enzymes of particular interest are (1) creatininase (creatinine iminohydrolase or deiminase) which catalyzes creatinine degradation to *N*-methylhydantoin and ammonium ion; and (2) creatinine hydrolase (creatinine amidohydrolase) which catalyzes the hydrolysis of creatinine to creatine. Creatininase is sometimes used in conjunction with an ammonium ion-selective electrode or with indicator dyes for the quantitation of ammonium. In one completely enzymatic assay creatinine is hydrolyzed to creatine, which is then measured using creatine kinase (CK).

### *Definitive method*

The definitive method for serum creatinine involves isotope-dilution mass spectrometry. A candidate reference method for creatinine uses isocratic ion-exchange HPLC with ultraviolet detection at 234 nm.

### *Creatine*

Creatine can be measured, but because it constitutes only a small fraction of the total NPN in plasma and urine, it has little clinical value for evaluating renal disease. Creatine is unstable at both acid and alkaline pH and rapidly undergoes conversion to creatinine. Creatine methods include (1) the condensation of creatine with diacetyl-l-naphthol to form a pink product; (2) the use of creatine kinase enzyme with a fluorometric assay; (3) the use of guanidine with ninhydrin in an alkaline solution to produce a fluorescent product, and (4) converting creatine to creatinine through heating the sample with acid in the presence of a heavy metal. Creatine is calculated using before and after procedure creatinine levels. The first 3 methods directly measure creatine and the 4<sup>th</sup> method measures creatine indirectly.

## **410. Uric acid, ammonia, and amino acid procedures**

Uric acid, ammonia, and amino acid procedures can also be considered renal function tests, although renal disease is usually a secondary affect from the disorders that cause abnormalities in these constituents.

### **Uric acid**

In humans, uric acid is the major product of the catabolism of purine nucleosides (i.e., adenosine and guanosine). Purines from catabolism of dietary nucleic acid are converted to uric acid directly. However, the bulk of purines ultimately excreted as uric acid in the urine originates from degradation of endogenous nucleic acids.

### *Physiology*

Uric acid production occurs primarily in liver and intestinal mucosa. Approximately 40% of daily uric acid formation comes from dietary nucleoproteins. The majority of uric acid in the body exists as

the urate ion. The only effective route for uric acid excretion is through the kidneys. Renal handling of uric acid is complex and involves 4 sequential steps: (1) glomerular filtration of virtually all the uric acid in capillary plasma entering the glomerulus; (2) reabsorption in the proximal convoluted tubule of about 98 to 100% of filtered uric acid; (3) subsequent excretion of uric acid into the lumen (organ cavity or tubule channel) in the distal portion of the proximal tubule; and (4) further reabsorption in the distal tubule. The net urinary excretion of uric acid is only 6 to 12% of the amount filtered.

### ***Clinical significance***

*Hyperuricemia* denotes elevated levels of uric acid in serum or plasma. The major causes of hyperuricemia are shown in the table below.

<b>Cause</b>	<b>Results</b>
Increased uric acid synthesis	Gout; intake of purine rich diet (organ meats); tissue catabolism (necrosis, radiation, chemotherapy, etc.); myeloproliferative and lymphoproliferative disorders; or Lesh-Nyhan syndrome.
Decreased renal excretion of uric acid	Renal failure; drugs (diuretics, small-dose aspirin); gout; metabolic acidosis; toxemia; or pregnancy.
Miscellaneous causes	Ethanol and methanol intoxication; endocrine disorders (hyperparathyroidism); hypertension; or psoriasis.

Increased nucleic acid turnover and consequent increase in catabolism of purines may be encountered in rapid proliferation of tumor cells or in massive destruction of tumor cells due to certain chemotherapeutic agents. Hyperuricemia is also attributable to primary defects of enzymes in the pathways of purine metabolism. We are going to look specifically at gout and renal function.

### ***Gout***

Gout is a disorder of purine metabolism or renal excretion. It occurs when monosodium urate precipitates from supersaturated body fluids, deposits in and around joints, bursae, periarticular cartilage, bone, and subcutaneous tissue. Usually single, specific joints of the extremities are involved. The symptoms can range from asymptomatic to a painful arthritis to severe crippling. The clinical signs of gouty arthritis are due to the urate crystals present in joint fluid, as well as the deposits of crystals (tophi) in the tissues surrounding the joint. The deposits may occur in other soft tissues as well, and wherever they occur they elicit an intense inflammatory response consisting of polymorphonuclear leukocytes and macrophages. Gout is more common in males (90% of all cases) and is uncommon in pre-menopausal females. It may be classified as primary or secondary. Primary gout is associated with essential hyperuricemia (i.e., due to metabolic overproduction of purines or to under-excretion of uric acid). Secondary gout is a result of hyperuricemia attributable to several other identifiable causes shown in the table above.

### ***Renal disease associated with hyperuricemia***

Renal disease associated with hyperuricemia may take one or more of several forms: (1) gouty nephropathy with urate deposition in renal parenchyma; (2) acute intratubular deposition of urate crystals; and (3) urate nephrolithiasis. Renal retention of uric acid may occur in any type of acute or chronic renal disease, or as a consequence of the administration of drugs (diuretics). Organic acidemia, due to increased acetoacetic acid in diabetic ketoacidosis or to lactic acidosis, may interfere with tubular excretion of urate. Medical treatment of sustained asymptomatic hyperuricemia is justified in order to prevent urate-induced renal damage.

### ***Hypouricemia***

Hypouricemia is defined as an abnormally low plasma uric acid. It is much less common than hyperuricemia. It can be seen in severe liver disease with decreased synthesis of purines. It's also

seen in hereditary deficiency of xanthine oxidase, administration of uricosuric drugs, and a defect in renal tubular reabsorption of uric acid (Fanconi's syndrome).

#### ***Laboratory procedures and limitations***

Serum, plasma, and urine can be used for uric acid testing. Current methods for determining uric acid fall into two groups: phosphotungstic acid methods (spectrometric) and uricase methods (enzymatic).

##### ***Phosphotungstic acid***

Phosphotungstic acid (PTA) methods rely on the development of a blue reaction (tungsten blue) as PTA is reduced by urate in an alkaline medium. The color is read spectrophotometrically at wavelengths of 650 to 700 nm. This reaction is nonspecific. Interference can be seen from glucose, ascorbic acid, glutathione, cysteine, and drugs (e.g., aspirin, acetaminophen, caffeine, and theophylline) that result in falsely elevated levels.

##### ***Uricase***

Uricase methods are essentially more specific because they have, either as a single step or as the initial step, urate oxidation catalyzed by the enzyme uricase. It has greater specificity and is more accurate than the PTA method. Uricase methods became feasible and popular as a result of availability of high-quality, low-cost preparations of the uricase bacterial enzyme.

##### ***Definitive methods***

HPLC methods using reversed-phase columns have also been used to measure uric acid, but the definitive method uses gas chromatography/mass spectrometry (GC/MS).

#### **Ammonia**

The major source of circulating ammonia is the gastrointestinal tract because of the bacterial action on amino acids. The hepatic portal vein carries blood rich in absorbed nutrients (proteins in the form of amino acids, etc.) from the intestines to the liver. After protein hydrolysis (splitting the compound into amino acids by the addition of water), the amino groups of the amino acids are converted to ammonia by oxidative deamination (removal of the amino group). Ammonia is converted to urea and, as stated earlier, urea is the nitrogenous end product of protein catabolism.

#### ***Physiology***

Ammonia is carried by the portal vein to the liver where it is converted to urea and, as mentioned above, is derived from the action of bacterial proteases, ureases, and amine oxidases on the protein contents of the colon. Portal vein plasma ammonia concentration is typically 5 to 10-fold higher than that in the general circulation. Under normal circumstances, most of the portal vein ammonia load is metabolized to urea in hepatocytes of the liver during the Krebs-Henseleit urea cycle. The ammonia enters the cycle by enzymatic conversion in the liver where it leaves the cycle as urea.

#### ***Clinical significance***

A number of animal and human studies have shown that excess ammonia has toxic effects on the central nervous system. Adequate conversion of ammonia to urea is extremely important because of this toxicity. Increased ammonia blood levels is known as hyperammonemia. Inherited enzyme deficiencies of the urea cycle is a major cause of hyperammonemia in infants. Inherited metabolic disorders involving the metabolism lysine and ornithine, and those involving the metabolism of organic acids, also produce hyperammonemia. Acquired hyperammonemia is commonly encountered in advanced liver disease. Severe liver failure whether acute (e.g., fulminate viral hepatitis) or chronic (e.g., cirrhosis) and altered blood flow to the liver can significantly impair normal ammonia metabolism. Hepatic encephalopathy can be a result of gastrointestinal bleeding, which enhances ammonia production by bacterial metabolism of the blood proteins in the colon and subsequently increases blood ammonia levels. Encephalopathy is any degenerative disease of the brain; and hepatic encephalopathy can lead to a coma. Impaired renal function may also accompany severe liver disease.

As urine output decreases, blood urea concentration increases, leading to increased excretion of urea into the intestine, where it is converted back to ammonia.

### ***Laboratory procedures and limitations***

Because ammonia procedures must be performed immediately after the venipuncture, plasma is the specimen of choice. EDTA and heparin (without ammonia salts) are acceptable anticoagulants. The blood collection must not be traumatic and the specimen must be placed immediately on ice to prevent in vitro metabolism of nitrogenous compounds. Methods for measuring ammonia can be divided into two major classes: two-stage procedures and one-stage procedures.

#### *Two-stage procedures*

The Conway technique is a two-stage procedure. The specimen is alkalinized in a closed system to convert all ammonium ions to ammonia, which is then trapped in an acid medium in a second chamber of a diffusion cell. Trapped ammonia may then be analyzed spectrophotometrically or back-titrated with a standard acid. Another two-stage procedure is flow-injection analysis. With this technique, an aliquot of the sample is injected into a flowing stream of alkaline solution from which the ammonia diffuses through a membrane into a pH-sensitive indicator stream. This method is fast, requires a minimal amount of specimen, and carries minimal risk of ambient ammonia contamination, but it requires dedicated equipment. In another two-stage assay, a cation-exchange resin is used to remove ammonium ions from its matrix, and then the ammonium ions are eluted from the resin. The eluate is reacted with Nessler's reagent or Berthelot's reagent prior to spectrophotometric quantitation.

#### *One-stage procedures*

One-stage procedures don't separate ammonium ions from the specimen before the analytical step. The enzymatic assay used in these procedures is generally based on the reaction as described by Mondzac and co-workers in 1965 and later modified by Van Anken and Schiphorst. In the presence of the enzyme glutamate dehydrogenase a change in absorbance at 340 nm is measured, as NADPH (the reduced form of nicotinamide-adenine dinucleotide phosphate) is transformed to NADP<sup>+</sup> (the oxidized form of nicotinamide-adenine dinucleotide phosphate). NADH may also be used as a substrate for glutamate dehydrogenase. However, falsely elevated results occur because NADH is also a substrate for many other serum enzymes. Using NADPH prevents this disadvantage. This method is also fast, doesn't require special equipment, and is readily automated. Direct methods using ammonia-selective electrode are also available but have not achieved widespread use at this time.

#### *Limitations*

To obtain accurate and reliable ammonia levels, the procedure must be carefully monitored and meticulously performed. Compliance with the following guidelines must be accomplished to avoid falsely elevated results.

1. Smoking. Smoking can cause ammonia contamination to both the patient and specimen. Therefore, the patient must refrain from smoking after midnight and put on fresh pajamas before the fasting blood specimen is drawn. If the patient is a heavy smoker, they should shower before the venipuncture. Also, the technician performing the test should be a nonsmoker.
2. Laboratory atmosphere. The laboratory atmosphere is also a source of ammonia contamination. Special precautions include performing the procedure in a designated area with restricted access and using chemically clean glassware.
3. Poor venipuncture. Using a heparin lock, probing for a vein, drawing blood into a syringe and transferring it to an anticoagulated tube, or partial filling of the evacuated tube can cause falsely elevated ammonia results.

4. Metabolism of nitrogenous constituents. Metabolism of nitrogenous constituents may occur once the specimen has been collected. The specimen must be put on ice and centrifuged and analyzed immediately. A delay exceeding 15 minutes between the venipuncture and centrifugation has been shown to increase ammonia concentrations even if the specimen is kept at 0°C.

### **Amino acids**

If you recall from your earlier studies, amino acids are organic compounds containing an amino ( $-\text{NH}_2$ ) and a carboxyl ( $-\text{COOH}$ ) group. Amino acids are the building blocks for proteins. Incredibly, it takes only 20 amino acids to build the large number of biologically active peptides (low molecular weight constituents of proteins) and proteins that exist in nature. There are 9 essential amino acids that are required for protein synthesis that cannot be synthesized by our bodies and must be obtained from our diet. They are histidine, isoleucine, leucine, lysine, methionine, phenylalanine, threonine, tryptophan, and valine. These are considered essential because they must be consumed in our diet for the maintenance of our health and/or growth. The 11 nonessential amino acids required for protein synthesis that are synthesized by our bodies and not specifically required in our diet are alanine, arginine, asparagine, aspartic acid, cysteine, glutamic acid, glutamine, glycine, proline, serine, and tryosine. Also, there are at least 150 other nonprotein amino acids found in nature. We are going to briefly look at the amino acids as a group, but specifically look at phenylalanine as it pertains to phenylketonuria (PKU).

### **Physiology**

Normal amino acid metabolism starts with proteolytic enzymes in the gastrointestinal tract that act on ingested proteins. Amino acids are released, then absorbed from the jejunum of the small intestines into the blood where they become part of the body pool of amino acids. The liver and other tissues draw on this pool for synthesis of plasma and intracellular proteins. The liver and kidneys are also actively involved in interconverting amino acids, by removing an amino group and forming a new keto acid and an amino acid. The amino acid formed is deaminated to produce ammonium ions, which are rapidly consumed in the synthesis of urea. Urea is then excreted by the kidneys.

### **Clinical significance**

Amino acids in the blood are filtered through the glomeruli and normally reabsorbed in the proximal tubules via specialized transport systems. High blood levels of amino acids result in significant renal excretion and, hence high urine amino acid levels. This condition is known as aminoaciduria. Abnormal concentration of amino acids in the plasma is known as aminoacidemia.

### ***Aminoaciduria and aminoacidemia***

In normal individuals, aminoaciduria is transient and correlates with protein intake above the amino acid requirements for restoring the body pool. Aminoaciduria and aminoacidemia are either primary or secondary depending on the nature of the underlying defect. Primary disease is due to an inherited enzyme defect, also called an inborn error of metabolism. These defects are in a specific or single amino acid metabolic pathway or in the specific renal tubular transport system where the amino acid is reabsorbed. Secondary disease affects different or many amino acids at the same time and is due to a disease of an organ (e.g., liver); generalized renal tubular dysfunction; or to protein-energy malnutrition.

### ***Phenylketonuria***

Phenylketonuria is a primary aminoaciduria and aminoacidemia disease. According to *Dorland's* phenylketonuria is the most severe manifestation of hyperphenylalaninemia due to phenylalanine 4-monooxygenase deficiency, with accumulation and excretion of phenylalanine, phenylpyruvic acid, and related compounds and inherited as an autosomal recessive trait; it is characterized by severe mental retardation, tumors, seizures, hypopigmentation of hair and skin, eczema, and a mousy odor,



all preventable by early restriction of dietary phenylalanine. In other words, PKU is due to a deficiency of a liver enzyme known as phenylalanine hydroxylase. This enzyme is required to convert phenylalanine to tyrosine. With its major utilization pathway blocked, phenylalanine accumulates in the blood. Abnormal levels lead to progressive mental retardation. However, if detected within the first few weeks or months after birth, the infant can be treated with a life-time dietary restriction of phenylalanine. This early detection can prevent mental deficiency or retardation.

### ***Laboratory procedures and limitations***

The most common specimens for amino acid evaluation are serum or plasma, urine, and CSF. Because plasma amino acid concentrations are higher in the afternoon and lower in the morning and vary during the day by about 30%, blood specimens should be collected at the same time each day. Amino acid excretion in urine varies with age due to maturation of the renal tubular function.

### ***Neonatal screening***

Genetic screening of newborns is commonly practiced in order to make the earliest possible diagnosis of potentially treatable inherited aminoaciduria and aminoacidemia. Most screening programs have been directed at early detection of PKU. One of the most widely used test for PKU screening is the Guthrie test. Since its introduction in 1961, the test methodology has been applied to the diagnosis of some of the other, rarer aminoacidurias or aminoacidemias. The Guthrie test is a semi-quantitative microbiological assay. Bacterial spores, usually *Bacillus subtilis*, are incorporated into an agar medium. A phenylalanine competitive growth inhibitor specific for phenylalanine is added to the medium. The patient sample, whole blood or urine, is collected onto a piece of soft filter paper. A standardized size or portion of the filter paper is punched out and laid on the agar surface. The plate is incubated and observed for bacterial growth. In the presence of elevated phenylalanine concentrations, the effect of the growth inhibitor is diminished or overcome, and zones of bacterial growth are seen. The Guthrie test method is designed to show growth only when the concentration of the amino acid of interest exceeds its upper reference limits.

**NOTE:** Proper neonatal specimen collection is critical for the Guthrie test. The spots on the filter paper must be filled completely on the front and the back. Clots and other biological substance should not be present on the filter paper. An automated chemical method is being used in some neonatal screening laboratories. It is based on the reaction of phenylalanine with alkaline ninhydrin, enhanced by the addition of the dipeptide L-leucyl-L-alanine. Copper is added after a 90°C incubation, and the product is measured by fluorescence spectroscopy.

### ***Other procedures***

Since hyperphenylalanine is not diagnostic of PKU, and because long-term diet restriction of phenylalanine can be harmful to some individuals without PKU, a positive Guthrie test must be confirmed by a more definitive method. These procedures include thin-layer chromatography, ion-exchange or high-performance liquid chromatography, and gas chromatography/mass spectrometry.

**NOTE:** This CDC is not meant to be an exhaustive review of renal function, but rather highlights clinical symptoms and laboratory procedures which help to distinguish between renal dysfunction and metabolic disorders. Although particular laboratory tests and approaches were discussed, detailed procedures were not given. If you would like more information on these methods, refer to the bibliography in this CDC.

### Self-Test Questions

After you complete these questions, you may check your answers at the end of the unit.

#### 409. Urea, creatine, and creatinine procedures

1. What are some of the problems with renal function testing?
2. What can the laboratory basically measure for renal evaluations?
3. What do practical renal evaluations include?
4. *Most* clinical laboratory information used to assess renal function is derived from what measurement?
5. On what does renal clearance depend?
6. What are the primary or reference substances used for measuring GFR and RPF respectively?
7. Why is the creatinine clearance test used in most clinical laboratories?
8. The creatinine clearance procedure includes what type of measurements?
9. What are the two main groups of nitrogen containing substances?
10. What are the NPN compounds?
11. From what and how is urea produced?
12. What is the result of waste product build-up?

13. What are the three classifications of azotemia?
14. What are the two laboratory procedures for urea and briefly describe each?
15. Where and how is creatine synthesized?
16. Why is an individual's creatinine excretion rate relatively constant?
17. Why does hemolysis cause falsely elevated creatinine results?
18. Creatinine methods are based on what reaction and what is used to improve the specificity of this method?
19. What are the two enzymes used in creatinine enzymatic assays?
20. What is the definitive method for measuring creatinine?

**410. Uric acid, ammonia, and amino acid procedures**

1. What is uric acid and where is it primarily produced?
2. What are the major causes of hyperuricemia?
3. What is gout and how does it occur?
4. What are the forms of renal disease associated with hyperuricemia?
5. Why is medical treatment justified in asymptomatic hyperuricemia?

6. Hypouricemia can be seen in what disorders?
7. What are the two groups of uric acid testing methods?
8. What is the definitive method for measuring uric acid?
9. What is the major source of circulating ammonia and what is it a product of?
10. What effect does excess ammonia have and is a major cause of hyperammonemia in infants?
11. Why is plasma the specimen of choice for ammonia procedures and which anticoagulants should be used?
12. Why is the specimen immediately placed on ice?
13. What can cause falsely elevated ammonia results?
14. What are amino acids and why are they important?
15. What is the cause of primary aminoaciduria and aminoacidemia?
16. What is phenylketonuria (PKU)?
17. How is PKU characterized?
18. How are these characterizations prevented?
19. What is the most widely used test for PKU screening and describe it?

20. How is a positive Guthrie test confirmed?

## 3-2. Renal Function Procedures on Urine Specimens

As stated earlier, no single body system is more important than the other and, in fact, they are interdependent. Likewise, analytes found in the blood and those in urine are related, whether they are considered normal or abnormal constituents. Because the physiology of most of these analytes has been discussed in the section above or in volume 2 of this course, just a brief review will be given in the following lessons. It may be helpful to review volume 2 before you begin this study on urine chemistry procedures in order to review the characteristics of the constituents found in urine.

### 411. Urine specimen processing and physical characteristics of urine

Along the way in this study we have discussed the urinary system and renal function, now it's time to look at their end product—urine. In every body fluid there are normal and abnormal constituents with standard levels for normal constituents. Urinalysis is the physical, chemical, and microscopic examination or analysis of urine. Let's look at the normal constituents of urine and then move on to its examination.

#### Normal constituents

We know how urine is produced and eliminated, but what is urine made of, or what is normally found in urine? During the production of urine, some chemical elements and compounds (analytes or constituents) are filtered and then reabsorbed, so what's left? Urine consists of 96% water and 4% dissolved solutes or substances. The dissolved substances are either derived from the food we have eaten or are the waste products of metabolism. The dissolved substance is made up of approximately 23% urea, 11% sodium chloride, 2.3% phosphoric acid, 1.3% sulfuric acid, and 0.5% uric acid. The rest, or 66%, is made up of small or trace amounts of hippuric acid, creatinine, ammonium, potassium, calcium, oxalate, phosphate, amines, amino acids, proteins, enzymes, purines, leukomaines, urobilin, sugars, cholesterol, hormones, fatty acids, vitamins, and metals. Urine also normally contains a few un-dissolved or formed elements which include epithelial cells, red and white blood cells, and a rare hyaline cast. Normal urine composition can vary a great deal and still remain within normal limits. Its composition depends on such factors as diet and nutrition, metabolism, and the general state or health of the body.

#### Specimen processing

Before we begin with the urinalysis, let's talk about specimen processing. As you can see there are a lot of different constituents found in urine. To obtain accurate results it is important for the urine to be mixed thoroughly before any procedures are done. If the specimen has been refrigerated it should warm-up to room temperature and if frozen, it must be completely thawed and thoroughly mixed.

#### Physical characteristics

The physical characteristics of urine include volume, color, appearance, odor, specific gravity, refractive index, osmolality, and pH. Let's briefly look at these characteristics.

#### *Urine volume*

Routine urinalysis procedures do not call for a volume measurement. However, the total amount of urine excreted in a 24-hour period is required for the calculation or formula of quantitative procedures. The total urine volume varies with diet, body size, fluid intake, fluid loss, and with the ingestion of diuretics (e.g., caffeine). For adolescents and adults, the average total daily volume is 1,200 to 1,500 ml with a range from 500 ml to 2 liters. This is considerably low when it is estimated

that 180 liters of fluid is filtered through the kidneys in a 24-hour period. Below are terms that describe abnormalities in urine volume and some of the possible causes.

### *Polyuria*

If the total urine volume is more than 2 liters in a 24-hour period the condition is called polyuria. Polyuria may be a physiologic response to increased fluid intake; ingestion of diuretic medications or liquids; chilling of the body; nervousness and anxiety; or the intravenous infusion of fluids. Polyuria also occurs in several disease conditions, particularly in diabetes mellitus and diabetes insipidus. It is a symptom of chronic renal disease and has been noted in patients with acromegaly, myxedema, and certain tumors of the brain and spinal cord. Polyuria may indicate the loss of concentrating ability by the kidneys.

### *Oliguria*

Oliguria is decreased urinary output of less than 500 ml in a 24-hour period. Physiologic forms of oliguria occur with decreased fluid intake, increased ingestion of salt, and excessive sweating. Oliguria occurs when there is excessive loss of body fluids as in vomiting, fever, bleeding hemorrhoids, and diarrhea.

### *Anuria*

The extreme form in which there is total lack of urine is called anuria. This is generally due to complete renal shutdown because of inflammation, poisoning, or cardiac failure.

### **Color and appearance**

Urine color and appearance varies considerably in healthy and disease states.

### *Color*

Normally, urine is some shade of yellow: almost colorless, yellow-green, straw, pale yellow, bright yellow, yellow-orange, light amber, or dark amber. Your laboratory should decide what terms signify a normal urine color; for instance using the terms *straw*, *yellow*, and *amber*. Variations in body metabolism and certain vegetable pigments from the diet can alter these colors, as well as certain drugs and pathological conditions. The normal color of urine results from the presence of three different pigments: urochrome, uroerythrin, and urobilin. The table below is a review of some of the more common colors of urine and the possible cause(s).

<b>Color</b>	<b>Possible cause(s)</b>	<b>Possible clinical condition</b>	<b>Disease association</b>
Yellow (straw and amber)	Urochrome (the chief pigment in normal urine) uroerythrin, and urobilin	Normal	None
Pale yellow to colorless	Dilute urine, low concentration of normal constituents	Polyuria	Diabetes mellitus, diabetes insipidus, chronic renal failure
Dark yellow to brown-red (amber)	Concentrated urine, food color, carotene, rhubarb	Oliguria, jaundice	Liver disease, dehydration, fever
Yellow-brown or yellow-green	Bilirubin or biliverdin	Jaundice	Liver disease, biliary obstruction
Orange-red or orange-brown	Urobilin	Jaundice	Liver disease
Bright orange	Phenazopyridine or Pyridium	Aminopyrine drugs	Treatment for urinary tract infections
Clear red	Hemoglobin	Increased red cell destruction	Hemolytic transfusion reaction, hemolytic anemia, G6PD deficiency, paroxysmal nocturnal

Color	Possible cause(s)	Possible clinical condition	Disease association
			hemoglobinuria, certain infections and drugs
Cloudy red	Red blood cell	Urinary system damage, menstruation	Tumors, trauma, glomerulonephritis, pyelonephritis, and renal calculi (stones)
Dark red or purple-red	Porphyrins, beets, food color, rhubarb	None	None
Clear dark red-brown	Myoglobin—a form of hemoglobin found in the muscles	Extensive muscle injury	Trauma or extreme exercise
Dark brown and black	Melanin, homogentisic acid, phenol poisoning	Urine is colorless when voided but upon standing turns black, results from serious conditions	Melanoma (tumor), alkaptonuria
Green, blue, orange	Drugs, medications, vitamins, and foodstuff	None	None

Generally, the deeper the color (darker) of urine the more concentrated it is; and the lighter it is, the less concentrated.

### *Appearance*

The transparency or appearance of urine is described as clear, hazy, cloudy, and turbid. *Clear* is defined as no visible particulate matter present. *Hazy* means some visible particulate matter is seen. *Cloudy* is the term used to signify that visible particulate matter is present and newspaper print is blurry if viewed through the specimen. *Turbid* denotes particulate matter present and newspaper print *cannot* be seen through the specimen. The appearance of urine is due to the presence of mucus, phosphates, bacteria, urates, pus, blood, fat, spermatozoa, casts, and/or crystals.

### *Foam*

Foam may also be seen in urinary specimens. A small amount of white-colored foam is normal. However, if the foam is abundant, similar to beaten egg whites, it is likely that protein (albumin) is present. A large amount of vivid yellow foam may indicate the presence of bilirubin or bile pigments.

### *Odor*

Urine definitely has a distinctive odor, even though it is difficult to define or describe. Although odor is usually not reported, the odor of the urine can indicate certain disease processes. Upon standing, urine acquires a strong ammoniacal odor due to the breakdown of urea to ammonia by the bacteria invariably present in the specimen. Foul- or putrid-smelling urine may indicate the presence of bacteria as a result of a urinary tract infection (UTI). A fruity- or sweet-smelling urine is associated with diabetes mellitus when ketones are present. The urine from patients with an amino acid metabolism error may have sweaty feet, cabbage or hops, mousy, rotting fish, maple syrup, or rancid odor. Food can also give urine a characteristic odor, such as after eating asparagus.

### *Specific gravity*

Specific gravity is the weight of a substance compared with that of an equal volume of another substance taken as the standard (i.e., water). More specifically, it is the ratio of density (weight per unit volume) of a solution compared to the density (weight per unit volume) of an equal volume of water at a constant temperature. From this definition, it is clear that the specific gravity of water is always 1.000. Since it is a ratio, specific gravity has no units. Specific gravity measures the density (amount) of dissolved solid substances present in urine or other solutions. The specific gravity of

urine is a useful indicator of the ability of the renal tubules to concentrate or dilute urine. The specific gravity of urine was traditionally measured with a urinometer. The urinometer is a glass float weighted with mercury. It is calibrated to float at the 1.000 graduation in deionized or distilled water when placed in a glass urinometer cylinder. The specific gravity of the urine is read directly from the graduated scale in the urinometer stem. A solution of potassium sulfate can be prepared with a specific gravity of 1.015 and used as control.

### ***Refractive index***

Refractive index is another measurement of solute concentration and correlates closely with the specific gravity. The refractive index of a solution is the ratio of the velocity of light in air to the velocity of light in solution. It is measured by a refractometer which is calibrated to give results in terms of specific gravity. The refractometry procedure requires that a drop of urine be placed in the appropriate spot and then the instrument held to a light source. The refractive index is read directly from the calibrated scale located in the eyepiece. Controls for the refractometer include deionized or distilled water and a known urine control.

### ***Osmolality***

Osmolality also measures solute concentration in urine. In volume 1, unit 6, it is defined as the particles dissolved in a solution. In other words, it is the measurement of the number of solute particles per unit amount of solvent and it is dependent only on the number of particles in solution. The measurement of urine osmolality is a valuable test when estimating the kidney's concentrating ability, as in the case of possible tubular disease or in the calculation of free water clearance. In volume 1, unit 6, the various types of procedures used to measure osmolality are described.

### ***pH***

The symbol *pH* relates to the hydrogen ion ( $H^+$ ) concentration or activity of a solution compared to a given standard solution. Numerically the pH is approximately equal to the negative logarithm of  $H^+$  concentration expressed in molarity. On a scale from 0.0 to 14.0, pH 7.0 is neutral; above 7.0 is considered alkaline; and below 7.0 is considered acidic.

## **412. Urinalysis—routine chemical procedures**

Routinely, chemical analysis of urine is performed by using one or more of the different dipsticks or reagent strips manufactured by various companies. These reagent strips consist of a plastic strip that contains one or more chemically impregnated reaction pads. A color reaction develops upon contact of the urine with the reagent pads. Reagent strips are a simple and fast way to semi-quantitatively test urine.

### **Reagent strips**

A 20<sup>th</sup> century invention, the urine reagent strip has all but replaced complicated individual chemical procedures for the determination of various analytes found in urine. The reaction or reagent pads contain the reagents required for the detection, reaction, and estimation of the analyte, and usually a buffer and other nonreactive components or chemicals.

### ***General procedure***

The reagent strip is dipped and completely immersed into the urine by a rapid deliberate motion and immediately removed. As the reagent strip is being removed, the edge of the strip is guided over the lip of the urine container to allow excess urine to run off the strip and back into the container. Follow the manufacturer's instructions for the incubation time sequence before comparing the test pad to the corresponding color chart. Timing of the reaction is critical! The incubation time for each analyte is different and may vary from one manufacturer to another.



### Color development

The color chart is provided by the manufacturer, and is usually found on the reagent strip bottle. The intensity of the reaction is indicated by the amount of color change. The color change of the reagent pad corresponds to a color on the color chart. The color change can represent a numerical value, positive or negative result, or a plus (+) grading scale (i.e., 1+, 2+, 3+, or 4+). Because each reagent strip may differ in its color change representation, a reference range isn't stated in these CDCs. Check the manufacturer's product insert or your laboratory's OI for the reference ranges.

**NOTE:** The use of a name or any specific manufacturer, commercial product, commodity, or service in this publication does not imply endorsement by the Air Force.

This reagent strip is used as an example only! The following analytes are listed in the same order as they are found on the back of the MILES, Ames N-Multistix®SG, Reagent Strips for Urinalysis container or bottle. The order of the reagent pad on the strip is based on the time required for a reaction or color development to take place. The order is shown in figure 3-1.

### Glucose

Glucose is one of a variety of reducing substances found in urine. Small amounts of glucose may be present in the urine after the ingestion of foods or beverages with a high sugar content. Although glucose is the sugar most commonly found in urine and the most clinically significant, other sugars such as lactose, fructose, galactose, and pentose (arabinose and xylose) may also be excreted. Normally all the glucose in the blood is freely filtered by the glomerulus and almost entirely reabsorbed by the renal tubules. Therefore, under normal conditions, the presence of glucose in urine is not normal, except for small amounts as mentioned above. *Glycosuria* (or glucosuria) is the term used to describe any condition in which glucose is found in the urine. Often glycosuria does not occur until the blood glucose level is considerably elevated. How high the blood glucose level will rise before glycosuria occurs depends upon the renal threshold of each individual. Glycosuria is generally detected in urine when blood glucose levels exceed 180 mg/dl. *Renal threshold* is the term used for any glucose level that exceeds the concentration that cannot be reabsorbed by the renal tubules and is excreted in the urine. Renal threshold is not an absolute number and can vary from individual to individual in a range of 160 to 200 mg/dl; at these levels the renal threshold for glucose is exceeded.

### Clinical significance of glycosuria

Screening urine specimens for glucose is the best method for detecting diabetes mellitus. Urine glucose levels can also be used to monitor the treatment of diabetic patients. You should know that diabetes is not the only cause of glycosuria. Kidney disease that affects the reabsorption capacity for glucose of the renal tubules will also produce glycosuria. Pancreatic disease, endocrine disorders, liver disease, and damage to the central nervous system may cause glucose excretion in urine. It may also be found during stress situations and pregnancy, after exercise, and/or associated with anesthesia.

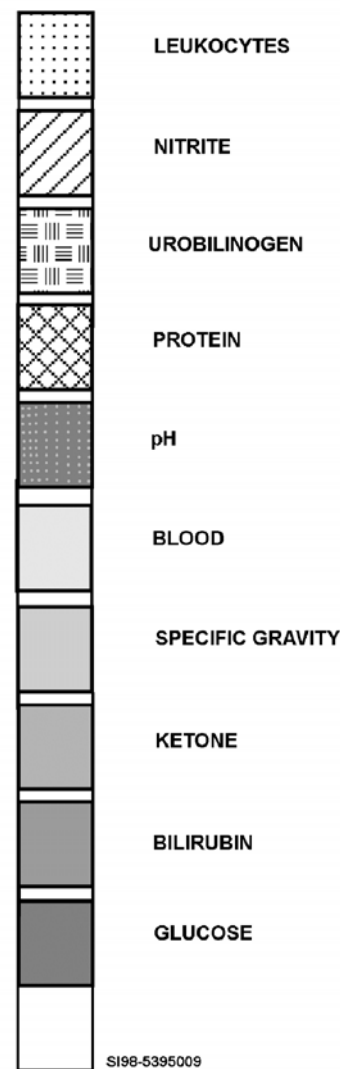


Figure 3-1. An illustration of the analyses on a reagent strip.

***Clinical significance of other reducing sugars***

Procedures that are based on the reducing ability of glucose are not specific for glucose. In these procedures, glucose is merely acting as a reducing agent, and any compound with a free aldehyde or ketone group will give the same reaction. Glucose is not the only reducing substance found in urine. Nonglucose reducing substances include creatine, uric acid, homogentisic acid, ascorbic acid, chloroform, formaldehyde, lactose, fructose, galactose, and pentose (arabinose and xylose). All of the aforementioned substances can reduce a heavy metal from a higher to a lower oxidation state, for example copper (II) to copper (I). Lactosuria, galactosuria, fructosuria, and pentosuria can be found in individuals with inherited metabolic defects. Let's look at these sugars separately.

***Lactose***

Lactose is often excreted in the urine of lactating women. This condition normally ceases when lactation is completed. It may be found in trace amounts in the urine of week old infants before their digestive systems have become fully developed and in other children and adults who are deficient in intestinal lactase.

***Galactose***

Galactose is found in the urine of infants afflicted with galactosemia. These children are deficient in the enzyme necessary for converting galactose into glucose. This is a severe condition that can be corrected by eliminating lactose and other sources of galactose from the diet. If not done, the infant will rapidly deteriorate physically and mentally to an early demise. Occasionally adults who ingest large quantities of milk or other lactose containing foods will show trace amounts of galactose in urine.

***Fructose***

Fructose sometimes occurs in the urine of patients with hepatic disorders. Its presence can be detected with the reagent test tablet. It can be identified by Selivanoff's test and by paper chromatography, neither of which are routine procedures.

***Pentose***

Pentosuria is associated with certain types of drug therapy and with some hereditary conditions. In both cases, its presence in urine is considered benign but can cause diagnostic problems. Pentoses may also be excreted after eating large amounts of fruits such as plums and cherries.

***Laboratory procedures and limitations***

It seems that urine tests for glucose have been around for many years. The Babylonians and Egyptians used the taste-test method; they would detect diabetes by tasting the urine for sugar or sweetness, which is different from its normal salty taste. Aren't you glad you weren't a laboratory technician in the good old days? Hindu physicians noticed that honey urine attracted ants and other sweet-loving insects. Let's move on to the more modern methods for determining glucose and other reducing substances.

***Copper reduction method***

The Benedict's reaction test or copper reduction is the classic method for detecting chemical reducing substance in urine. Benedict's qualitative reagent contains copper sulfate, sodium citrate, and sodium carbonate. In this alkaline solution, any compound that contains free aldehyde or ketone groups will reduce cupric ions ( $\text{Cu}^{++}$ ) to cuprous ions ( $\text{Cu}^{+}$ ) upon boiling. A commercial test tablet that contains copper sulfate, sodium carbonate, sodium citrate, in addition to sodium hydroxide, is mixed with a predetermined amount of urine and water. The cupric ion ( $\text{CuSO}_4$ ), in the presence of NaOH (a base), reacts with the reducing substances in urine to produce cuprous ions plus heat. The approximate amount of reducing substances present is indicated by a color comparison of the reaction with a color chart supplied by the manufacturer. The reaction must be observed as it occurs, so that a pass-through

reaction isn't missed. This procedure measures glucose, other reducing substances, and some pharmaceutical agents.

#### *Reagent strip*

Because of the false-positive glucose results in copper-reduction methods due to the presence of other reducing substances, a more specific test for glucose was required. Hence, the glucose oxidase method, an enzymatic assay, was developed. The reagent strip employs this methodology. In this method, glucose oxidase catalyzes the formation of gluconic acid and hydrogen peroxide ( $H_2O_2$ ) from the oxidation of glucose. Peroxidase (horseradish), a second enzyme, catalyzes the reaction of hydrogen peroxide and potassium iodide (a chromogen). The chromogen produces colors ranging from green to brown. Sugars such as lactose, fructose, galactose, and pentose are not substrates for glucose oxidase and therefore, do not react with this test. Also, glucose oxidase does not react with the reducing metabolites of drugs (e.g., salicylates and nalidixic acid). At this time there are no false-positive interferents. Large amounts of ascorbic acid from vitamin C or drugs (tetracyclines) may inhibit or delay color development, hence a false-negative reaction is obtained. Ketones may reduce the sensitivity of this test and result in false-negative reactions. A fresh, well-mixed urine sample at room temperature should be used because refrigerated specimens will give falsely low results due to decreased enzyme activity.

#### *Confirmatory test*

Confirmatory testing for all urinary analytes may include reagent test tablets, semi-automated techniques, and/or completely automated methods. The urinalysis OI in your laboratory should state which confirmatory procedure you use if it is required. For the purpose of this CDC, a general laboratory confirmatory test or procedure is presented.

### **Bilirubin**

Remember from your earlier studies that bilirubin is the principal pigment in bile and is produced from the breakdown of red blood cells. Review volume 2, unit 2, for the physiology of bilirubin. *Bilirubinuria* is the term used for the presence of bilirubin in the urine. Normally, bilirubin is excreted from the body by the liver by way of the intestine. Unconjugated bilirubin is water insoluble, therefore, cannot be excreted by the kidneys. However, during certain pathological conditions, conjugated bilirubin enters the bloodstream and, because it is water soluble, can be excreted by the kidneys.

#### *Clinical significance*

The presence of bilirubin in the urine is an important early indicator of liver dysfunction and/or biliary obstruction. Bilirubinuria may even be diagnosed before clinical jaundice appears.

#### *Laboratory procedures and limitations*

There are several tests used to detect bilirubin in urine. One of the oldest methods was the foam test. The Greeks would shake the urine specimen and look for the vivid yellow foam produced by the presence of bilirubin. They used this method to help determine the cause of clinical jaundice. Unfortunately, this simple and inexpensive test is not adequate today.

#### *Reagent strip*

The reagent strip is the simplest semi-quantitative test for the determination of bilirubin. Reagent strips are impregnated with stabilized 2,4-dichloroaniline diazonium salt that reacts with bilirubin in a strongly acid medium to form azobilirubin. Positive reactions produce various shades of tan. The reaction must be carefully compared with the color chart supplied by the manufacturer. False-positive results can be seen in highly pigmented urine as a result of the presence of certain drugs (e.g., Pyridium). Specimens must be absolutely fresh because bilirubin rapidly breaks down when exposed to light and due to the oxidation of bilirubin to biliverdin. Results will be significantly lower or falsely negative.

### *Confirmatory test*

Reagent tablets can be used to confirm reagent strip results and if a more sensitive test is required. The tablets usually contain similar diazotized dyes which result in a blue or purple color. The bilirubin in the sample reacts with 2,4-dichlorobenzenediazonium tetrachlorozincate and sulfosalicylic acid to produce a color change. False-positive results can also be due to highly pigmented urine. False-negative results can occur if the urine contains large amounts of ascorbic acid or nitrites (released from bacteria).

### **Ketone**

Ketone bodies are normal products of fat metabolism and are formed in the liver. In normal metabolism, the body completely metabolizes fat to carbon dioxide and water; therefore, ketone bodies are not found in the blood or urine. In certain conditions, especially diabetes and starvation, carbohydrates are not utilized effectively or are used up. Hence, fat is used as a major source of energy. Ketone bodies, products of fat metabolism, are in turn found in the blood (*ketosis*) and in the urine (*ketonuria*). The ketone bodies are acetoacetic acid,  $\beta$ -hydroxybutyric acid, and acetone (the last two are derivatives of acetoacetic acid). In the urine, acetoacetic acid accounts for about 20% of the ketone bodies,  $\beta$ -hydroxybutyric acid approximately 78%, and acetone around 2%.

### *Clinical significance*

Ketones are excreted in the urine, together with basic ions, depleting the body of its anions and thus producing acidosis. Acidosis is a pathological condition resulting from the accumulation of acid, of which ketone bodies are comprised, and is considered serious. Before commercial insulin was developed, acidosis was the cause of death in over 50% of diabetes mellitus patients. Also, acetoacetic acid and acetone have been found to be toxic to brain tissue when elevated in the blood. (No, I don't have elevated levels of these in my blood.) Ketosis and/or ketonuria can occur in starvation, anorexia nervosa, prolonged fever, vomiting and diarrhea (dehydration), fat feeding or low carbohydrate diets, and uncontrolled diabetes; starvation and diabetes mellitus are the most common causes of ketone accumulation. Occasionally, liver damage may also result in ketosis. Because most carbohydrate is stored in the liver as glycogen and is absent in liver damage, the body must resort to fat for energy. In ketogenic diets, used by some physicians to treat epilepsy, carbohydrate intake is very low and fat intake is high. The body must, again, resort to fat as an energy source instead of carbohydrates.

### *Laboratory procedures and limitations*

As with the above test, the urine must be fresh. In the formation of ketone bodies, acetoacetic acid is produced first and then  $\beta$ -hydroxybutyric acid and acetone. In a like manner, if the urine sample contains all three ketone bodies and is allowed to stand, acetoacetic acid and  $\beta$ -hydroxybutyric acid are converted to acetone. Because acetone is volatile, it will eventually disappear from the sample and a false-negative result is obtained.

### *Reagent strip*

The reagent strip pad contains sodium nitroprusside. When this reagent comes in contact with acetoacetic acid a purple color develops. Negative results are a light-tan or a buff-pink. Some reagent strips will only detect acetoacetic acid, not  $\beta$ -hydroxybutyric acid and acetone. False-positive results may occur with highly pigmented urine caused by various compounds. Occasionally low levels of ketone bodies may be detected in the urine during fasting, pregnancy, or frequent strenuous exercise.

### *Confirmatory test*

A reagent tablet is also used as a confirmatory test for ketone bodies. It is more sensitive than the reagent strip and it detects both acetoacetic acid and acetone. The tablet also contains sodium nitroprusside with lactose which enhances color differentiation. False-positive reactions may occur with certain dyes or drugs that produce highly pigmented urine. False-positive reactions can be

observed if compounds that contain sulfhydryl groups are present in the sample. False-negative results can be seen in improperly stored urine samples because of the breakdown, as mentioned above.

### **Specific gravity**

Waste product excretion and reabsorption of vital substances are the most important functions of the kidneys. As the previous lesson stated, specific gravity is a useful indicator of the ability of the renal tubules to concentrate or dilute urine, or measure their reabsorption capacity.

### ***Clinical significance***

Properly functioning kidneys are able to alter the specific gravity of urine in a range from 1.003 to 1.035. First-morning or 8-hour specimens are more concentrated than random samples and the specific gravity ranges from 1.020 to 1.025. Random samples with a specific gravity above 1.025 can be seen in patients with congestive heart failure (CHF), severe liver damage, dehydration, or severe water loss from vomiting or diarrhea. Low specific gravity results ( $<1.010$ ) are obtained in patients with severe end-stage renal disease and diabetes insipidus. Low values may also be seen after ingestion of large amounts of water or after eating.

### ***Laboratory procedures***

The fastest and most convenient method for measuring specific gravity is the reagent strip. The reagent strip estimates the specific gravity by a colorimetric (color chart comparison) method. The reagent strip pad contains polymethylvinyl ether/maleic acid, bromthymol blue, and sodium hydroxide. This indirect test method is based on the principle that a change in ionic concentration of a solution initiates a change in the dissociation constant (pK) of pretreated polyelectrolytes (polymethylvinyl). This ionic change is detected by the bromthymol blue (pH indicator). Colors range from blue-green in low ionic concentrations of urine to green-yellow in high ionic concentrations. Confirmatory testing is performed using a urinometer or refractometer.

### **Blood**

*Hematuria* is the term used to describe the presence of intact red blood cells in urine. If the red blood cells have been lysed, hemoglobin is released into the urine and the term *hemoglobinuria* is used to describe this condition. The presence of myoglobin in urine is known as *myoglobinuria*. A distinction between the three is clinically significant, as you will see.

### ***Clinical significance***

Hematuria is an important indicator of diseases involving the urogenital system. Possible causes include bladder and kidney tumors; trauma to the kidneys; glomerulonephritis; pyelonephritis; renal calculi (kidney stones); and bleeding disorders. The bleeding may come from a variety of areas: kidneys, ureters, bladder, urethra, prostate gland, and uterus and/or vagina. Hematuria can generate various colors including bloody, pink, amber, red-brown, and brown.

### ***Hemoglobinuria***

Hemoglobinuria may result from trauma; intravascular hemolysis due to transfusion reactions, hemolytic anemias, or paroxysmal nocturnal hemoglobinuria; severe infections such as malaria, etc.; severe burns; poisoning with strong acids or mushrooms; or trauma from neoplastic diseases. Usually the color of urine from hemoglobin, as well as myoglobin, is brown or red-brown. Sometimes the term *occult blood* is associated with hemoglobinuria. Occult means obscure or hidden.

### ***Myoglobinuria***

Myoglobin is the heme protein of striated muscle and results from muscle fiber necrosis. Myoglobinuria is seen in traumatic muscle injury acquired from automobile accidents, beatings, or crush injuries; excessive unaccustomed exercise; exposure to toxic drugs or substances; and rare hereditary disorders.

### ***Laboratory procedures and limitations***

Distinguishing between hematuria, hemoglobinuria, and myoglobinuria may be difficult. It is based on a combination of gross observations and chemical procedures. If a blood specimen is drawn at the same time the urine is collected, an observation of serum color may be helpful. If the urine contains only intact red blood cells, the serum would mostly be yellowish or normal. If intravascular hemolysis has occurred, the serum appears light pink or red. With the acute destruction of muscle fibers (rhabdomyolysis), myoglobin is released in the blood stream but is rapidly excreted by the kidneys; consequently, the serum appears normal in color. In hematuria, intact red blood cells would be seen during microscopic examination of the urine. In hemoglobinuria and myoglobinuria, intact red blood cells are absent, or only a few are seen microscopically. If myoglobin is present in the blood, markedly elevated serum creatine kinase (CK) are expected. Some texts suggest performing LD isoenzymes, because LD<sub>1</sub> and LD<sub>2</sub> are predominate in hemolysis, whereas LD<sub>4</sub> and LD<sub>5</sub> are predominate in rhabdomyolysis.

### ***Reagent strip***

The reagent strip detects red blood cells, hemoglobin, and myoglobin. The reagents include diisopropylbenzene dihydroperoxide; 3,3',5,5'-tetramethylbenzidine, and a buffer. This method is based on the peroxidase-like activity of hemoglobin, which catalyzes the reaction of diisopropylbenzene dihydroperoxide and 3,3',5,5'-tetramethylbenzidine to form a color change. The color ranges from an orange-yellow to a dark green. A bluish color may be seen in the presence of high levels of blood.

**NOTE:** Intact red blood cells are lysed when they come in contact with the reagent pad. This releases the hemoglobin which is required for the reaction. If this is the case, a speckled-pattern on the reagent pad is observed. False-positive results may be produced with certain oxidizing contaminants (e.g., hypochlorite and microbial peroxidase). False-negative results may be seen in urine samples with an elevated specific gravity and the drug Captopril. With some reagent strips, ascorbic acid can also cause false-negative results. You should read the manufacturer's product insert and your laboratory's OI to see if ascorbic acid interferes with the test method utilized by your laboratory.

### ***Confirmatory test***

Other techniques can be used to detect hemoglobin when interfering substances are present. Direct chemical analysis using spectrophotometer can be used as a confirmatory test.

### **pH**

The pH of freshly voided, normal urine varies from 4.5 to 8.0 with a mean between 5.0 and 6.0. Do you know why urine is more acidic than alkaline? Go back to the previous lesson in the *normal* constituents area. Did you notice all of the various acids present in urine? If you were able to conclude that these bring about a more acidic fluid, then you get a star for the day! When urine specimens are allowed to stand at room temperature, they become less acidic and more alkaline. This is due to the growth of bacteria and the breakdown of urea to ammonia. The concentration of ammonia in old urine can become so high its distinctive odor is noticeable.

### ***Clinical significance***

The kidneys, along with the lungs, are prime regulators of acid-base balance (a part of homeostasis), which was discussed in volume 2, unit 1. As stated, the blood pH ranges from 7.35 to 7.45. The pH range compatible with life is 6.80 to 7.80. The carbon dioxide produced in normal metabolism results in a tremendous amount of acid accumulation. The acid must be removed or eliminated from the blood and interstitial fluid. This is done through the lungs and kidneys. Nonetheless, routine urinalysis is not necessarily used to monitor this function, but the pH value is helpful in identifying various formed elements found in urinary sediment and some pathological disorders. Persistently acidic urine occurs in metabolic disorders such as diabetes. Persistently alkaline urine is associated with bacterial infections, metabolic disorders, and certain drugs. Occasionally it may be required to

control or alter the patient's urinary pH in order to manage kidney infections, renal calculi, or for the administration of certain drugs. This can be done by regulating the diet: high meat intake diets generally produce acidic urine and high vegetable intake diets produce alkaline urine.

#### *Reagent strip*

The reagent strip method is based on the double indicator principle that gives a broad range of colors covering the entire urinary pH range. The strip contains methyl red and bromthymol blue. Methyl red is used to indicate an acid pH with color changes from red to yellow. Bromthymol blue indicates an alkaline pH and the colors range from yellow to blue. No interferences are known.

#### *Confirmatory test*

The most accurate measurement of pH is made with a pH meter. Its principle was discussed in volume 1, unit 6, under electrochemistry, potentiometric methods.

### **Protein**

Only a small amount of protein is excreted in the urine on a daily bases. Proteinuria is the occurrence of increased protein levels in urine. This condition is probably the most significant pathological condition found in routine urinalysis. Urinary protein is the single most important finding in the detection and diagnosis of renal disease. Proteinuria is a result of glomerular damage, tubular damage, or overflow from excessive production of low-molecular weight proteins. The protein excreted by the kidneys is derived from plasma proteins, with albumin as the primary protein fraction normally excreted. It is theorized that more albumin is excreted by the kidneys because of its relatively low-molecular weight and because glomerular filtration is impermeable to large molecules.

#### *Clinical significance*

Proteinuria can be derived from the urinary system or from nonurinary system disorders. Let's look at both.

##### *Urinary system*

The implication of protein in the urine is extremely serious. Extensive renal destruction is incompatible with life, and any renal destruction is permanent. As stated in previous lessons, the glomerular filtrate is the initial stage in the formation of urine. The glomerular filtrate is an ultrafiltrate of blood plasma without cells, large protein molecules, and certain fatty substances. The normal glomerular membrane allows the passage of proteins with molecular weights of 50,000 to 60,000, or less. Albumin has a molecular weight of 67,000 (which is a small molecule), so some albumin is normally filtered through the glomerulus. Nonetheless, this is normally filtered, then reabsorbed by the convoluted tubes. As you can see, this makes albumin a very sensitive marker in early stages of glomerular and/or tubular damage. There is a correlation between the presence of protein in urine and urinary casts found in urinary sediment since casts are made of precipitated protein. Tamm-Horsfall mucoprotein is a type of protein that is normally secreted by the renal tubules. It is a product of the kidney and not present in the plasma. This protein forms the matrix of most urinary casts. The occurrence of casts with proteinuria distinguishes an upper urinary tract (kidney) disorder from a disorder of the lower urinary tract. Bacterial infections are often indicated by the presence of white blood cells and bacteria in the urinary sediment, in addition to protein in the urine. White blood cells and bacteria *without* urine protein indicate a lower UTI without renal involvement.

##### *Nonurinary system*

This is really a catchall category for proteinuria that isn't derived from the urinary system. Proteinuria may accompany generalized nonurinary system conditions, such as hemorrhage, systemic infections, heart failure, dehydration, and starvation. In the urine of patients with multiple myeloma, large quantities of a low-molecular weight globulin known as Bence Jones protein may be found. Bence Jones protein replaces albumin as the predominant protein found in urine in these patients. Bence

Jones protein may also be present in malignant lymphoma and macroglobulinemia. Normal persons may excrete abnormal quantities of protein, mainly albumin, in some situations; such as during strenuous physical exercise, pregnancy, exposure to extreme cold, and psychological stress. Orthostatic proteinuria is a condition mainly found in adolescents and young adults, in which increased quantities of protein are found in the urine when the patient is in the vertical position (standing); yet the proteinuria disappears when the patient is horizontal (resting or lying down).

#### ***Laboratory procedures and limitations***

Protein procedures should be performed on first-morning specimens, because random specimens may experience the dilutional effects of food and water. This effect produces false-negative results.

#### ***Reagent strip***

The reagent strip uses a colorimetric methodology based on the concept of the protein error of indicators. This method utilizes a physicochemical property of protein that causes certain indicators to change color in the presence of varying concentrations of protein when the pH is kept constant. The indicator in this method is tetrabromophenol blue. The dye-binding properties of protein make the reagent strip the most sensitive to the presence of albumin. The reagent strip responds semi-quantitatively to increasing concentrations of urinary albumin, and fails to react with globulin, hemoglobin, Bence Jones protein, and mucoprotein. If albumin is absent in the urine, but the others are present, a false-negative result will be obtained. Depending on the patient population, both reagent strip and precipitation methods may be required on all urine samples. Protein determinations using reagent strips are not very accurate and cannot be used in a quantitative way. A positive or faintly positive result should be confirmed with a more specific test.

#### ***Confirmatory test***

The most important reason for protein testing is the diagnosis of kidney disorders. Consequently, most authorities recommend that all positive urine protein screening tests be confirmed. Confirmatory testing, in this instance, is done for two reasons; the clinical importance of the results and the limitations of any single test. Precipitation methods use either a chemical (strong acid) to precipitate out the protein or heat to coagulate the protein out of urine. The turbidity of the reaction is compared to standards of known protein concentrations. The reaction can be measured macroscopically (visually), or by using a spectrophotometer or nephelometer. The most commonly used precipitation procedure is the sulfosalicylic acid method. The sulfosalicylic acid test detects albumin, globulins, glycoproteins, and Bence Jones protein. To identify the actual type of protein present, electrophoresis, immunoelectrophoresis, immunodiffusion, or ultracentrifugation techniques may be necessary.

#### **Urobilinogen**

Urobilinogen is a product of hemoglobin metabolism (red blood cell degradation) and is formed by the intestinal reduction of bilirubin by bacteria. About 50% of the urobilinogen is excreted in the feces and the other 50% is reabsorbed into the circulation from the intestines. As the blood is filtered by the kidneys, most urobilinogen re-enters the blood, only a small amount is excreted in the urine.

#### ***Clinical significance***

Plasma and urine urobilinogen levels increase when bilirubin levels increase. Increased bilirubin production is due to severe liver disease and is present in hemolytic disorders. Under certain circumstances urobilinogen is completely absent from the urine and feces (e.g., in biliary blockage). This is due to the fact that bilirubin doesn't reach the intestines where it is broken down into urobilinogen. However, bilirubin is increased in both plasma and urine. Therefore, the presence of urobilinogen can distinguish between liver or hemolytic disease and biliary obstruction.

#### ***Laboratory procedures and limitations***

The urine sample must be fresh and protected from light, since urobilinogen will be converted to urobilin upon standing.



*Reagent strip*

Urobilinogen and other bile pigments are detected by means of the Ehrlich reaction in which urobilinogen reacts with para-dimethylaminobenzaldehyde in a strongly acid medium to form a pink-red color. Reagent strips are impregnated with para-dimethylaminobenzaldehyde and an acid buffer solution. They react with urinary urobilinogen, porphobilinogen, and para-aminosalicylic acid; and sulfonamides to form colored compounds. Atypical colors can be obtained if high concentrations of para-aminobenzoic acid are present. False negative results are seen in urines that are preserved with formalin.

*Confirmatory test*

Various semi-quantitative methods are available to confirm a positive reagent strip reaction. Quantitative methods utilize spectrometric techniques.

**Nitrite**

Most of the bacterial species isolated from urine cultures have the ability to reduce urine nitrate (an abundant constituent of urine) to nitrite.

*Clinical significance*

The nitrite test is a rapid screening method used to detect the presence of bacteria in urine. The presence of bacteria in urine is known as bacteriuria. The most common organisms associated with UTIs that reduce nitrate to nitrite are *Escherichia coli*, *Klebsiella*, *Proteus*, *Pseudomonas*, and *Staphylococcus* species. The nitrite test, in conjunction with the leukocyte esterase test, is very useful in detecting asymptomatic infections. A positive nitrite test may alert the physician to order a urine culture, if one was not done with the urinalysis. The nitrite test is not designed to replace urine culture or sensitivity studies. UTIs require early detection and treatment in order to prevent bacteria from reaching the kidneys. If UTIs are left untreated, the subsequent infection of the kidneys can lead to renal failure.

*Laboratory procedures and limitations*

The reagent strip pad is impregnated with para-arsanilic acid (an aromatic amine), and 1,2,3,4-tetrahydrobenzo(h)-guinolin-3-ol. Nitrite reacts with the para-arsanilic acid to form a colored diazonium compound that, in turn, reacts with 1,2,3,4-tetrahydrobenzo(h)-guinolin-3-ol to produce a pink color.

**NOTE:** First of all, the test depends upon the conversion of dietary nitrate to nitrite by the action of bacteria in the urine. Also remember non-nitrite producing microorganisms can cause a UTI and will not be detected by this test. False-positive reactions may be seen with highly pigmented specimens. False-negative results may be obtained from samples with high levels of ascorbic acid or urobilinogen, a diet deficient in vegetables (provides nitrate for conversion), and the presence of antibiotics in the urine.

**Leukocyte esterase**

As stated above, the leukocyte esterase test, in conjunction with the nitrite test, can successfully detect asymptomatic UTIs. Leukocyturia or pyuria is used to describe the presence of white blood cells in urine.

*Clinical significance*

The presence of pyuria is an excellent indicator of urinary tract inflammation. Infections or inflammation can result from various microorganisms such as bacteria, trichomonads, and chlamydiae. Polymorphonuclear neutrophils (PMNs) are almost always present in infections and inflammation of the kidneys and lower urinary tract. Leukocytes of the granulocytic series contain esterase whereas lymphocytes and epithelial cells do not. A positive leukocyte esterase test may be seen in patients with pyelonephritis, cystitis, urethritis, renal calculi, interstitial nephritis, and glomerulonephritis.

**Laboratory procedures and limitations**

The reagent strip is based on the principle that leukocyte esterase catalyzes the hydrolysis of the derivatized pyrrole amino acid ester to liberate 3-hydroxy-5-phenyl pyrrole. This pyrrole then reacts with a diazonium salt to produce a purple end product. The reagent strip contains derivatized pyrrole amino acid ester and a diazonium salt. The leukocyte esterase test is positive whether the white blood cells in the urine are lysed or intact. If a urine sample is allowed to stand for a long time, the granulocytes may lyse and will not be seen during microscopic examination of the sediment. False-positive results are rarely seen, except if oxidizing reagents are present in the urine or the urine is contaminated with vaginal secretions. False-negative results may be seen in urine containing high levels of ascorbic acid, formalin, or albumin.

**REMEMBER:** The results of the reagent strip can indicate the type of formed elements that may be present in the urinary sediment, so make a mental note of these results.

**413. Urinalysis—microscopic procedures**

Sometimes the physicochemical or macroscopic examination of urine does not provide adequate information for the assessment of renal function or therapy. A microscopic examination of urinary sediment frequently provides valuable information for the physician in the diagnosis and treatment of urinary system disorders.

**General considerations**

NCCLS, Approved Guideline, GP16–A2 states that numerous journal articles and texts address the need for a urine sediment microscopic examination. However, with the addition of the nitrite and leukocyte esterase tests to reagent strips, the cost effectiveness of the microscopic examination on urine specimens with normal physicochemical (reagent strip) results has been questioned and debated. The decision to perform microscopic examinations should be made by each individual laboratory based on its specific patient population. Usually, microscopic examination is performed under the following circumstances.

- When requested by the physician.
- When determined by laboratory protocol (e.g., immunosuppressed, urology-nephrology, diabetic, or pregnant patients).
- When any abnormal physicochemical or reagent strip result is obtained.

**Specimen processing**

GP16–A2 also states that the type and quality of the urine specimen greatly affect the results of the microscopic examination. As with the macroscopic examination, the specimen of choice for microscopic examination is the first-morning specimen because it is the most concentrated. This maximizes the recovery of urinary sediment elements. Urine specimens more than 2 hours old, without refrigeration, are not acceptable for microscopic examination. Red blood cells, white blood cells, and casts are especially susceptible to lysis in urine specimens with a low specific gravity (<1.010) and/or an alkaline pH (>7.0).

**Volume**

Each laboratory should establish and standardize the volume of urine centrifuged to obtain the sediment. Commonly used volumes are 5, 10, 12, or 15 ml. If a smaller volume is used, for example with neonates or pediatric patients, a notation should be made on the final report.

**Time and speed of centrifugation**

To ensure equal sedimentation of all urine specimens, a standardized time and speed for centrifugation is critical. This may be different for each laboratory depending on the equipment used. However, centrifugation of 10 to 12 ml of urine for 5 minutes at a relative centrifugal force (RCF) of 400 to 500 is usually standard practice. This ordinarily produces a concentrated sediment in which all

elements present may be easily found yet are not artificially distorted. See your urinalysis section's OI for the time and speed established by your laboratory.

### ***Concentration and volume of sediment***

All urine specimens should be concentrated to the same volume, and a standardized volume of the concentrated sediment for examination should be used. For example, the specimen is concentrated to a volume of 0.5 ml and from that 0.1 ml of the sediment is placed on the slide for examination (amounts are for illustration purposes only). Figure 3-2 portrays a specially manufactured centrifuge tube used to standardized the volume of specimen used, the concentration of the sediment, and the volume of sediment examined. Urinary elements settle into the shaded area of the tube, the urine supernatant is discarded. The shaded area is placed in a customized holder that is attached to the microscope stand. This system standardizes the volume of sediment examined. Also, each piece of equipment used throughout the procedure should be standardized; from collection containers to slides and coverslips.

### ***Reporting results***

Every person in your laboratory who performs microscopic examinations must use the same terminology, reporting format, and reference intervals. For example, your laboratory may report the number of different elements seen per high-power field (HPF) or low-power field (LPF). Another method involves using a hemocytometer to quantify the number of cells and casts present. The count is reported as number of cells per microliter (cells/ $\mu$ l). For the purpose of these CDCs we'll use the number of elements seen per HPF or LPF. Also, your laboratory should have an established reference range for urinary elements, along with panic or alert values.

### ***Microscopic methods***

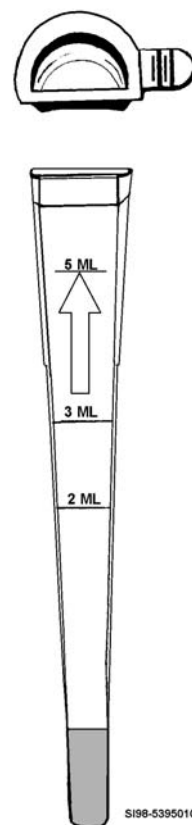
Before we actually look into the microscope to identify urine sediment elements, we need to know a little bit about the microscope we are using. Unit 1 discussed the principle and the various microscopic methods, so we'll just mention the most popular methods used in urinalysis. The traditional method of microscopic examination of urine utilizes wet mounts and brightfield microscopy. Phase-contrast and interference-contrast microscopy are recommended for identifying abnormal sediment elements.

### ***Staining the sediment***

Staining the sediment can also be helpful in the identification of cells and casts. Common supravital stains appropriate for wet mounts include Sternheimer-Malbin (crystal violet and safranin) and 0.5% Toluidine blue (nuclear stain). Supravital staining is not sufficient for the identification or confirmation of all urine sediment elements. Other stains that may be required are Oil red O and Sudan III for fat and oval fat bodies; Gram stain and Papanicolaou for bacteria; Hansel, Wright-Giemsa, or Papanicolaou for eosinophils; and Prussian blue for hemosiderin granules. Papanicolaou is the stain of choice for characterizing epithelial cells and Hansel stain is suggested for eosinophiluria and possible allergic nephritis.

### ***Most importantly***

The most important consideration in the microscopic examination of urinary sediment is to be able to distinguish normal sediment elements from abnormal sediment elements. In this lesson we'll describe



**Figure 3-2. An illustration of a standardized urine examination system.**

the different types of formed elements seen in urinary sediment and identify those elements as normal or abnormal. It should also be noted that some of these elements are normal in small numbers, but increased numbers indicated a pathological condition. Unless stated otherwise, the element is described as seen under brightfield microscopy in a wet mount.

### **Epithelial cells**

Let's look at the various types of epithelial cells that can be seen in urinary sediment.

#### ***Squamous epithelial cells***

Squamous epithelial cells line the distal portion of the lower urinary tract and the female genital tract. Squamous cells are the largest of the epithelial cells found in urine. They have abundant flat cytoplasm with small nuclei. Frequently, one or more corners of these cells are folded as shown in figure 3-3. Squamous cells in urine usually indicate vaginal contamination or squamous metaplasia of the bladder and represent the least significant type of epithelial cell found in urine.



**Figure 3-3. Squamous epithelial cells seen in clumps.**

#### ***Transitional epithelial cells***

Transitional epithelial cells originate in the transitional epithelium lining the renal pelvis and calyces, ureter, urinary bladder, and the proximal  $\frac{2}{3}$  of the urethra. They are round to oval and have a large, centrally located nucleus. The cytoplasm borders of these cells appear thickened and crisp. See figure 3-4. When the nuclei of transitional cells become enlarged or irregular, cytological techniques should be suggested for the purpose of detecting a urinary system malignancy. A few transitional epithelial cells may be found in normal urine. Inflammatory conditions, catheterization, or a pathological process such as malignancy may be indicated by large numbers of these cells.

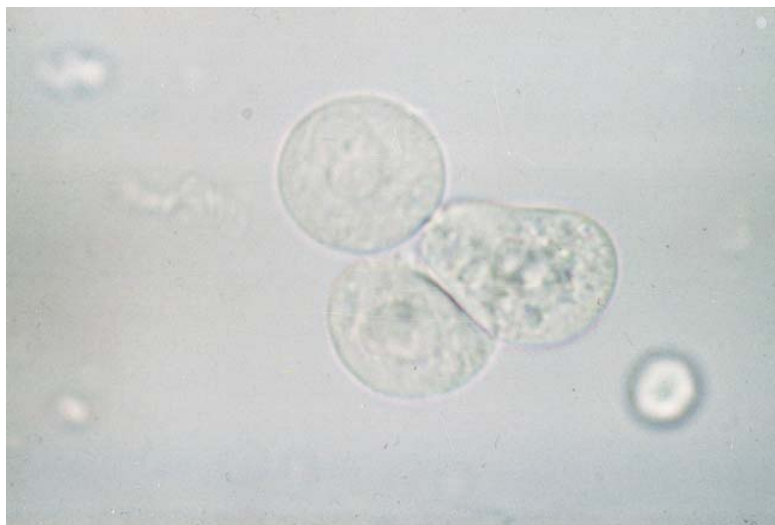


Figure 3-4. Transitional epithelial cells, which are nearly spherical.

### ***Renal epithelial cells***

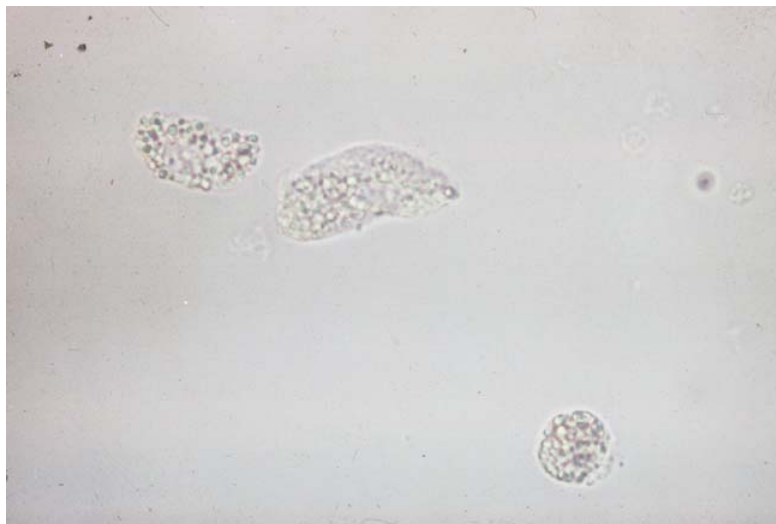
Renal epithelial cells originate from the epithelial lining of renal tubules within the nephron. Renal epithelial cells undergo a constant process of regeneration, with the more mature or older cells being sloughed or exfoliated into the urine. If there are more than 2 to 3 of these cells per HPF in urine, it indicates renal tubular damage. Considerable difficulty exists in the accurate identification of renal epithelial cells, particularly in distinguishing them from other mononuclear cells commonly found in urine. By brightfield microscopy, they are polygonal and slightly larger than leukocytes as shown in figure 3-5. Their nuclei may be eccentric (off center) and displaced toward one end of the cell. For accurate identification of the various types of renal tubular cells, sheets, or fragments; cytological techniques are required. Quantifying renal epithelial cells is important in documenting progressive or regressive changes in several renal parenchyma diseases; such as acute tubular necrosis, inflammation, or kidney transplant rejection.



Figure 3-5. Renal epithelial cells with nuclei approximately the same size as the adjacent neutrophil.

**Oval fat bodies**

Oval fat bodies are renal tubular epithelial cells that are filled with absorbed globules of lipid. The lipid globules or fat droplets are usually within the renal tubular epithelial cell that has undergone degenerative cellular changes as shown in figure 3–6. However, some oval fat bodies may be macrophages (leukocytes) that have phagocytized fat droplets. The fat droplets within the cells vary greatly in size, are usually spherical, highly or double refractive, and can obscure the nucleus of the cell.



**Figure 3–6. Renal epithelial cells with cytoplasmic lipids known as oval fat bodies.**

***Lipid globules or fat droplets***

Lipid globules or fat droplets can be free-floating (found alone) in urinary sediment as highly refractive droplets of various sizes. The term *lipiduria* is used when fat is present in the urine. Although rare, fat droplets may be due to contamination.

***Fatty cast***

Fatty casts are discussed at this time because of their relationship to lipid globules and oval fat bodies. Fatty casts contain either free fat or oval fat bodies in which lipid is visibly apparent. The free fat or oval fat bodies can be within the cast or on the cast and can be solidly packed together or sparse (loosely packed). Fatty casts are easily recognized because they contain globular lipids that have a high refractive index, vary greatly in size and shape, are birefringent, and are yellowish-tan in color. When lipid globules or fat droplets (within cells and/or casts, or free-floating) are present, they indicate a serious pathological condition implying severe renal dysfunction exists. These elements may also be seen in patients with heavy metal poisoning, glomerular injury, or diabetes mellitus.

**Blood cells**

The different types of hematopoietic or blood cells seen in urinary sediment are red blood cells (erythrocytes) and white blood cells (leukocytes).

***White blood cells***

As with some elements already discussed, a few white blood cells (3 to 5 per HPF) may be present in normal urine. This number may be different from each laboratory depending on the established reference range (normal values). The white blood cell most frequently found in urinary sediment is the polymorphonuclear neutrophil, but lymphocytes, monocytes, and eosinophils may also be present.

White blood cells are quite easily distinguished from red blood cells on the basis of size and the presence of internal structures, as shown in figure 3-7. They are approximately 12 to 15  $\mu\text{m}$  in diameter and contain cytoplasmic granules. The nucleus may or may not be distinct, but the center of the cell appears granular. Cytocentrifugation techniques and staining procedures are usually required to accurately identify these cells. Increased numbers of leukocytes ordinarily indicate inflammatory conditions involving the kidney or lower urinary tract, as seen in figure 3-8 (note the presence of bacteria). *Glitter cells* are PMNs in which the cytoplasmic vacuoles are large and in motion (ameboid movement) or are phagocytizing microorganisms. They appear to be twinkling when observed in fresh urine, hence the name glitter cells. White blood cells are fragile and will disintegrate or lyse in old, dilute, alkaline urine. Phase-contrast microscopy is especially useful in the identification of white blood cells.



Figure 3-7. Neutrophil with two red blood cells.

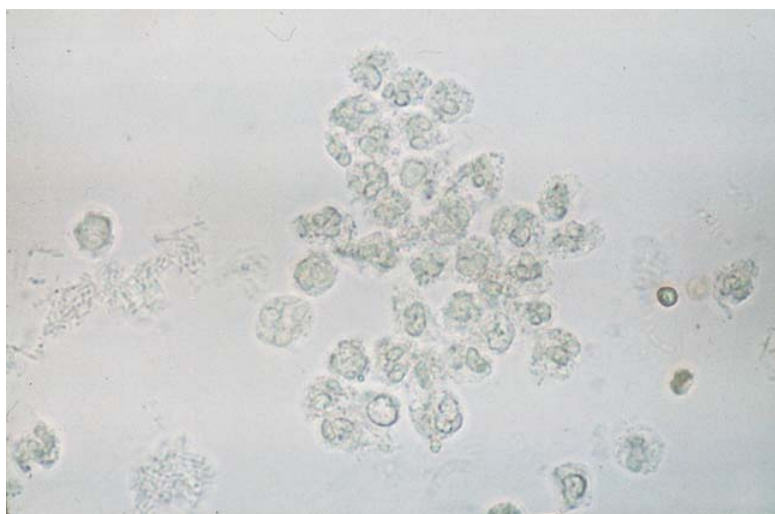


Figure 3-8. Numerous PMNs with bacteria.

### ***Red blood cells***

Red blood cells are normally present in urinary sediment in small numbers (0 to 3 per HPF). Ordinarily, reagent strips are not sensitive enough to detect these small numbers. If hematuria is present in a specimen from a premenopausal female, the urine may be contaminated with menstrual blood. If there are so many red bloods that they are too numerous to count (TNTC) and obscure other



elements, a drop of acetic acid can be used to lyse the red blood cells. A properly collected, subsequent or repeat specimen for urinalysis will ordinarily produce a normal number of red blood cells in the sediment. Sometimes, hematuria is seen in patients involved in contact sports or strenuous exercise. Within 48 hours, if the sport or exercise is discontinued, the hematuria will disappear and the red blood cell count is normal. Red blood cells appear in fresh urine, much like they do in dilute whole blood, as biconcave disks with a smooth appearance, as shown in figure 3-9. However, the urine may be more or less concentrated than the blood and depending on the osmotic gradient of the urine surrounding the red blood cells, they may be swollen or crenated.



**Figure 3-9. Red blood cells in urine.**

#### *Swollen and ghost red blood cells*

In hypotonic or dilute (low specific gravity) urine, red blood cells swell due to the surrounding fluid moving into the cells. This is because the ion concentration of the urine is less than the ion concentration of the fluid within the red cell. The red blood cells become a little larger and round, not biconcave. When the urine is dilute and alkaline, the red blood cells will often appear as ghost or shadow cells. Ghost and shadow cells result from the red blood cell bursting and releasing its hemoglobin and all that remains is the faint, colorless, cell membrane. Ghost cells may also appear in old urine.

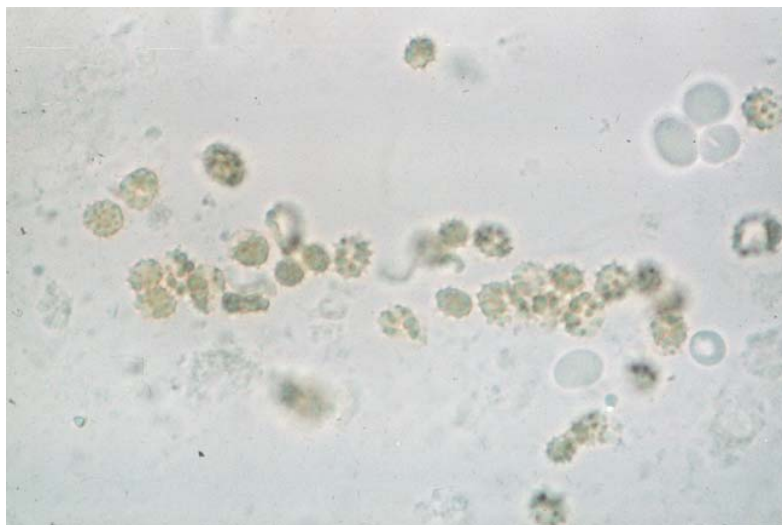
#### *Crenated red blood cells*

In a hypertonic or concentrated (high specific gravity) urine, the red blood cells are crenated or are prickly because the fluid within the cells moves into the surrounding fluid (urine). This occurs when the ion concentration of the urine is higher than the ion concentration within the cell. As the fluid moves out of the cell, the cell shrinks and the membrane becomes prickly, as shown in figure 3-10. The cells still show hemoglobin pigmentation but their biconcavity is more difficult to discern.

#### *Elements confused with red blood cells*

Occasionally, to the inexperienced microscopist, red blood cells are confused with bubbles, oil droplets, or yeast. Bubbles and oil droplets vary greatly in size and are highly refractile. Red blood cells range from 6 to 7  $\mu\text{m}$  in diameter, and vary little in size. If red blood cells are present, they are useful in making size comparisons when identifying other cells and elements. Yeast is usually smaller than red blood cells, varies in size, is spherical rather than biconcave or slightly flattened, and produce buds or little outgrowths.

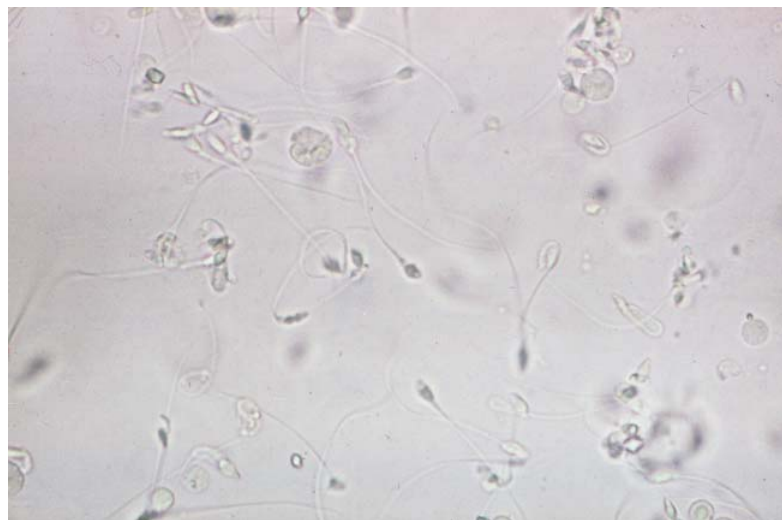




**Figure 3–10. Many crenated red blood cells in the urine.**

### **Spermatozoa**

Spermatozoa may also be seen in urinary sediment. They are motile or nonmotile depending on the time of ejaculation in reference to the collection of the urine sample and time of examination. Urine is toxic to spermatozoa, so the absence of motility isn't clinically significant. They are easily recognized by their oval body and long delicate tail, as shown in figure 3–11. They can be normal in both males and females. Your laboratory's OI should have established guidelines for reporting the presence of spermatozoa in a urine sample. Age and sex of the patient, fertility studies, and abnormal spermatozoa morphology should be considered when developing these guidelines.



**Figure 3–11. Large number of spermatozoa in urine.**

### **Microorganisms**

Although urine is normally free of microorganisms, bacteria and yeast are often found in normal urine because the specimen is not collected under sterile conditions. Urine samples can be contaminated with microorganisms from the vaginal or lower urinary tract, by the use of nonsterile collection equipment, or by airborne contaminants. Because the urine is an excellent culture medium, if it isn't examined immediately, these contaminants can multiply rapidly and obscure the true microscopic analysis.

**Bacteria**

Morphologically, the bacteria will take on either a bacillary, as shown in figure 3-12, or coccal form. If the sediment is gram-stained, they will exhibit gram-positive or gram-negative staining. The presence of bacteria with white blood cells and measurable amounts of protein is considered important and clinically significant.



**Figure 3-12. Bacteria seen with squamous epithelial cells.**

**Yeast**

Fungi can be contaminants or pathogens. Yeast may be confused with fat globules and red blood cells, so careful scrutiny is required for all spherical elements. As described above, yeast are usually smaller than red blood cells and vary in size. If the urine has been at room temperature for a long period of time before examination, buds, as shown in figure 3-13, and hyphal forms can be observed. As with bacteria, if white blood cells or other inflammatory symptoms are not present, the fungi are probably contaminants.



**Figure 3-13. A squamous epithelial cell with budding yeast.**

**Mucus**

Mucus is a common element found in urinary sediment. It is usually more frequent in women than in men. If large amounts of mucus are present, possible contamination of vaginal secretions should be considered. Mucus is formed by glandular cells lining the lower genitourinary tract and the vaginal

epithelium. Recent immunological studies have shown that at least some of the mucus is Tamm-Horsfall protein, mentioned earlier. Mucus is thin, fibrous, wavy bands or strands that take on a variety of shapes and forms. They indiscriminately stretch across the microscope field of view and can entrap red blood cells, white blood cells, and other urinary sediment elements as shown in figure 3-14. Mucus is often mistaken for hyaline casts. You should note that mucus strands take on many different shapes while hyaline casts typically have smooth parallel sides, a cylindrical shape, and a specific size. Mucus has a low refractive index, like hyaline casts, and may be missed by ordinary brightfield microscopy. However, the strands are easily seen when phase-contrast microscopy is utilized. The absence or presence of mucus is not clinically significant.



Figure 3-14. Mucus strand with two entrapped neutrophils and erythrocytes.

#### 414. Identifying urinary crystals and casts

As with the other urinary sediment elements discussed in the previous lesson, normal and abnormal crystals and casts can be observed microscopically. It is essential that you are able to accurately identify crystals and casts because of their diagnostic value. For this lesson, it would be quite helpful to refer to the microscopic anatomy of the kidney and nephron in order to visualize the area of origin or damage.

##### Crystals

There are many different types of urinary crystals, some of which are produced by healthy individuals, and others produced by pathological processes. The formation of crystals is related to the concentration of salts in the urine, and this, in turn, is related to the patient's metabolic state, diet, and fluid intake. If you recall, the kidneys excrete the products of metabolism and if these products are in high concentrations, they tend to precipitate and form crystals. Whether the crystal is normal or abnormal, each crystal is usually found in a specific pH. This seems to be the best way to group or categorize crystals. So let's begin with normal crystals found in acid urine.

##### *Normal urinary crystals in acid urine*

Finding crystalline forms in urinary sediment is normal if there is a delay in the microscopic examination.

##### *Uric acid*

From our earlier studies, we know that uric acid, a purine derivative, is a metabolite of protein (i.e., nucleic acids or nucleoprotein). Uric acid crystals are probably the most common crystals encountered in urinary sediment. If an acidic urine is allowed to stand at room temperature for a sufficient length of time, uric acid crystals are observed. These crystals are the most pleomorphic of

all the urine crystals. They may be in the shape of rods, cubes, rosettes, six-sided plates, rhombi, stars, spears, needles, barrels, and whetstone. They appear colorless, pale yellow, pinkish-brown, or reddish-brown as in figure 3-15. Uric acid crystals are insoluble in alcohol, acetic acid, and hydrochloric acid. They are slightly soluble when heated, but become readily soluble upon the addition of an alkali such as sodium hydroxide. Ammonium hydroxide should never be used because its reaction with uric acid promotes the formation of ammonium urate crystals. Uric acid crystals should only be considered pathologic when found in fresh, warm urine.



Figure 3-15. Variegated uric acid crystals.

#### *Amorphous urates*

Amorphous urates are salts of uric acid and appear as fine, brownish tan granules. These salts usually precipitate in neutral or acid urine that has been refrigerated or cooled to room temperature. Amorphous urates may obscure other formed elements and should be cleared or removed before sediment examination. They can be cleared by centrifuging the urine, decanting the supernatant, replacing supernatant with an equal volume of warm saline, re-centrifuging, and examining the sediment. Amorphous urates as a rule have no clinical significance.

#### *Calcium oxalate*

Calcium oxalate is the second most frequently seen crystal. They are found in acid, neutral, or slightly alkaline urine. Calcium oxalate crystals have two basic shapes: octahedral and dumbbell. They are colorless and birefringent. They are readily identified by their characteristic octahedral shape which appear as an envelope. See figure 3-16. Occasionally they are confused with triple phosphate crystals. The two can be distinguished by their solubility properties. Calcium oxalate crystals are insoluble in acetic acid, whereas triple phosphates are soluble. However, calcium oxalate crystals are soluble in dilute hydrochloric acid and in 90% ethyl alcohol. Calcium oxalate crystals may be found in the urine from normal persons, especially if they eat foods rich in oxalic acid (e.g., tomatoes, asparagus, apples, oranges, and carbonated beverages), or ingest high doses of ascorbic acid. They may also be found in patients with diabetes mellitus, heart-lung disease, diseases of the nervous system, and organic liver disease. Calcium oxalate crystals have a high potential for forming renal calculi.

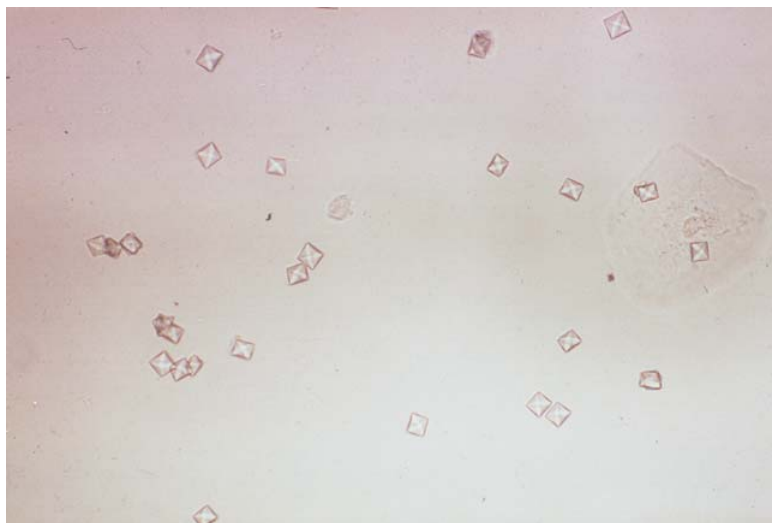


Figure 3-16. Calcium oxalate crystals with a squamous epithelial cell.

### *Normal urinary crystals in alkaline urine*

In this area we'll discuss only the most common crystals found in alkaline urine.

#### *Amorphous phosphates*

Amorphous phosphates appear in neutral to alkaline urine. They are fine, colorless, or slightly brown granules that tend to aggregate into groups or clumps, especially after refrigeration. See figure 3-17. They also produce a white precipitate in the bottom of the centrifuge tube. Amorphous phosphates also obscure other formed elements within the sediment and, therefore, must be cleared before examination. They can be cleared with a few drops of acetic acid, but the sediment should be examined for the presence of red blood cells, since they will be lysed by the acetic acid. The addition of acetic acid helps to differentiate amorphous phosphates from amorphous urates. These crystals usually appear in the urine after ingestion of a heavy meal, but have no real clinical significance.

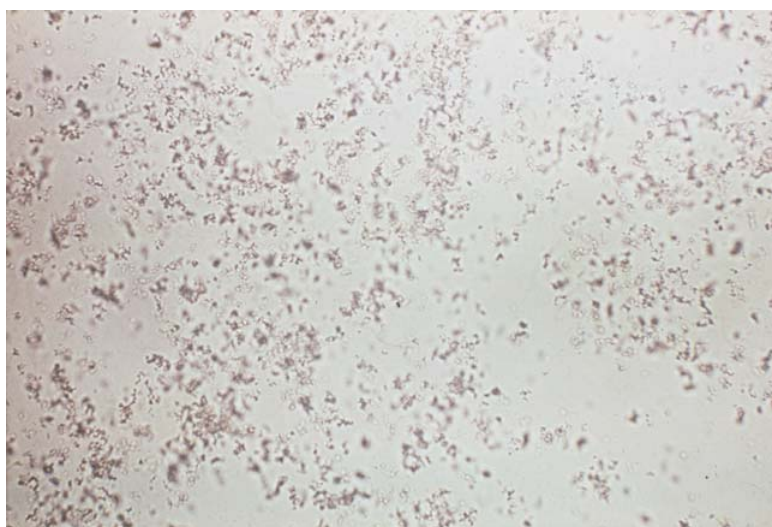


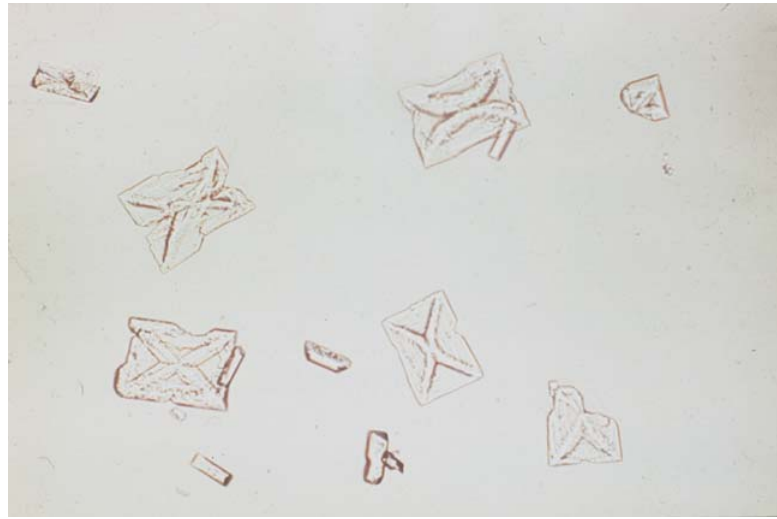
Figure 3-17. Amorphous phosphate crystals.

#### *Triple phosphate*

Triple phosphate (ammonium-magnesium phosphate) crystals are present in alkaline or neutral urine samples. They are colorless, birefringent, vary in size, and usually have a prismatic form that is described as a coffin lid. Triple phosphate crystals are soluble in acetic acid. They may also assume a



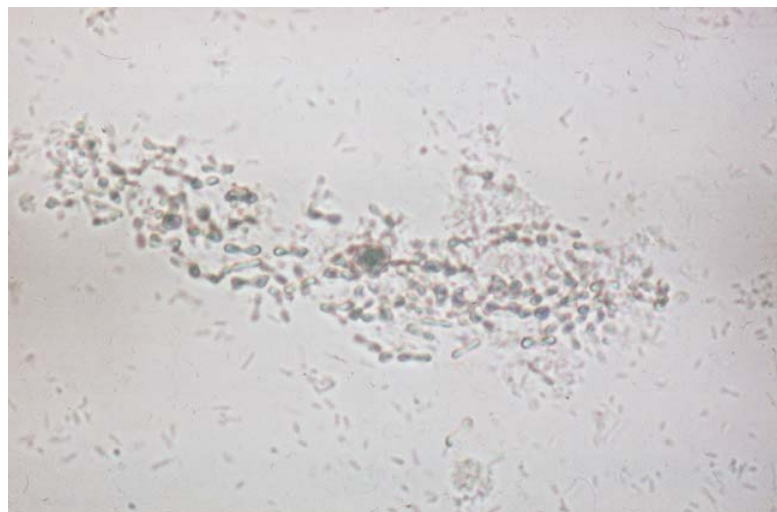
feathery or leaf-like appearance upon going into solution, as shown in figure 3-18. Generally, they have no clinical significance but if present in fresh urine specimens, they may be associated with a UTI or obstruction.



**Figure 3-18. Feathery form of triple phosphate crystals.**

#### *Calcium carbonate*

Calcium carbonate crystals are small, colorless, granules or spherules of dumbbell-shaped, as shown in figure 3-19, birefringent crystals found in alkaline urine. Occasionally they are confused with bacteria because of their small size. They are soluble in acetic or hydrochloric acid and, when the acid is added to the sediment, they produce bubbling due to the release of carbon dioxide. They are seen in normal urine samples from patients who ingest large quantities of vegetables and are of no clinical significance.



**Figure 3-19. Calcium carbonate crystals with bacteria for comparison.**

#### *Ammonium urate*

Ammonium urate crystals are the only urate crystals that appear in alkaline urine. They are yellowish-brown or golden brown. They have two basic forms that often occur together: spheroids or spheres with radiating spicules, as shown in figure 3-20. Ammonium urate crystals are described as crab-like,

thorn-apple, and scorpion-like. They are soluble in sodium hydroxide, and produce ammonia gas as they dissolve. If acetic or hydrochloric acid is added to the urine, these crystals slowly dissolve and are replaced by uric acid crystals. They also dissolve slowly when heated, but re-crystallize upon cooling. Ammonium urate crystals are thought to be of no clinical significance, but can form when ammonia is present in the urine in bacterial cystitis.



Figure 3-20. Golden brown ammonium urate crystals with radiating spicules.

#### ***Abnormal urinary crystals in acid urine***

It is important to note that abnormal crystals are found only in urine with an acid or neutral pH. Now that you have an idea of the normal crystals found in acid urine, you can readily identify the abnormal crystals.

#### ***Tyrosine***

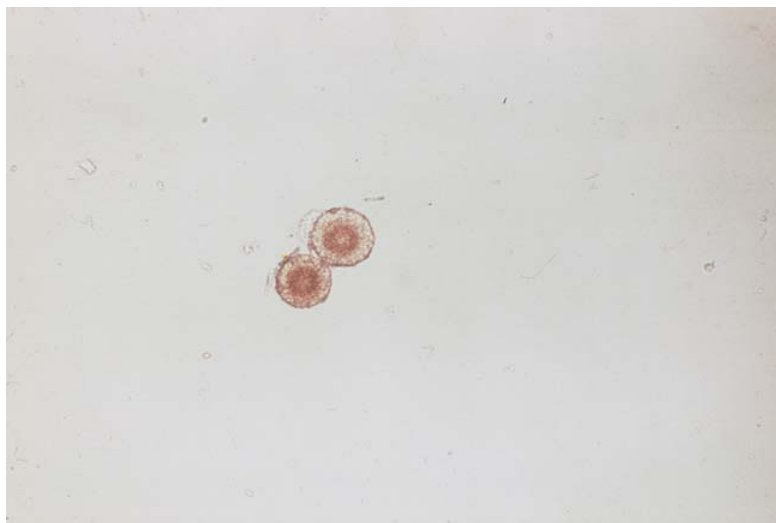
Tyrosine crystals are rarely found and are difficult to see. They have a relatively low refractive index and are birefringent. Tyrosine crystals are colorless to yellowish-brown and have a fine, delicate, silky needle-shaped appearance, as shown in figure 3-21. The needles may be single or arranged in clusters or sheaves. They are insoluble in acetic acid, alcohol, and ether; but soluble in hydrochloric acid, sodium hydroxide, and boiling water. Tyrosine and leucine crystals are often found together and both are products of protein metabolism. They are associated with tissue degeneration or necrosis; especially severe liver disease.



Figure 3-21. Tyrosine crystals.

### *Leucine*

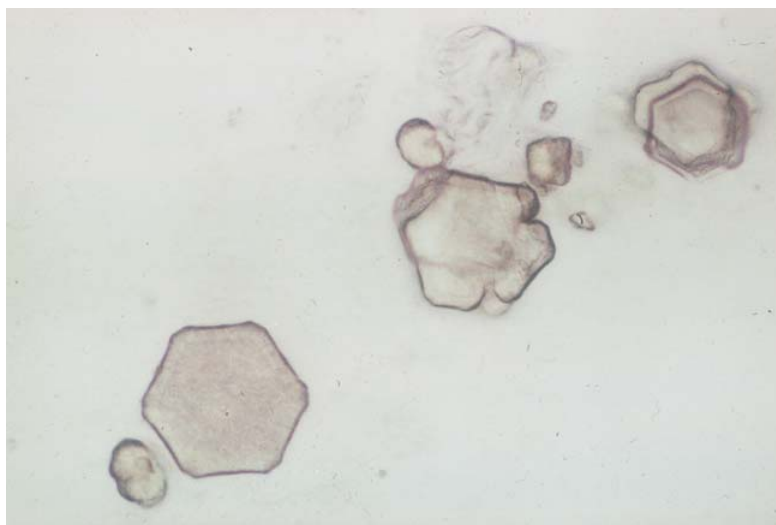
Leucine crystals are highly refractile and appear as spheroids with a dense central area or nidus surrounded by concentric bands, as shown in figure 3-22. Spokes radiate from the nidus and cross the concentric bands. They are yellowish-brown and sometimes oily looking; don't confuse them with free fat globules or pollen grains. Leucine crystals are insoluble in acetic acid, hydrochloric acid, and ether; but soluble in sodium hydroxide, boiling water, and hot acetic acid. Leucine crystals are rarely found in urine and are associated with severe liver disease (e.g., fulminate hepatitis, advanced cirrhosis, congenital metabolic conditions, and hepatocellular poisons).



**Figure 3-22. Leucine crystals.**

### *Cystine*

Cystine is an amino acid and is rarely a constituent of urine. Cystine crystals are usually flat, thin, six-sided (hexagonal) plates that are clear and colorless, as shown in figure 3-23. The sides may or may not be the same length. The crystals can join together, overlie, or overlap each other, or fuse into a rosette configuration. They display birefringence with polarized light. Cystine crystals are insoluble in acetic acid, ether, alcohol, and boiling water. They are soluble in hydrochloric acid, sodium hydroxide, and ammonium hydroxide. Cystine crystals are most often seen in the urine of patients with inherited metabolic disorders involving the reabsorption of cystine by the epithelial cells of the nephron.



**Figure 3-23. Cystine crystals.**



### *Cholesterol*

Cholesterol crystals are found in acid and neutral urine; frequently after refrigeration. They are large, flat, transparent rectangular plates with irregular sides that often show a missing corner or staircase pattern. They are birefringent and often appear together and overlap each other. See figure 3-24. Cholesterol crystals are insoluble in dilute acids or alkalis; and are soluble in chloroform, ether, and boiling alcohol. These crystals are always considered pathogenic and are found in the urine of patients with various renal diseases, the nephrotic syndrome (edema, lipiduria, lipidemia, and hypercholesterolemia), and lymphatic obstruction due to neoplasms or parasitic disease (filariasis).

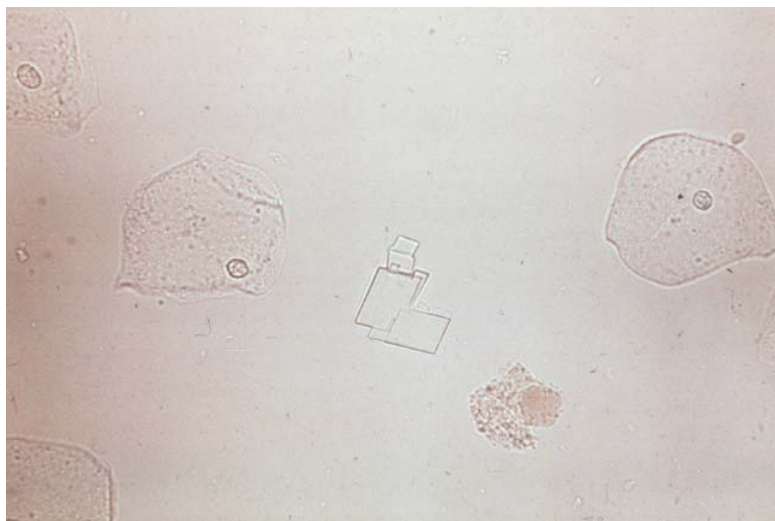


Figure 3-24. Cholesterol crystals with squamous epithelial cells; note missing corner.

### *Sulfadiazine*

Although most sulfanilamide drugs are currently manufactured with water solubility in mind, you may occasionally observe sulfonamide crystals in acid urine. The shape and color of these crystals can vary, depending on the specific sulfonamide being administered. The most common is sulfadiazine crystals which are birefringent, brownish-yellow, and assume various needle-like shapes. They often appear as fan-shaped bundles or sheaves of wheat, as shown in figure 3-25. Other sulfonamide crystals range in shape from amorphous granules to well-formed plates, whetstones, and hexagons. These shapes are morphologically indistinguishable from uric acid crystals. Sulfonamide crystals are insoluble in dilute acetic acid, but are soluble in acetone, strong acids (e.g., hydrochloric acid), and strong alkalis (e.g., sodium hydroxide).

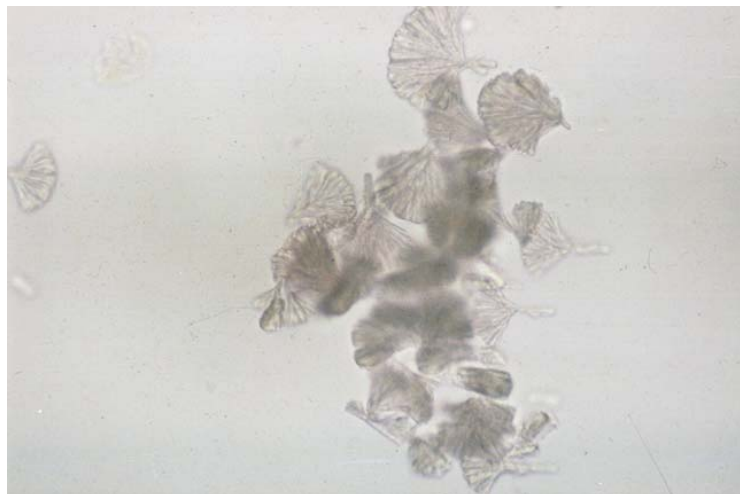


Figure 3-25. Sulfa crystals.

## **Casts**

Urinary casts are defined as cylindrically shaped, formed, microscopic elements that form in the renal tubule (usually the distal or collecting tubule). They occur in the urine of both healthy persons and those with renal disease or other pathological conditions. In normal individuals, the cylinders that may be present are hyaline and granular casts. If other casts are seen, they should be considered abnormal or clinically significant. Urinary casts can be categorized or grouped on the basis of their microscopic appearance and whether or not they contain cellular constituents. Therefore, casts are classified as acellular or cellular.

### ***Formation of casts***

The phenomenon of cast formation isn't completely understood, yet it is known that alterations in urine flow, pH, ionic strength, concentration, and salinity of the glomerular filtrate play a role. As stated earlier, the matrix of all casts is primarily composed of the kidney-specific protein called Tamm-Horsfall mucoprotein (THM), and other immunoproteins (e.g., IgG and IgM). As THM is excreted by the cells that line the distal and collecting tubules, certain events in the glomerular filtrate cause this soluble protein to become insoluble. This insoluble protein forms thin proteinaceous bands that can be molded into casts. As gelatin assumes the exact shape of the container, likewise, the cast thus formed represents an exact model of the shape of the lumen of the renal tubule in which it originates. Because of this, they often mirror particular diseases of the kidneys and can accurately reflect the course and prognosis of a specific renal disease.

### ***Acellular casts***

As the name implies, acellular casts do not contain cellular elements.

#### ***Hyaline***

Whether from a healthy individual or a patient with renal disease, hyaline casts have the same basic microscopic appearance. They are typically translucent, colorless, cigar-shaped, and are somewhat difficult to see because of their low refractive index. Hyaline casts have a smooth, wavy, or finely wrinkled surface and a smooth outer perimeter, as shown in figure 3-26. Their ends are usually curved and smooth, but occasionally one end may be drawn out into a tail or point. In the past, hyaline casts with a tail were called cylindroids. This term is considered out-of-date and should not be used. Sometimes various types of inclusions (granules or cells) may be present within or on the cast. One or two of these inclusions should not change the nomenclature—it's still a hyaline cast. The hyaline cast can be regarded as the prototype for all the other casts because of its transparency, cigar-shape, and cylindric gel of protein that has a smooth or finely wrinkled surface.



**Figure 3-26.** Hyaline cast with a drawn out end.

- Normal or abnormal. As stated earlier, hyaline casts are considered normal or abnormal. Strenuous physical exercise and stressful emotional situations can invoke cylindruria (increase of numbers of casts in the urine). Hence, if the patient has undergone physical or emotional stress within the past 24 hours, cylindruria should not be considered pathological. In these situations, the urine reverts to a normal state within 24 to 48 hours after physical exercise is stopped or the emotional situation has passed. In disease states, hyaline casts have the same microscopic appearance as in normal states. However, if broad (wider) hyaline cast forms are seen, it is usually an indication of serious renal disease.
- Staining procedure. By using an ordinary brightfield microscope and not adjusting the contrast, many inexperienced microscopists miss these elements. Therefore, some authorities suggest the routine use of a staining procedure or a phase-contrast microscope for urinary sediment examination.

#### *Finely granular*

Granular casts are small or large (broad). The granules are diffusely scattered over the entire surface or concentrated in one or more sections of the cast. Depending on the granules size and shape they are termed finely granular or coarsely granular. Granular casts can also be seen in normal urine under the same circumstances as mentioned above. The finely granular cast is the one usually associated with normal states. Although granular size has long been a topic of debate and discussion, if the granules are small, regular, and difficult to distinguish from one another they are considered finely granular. See figure 3-27. Because of the granules, these casts have a slightly higher refractive index than do hyaline casts and are easier to see with brightfield microscopy. Fine granular casts are seen in patients with any type of intrinsic renal disease.



**Figure 3-27. Finely granular cast; note uric acid crystal.**

#### *Coarsely granular*

The granules of the coarse granular cast are large and vary greatly in size and shape. See figure 3-28. These casts are, as a rule, seen in renal diseases where extensive cellular damage and death have occurred. The origin, within the renal tubules, of granular and hyaline casts is still not clear.



**Figure 3-28. Coarse granular cast with numerous red blood cells and an occasional white blood cell.**

### *Waxy*

Of all the urinary casts, waxy casts are the easiest to identify under ordinary brightfield microscopy. They are characterized by blunt, broken-off or cut-off ends (not rounded), notched or indented sides that are parallel, tallow-wax surface, and high refractive index, as shown in figure 3-29. Waxy casts may be narrow or broad; any broad size or shaped cast usually denotes a relatively severe renal abnormality. They can be colorless, pale yellow, or tan in appearance with a smooth or hard-looking surface. The waxy cast surface may also contain a few granules or cellular elements. The origin of the waxy cast is also unclear. The presence of large numbers of waxy casts in urinary sediment indicates severe, chronic renal disease and is associated with nephron destruction, oliguria, and renal failure.



**Figure 3-29. Typical waxy cast.**

Figure 3–30 is an example of a mixed (waxy and fatty) cast. The identification of mixed casts may require staining procedures, phase-contrast microscopy, and/or chemical analysis before making a definitive identification, especially with fatty casts, and fat globules.

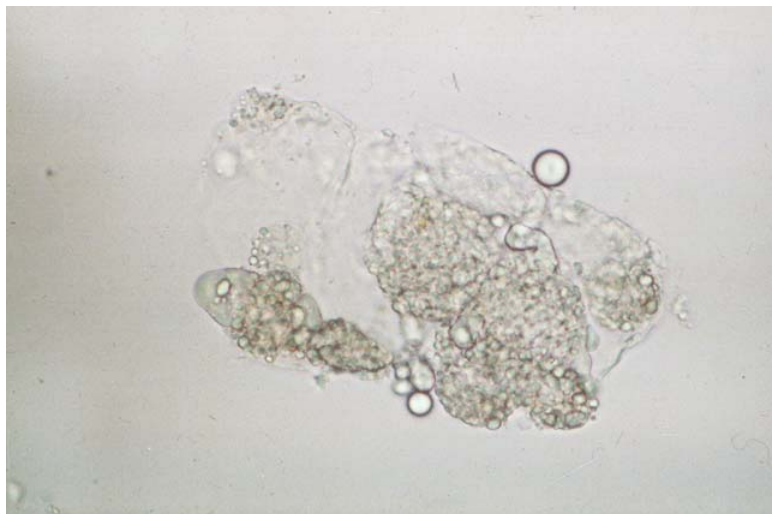


Figure 3–30. Mixed cast (waxy and fatty) with fat globules and granules superimposed.

### ***Cellular casts***

Depending on the location, duration, type, and cause of nephron damage, cellular elements may leak or be sloughed into the urine. As these elements enter the urine, they may be incorporated into the cast as it forms. Let's look at the various types of cellular casts observed in urinary sediment.

#### ***Red blood cell***

Most texts agree that the red blood cell cast is the most diagnostically significant of all the formed elements found in urinary sediment. The presence of red blood cell casts ordinarily indicates damage to the glomerular basement membrane (GBM). However, they may also enter the urine through the disruption of the tubular basement membrane (TBM). Red blood cell casts are, in essence, hyaline casts with red blood cells attached to the matrix by thin fibrils. It is thought that the Tamm-Horsfall mucoprotein serves as a type of glue that attaches the cells to the surface of the cast. It seems apparent that the red blood cells attach after the cast has been formed in the distal tubule or collecting duct. Because red blood cells contain hemoglobin, they appear tan or brown in color and, therefore, are easily recognized. Keep in mind that the red blood cells may be crenated or show degeneration and can be sparsely or closely packed on the cast. See figure 3–31. The cast can show dysmorphism, and appear distorted or twisted. Red blood cell casts are seen in patients with glomerulonephritis, lupus nephritis, renal infarction, and pyelonephritis. The first two are associated with GBM damage and the later are associated with TBM damage.



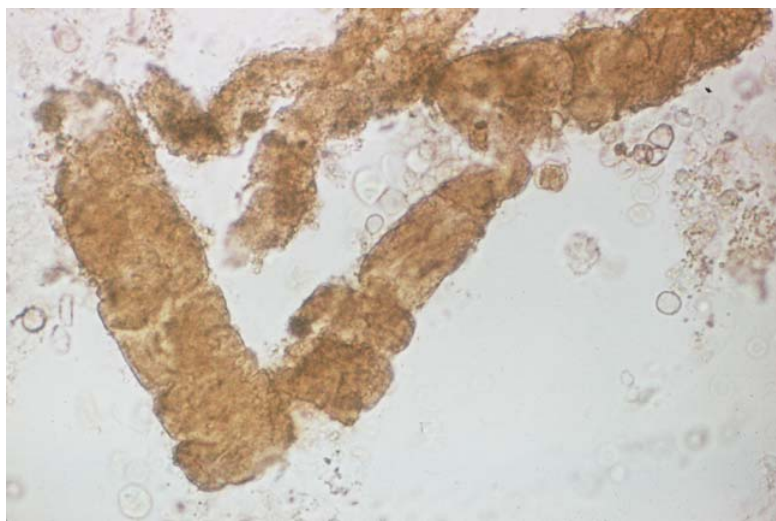


**Figure 3-31. Red blood cell cast with intact, sparsely packed cells.**

**NOTE:** Hyaline casts are the prototype for all casts and occasionally have cellular elements attach or caught-up in the casts. So how many red blood cells must be present to call it a true red blood cell cast and not a hyaline cast? This question is not easily answered and each text presents its own rule of thumb. Nonetheless, each text agrees it is more than 1 or 2 cells, and that there shouldn't be any doubt of the diagnosis. One text stated at least  $\frac{1}{3}$  of the cast surface should be composed of the cellular element. Hopefully your laboratory's OI can provide some guidance or answers to this question.

### *Hemoglobin*

As red blood cell casts pass through the nephron, they may be disrupted enough to leak or release their hemoglobin into the surrounding cast. In this instance, only a few or no intact red blood cells may remain on the cast. The only elements left are the membranes of the red blood cells and the brownish-red hemoglobin pigment in the cast. Hemoglobin casts vary greatly in shape and size, have a high refractive index, and are a brownish-red or a brownish-tan in color, as shown in figure 3-32.



**Figure 3-32. Hemoglobin casts surrounded by abnormal red and white blood cells.**

### *White blood cell*

White blood cell casts found in urinary sediment indicate a pathological condition and, consequently, are not present in normal urinary sediment. White blood cells are attached to the cast much the same way as red blood cells—by fibrillar protein bands. They enter the urinary stream through two primary pathways—transglomerular or transtubular. In the transglomerular pathway, white blood cells migrate from the bloodstream across the GBM and into the Bowman's capsule space as a component of the glomerular filtrate. In the transtubular pathway, white blood cells move by ameboid motion through the TBM into the tubular lumen. The transglomerular mechanism is seen in glomerular damage that induces an immune response; as in lupus nephritis, polyarteritis nodosa, and acute glomerulonephritis (e.g., streptococcal AGN). The transtubular mechanism is seen in intrinsic renal infections; as in acute pyelonephritis and interstitial nephritis. White blood cell casts are also easy to identify. They appear on the cast much like they do in blood or in urine. See figure 3-33. The cast normally consists of PMN, but rarely may be composed of lymphocytes or monocytes. Sometimes the white blood cells show marked degeneration in which the nucleus disappears, the cell membrane is disrupted, and the cast seems granular. White blood cell casts are associated with kidney infections and may be seen in bacteriuria, if the inflammation is initiated by bacteria.



**Figure 3-33. White blood cell cast with intact and easily identified cells.**

### *Epithelial*

Epithelial cells originate from renal tubular epithelium lining the nephron, and vary morphologically in each segment of the nephron, as well as through the urinary system (i.e., upper or lower urinary tract). But, because casts form only in the kidney, it is sufficient to identify these casts as epithelial in origin, and not by the specific type of epithelial cell, as stated in the previous lesson. Epithelial cells are often sloughed off the tubule into the urinary stream and then attached to the cast matrix the same way red blood cells and white blood cells are secured. See figure 3-34. The cells of the epithelial casts have a distinct cell membrane, usually a large nucleus, a sparse amount of agranular cytoplasm, and a polyhedral, elongate, or columnar shape. Epithelial casts are often confused with white blood cell casts, but remember, white blood cells are smaller, have a granular cytoplasm, and have multilobed nuclei. Epithelial casts are easily noticed because of their high refractive index. The casts can be narrow or broad and the cells may also undergo degeneration. Epithelial casts are not found in the urinary sediment from healthy individuals, so their presence is clinically significant. They are present in a wide variety of renal diseases, but are most prominent in nephrotic syndromes (e.g., ingestion of nephrotoxic substances).



**Figure 3–34. Epithelial cast with cell imbedded in a hyaline cast matrix.**

#### *Miscellaneous casts*

It is important to remember that there are other casts, as well as crystals, than those discussed in this lesson. Crystal casts, bacterial casts, and bilirubin pigmented casts; and calcium phosphate, bilirubin, and hemosiderin crystals may be observed in urinary sediment. Various therapeutic or diagnostic compounds can also produce many different crystals.

#### **Miscellaneous elements found in urinary sediment**

At this time, we are going to talk about some of the unusual elements that are found in urinary sediment. They are normal or abnormal findings and may be confused with the aforementioned elements.

#### ***Parasites***

Although rarely found, certain parasites may be present in urinary sediment.

##### *Trichomonas vaginalis*

In the United States, *Trichomonas vaginalis* is the most frequently encountered parasite found in urinary sediment. It is ordinarily a contaminant from the vagina. *T. vaginalis* is an organism that inhabits the distal urethra of men and women, the vagina, and rarely, the prostate gland. It is spherical or pear-shaped and is 10 to 30  $\mu\text{m}$  in length. The organism has four anterior flagella and an undulating membrane that covers about half of its length. *T. vaginalis* is very motile in freshly voided urine; it can move in a rapid, jerking motion or a slow, gentle rotation. These organisms can be mistaken for PMNs, because they are usually accompanied by a marked exudation of PMNs. Their elliptical shape, flagella, and motility should help you distinguish between the two elements. See figure 3–35.

##### *Enterobius vermicularis*

*Enterobius vermicularis* (pinworm) is a parasitic organism commonly found in children. During the night, the adult female worm migrates to the perirectal region and deposits her eggs. If the urine specimen is improperly collected or is contaminated by fecal material, pinworm ova may be found in the urinary sediment. The eggs or ova measure approximately 25 by 50  $\mu\text{m}$ . They are oval in shape with one side slightly flattened, as shown in figure 3–36.



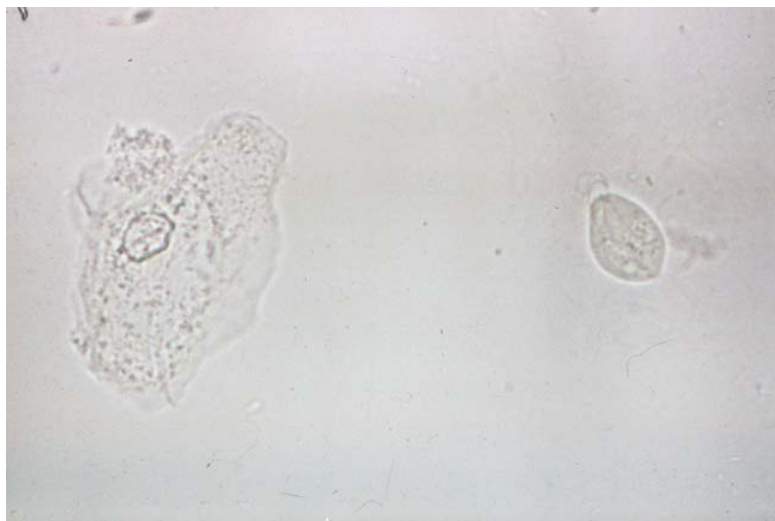


Figure 3–35. *Trichomonas vaginalis* with a squamous epithelial cell.



Figure 3–36. *Enterobius vermicularis* ova seen in urine.

### *Schistosoma hematobium*

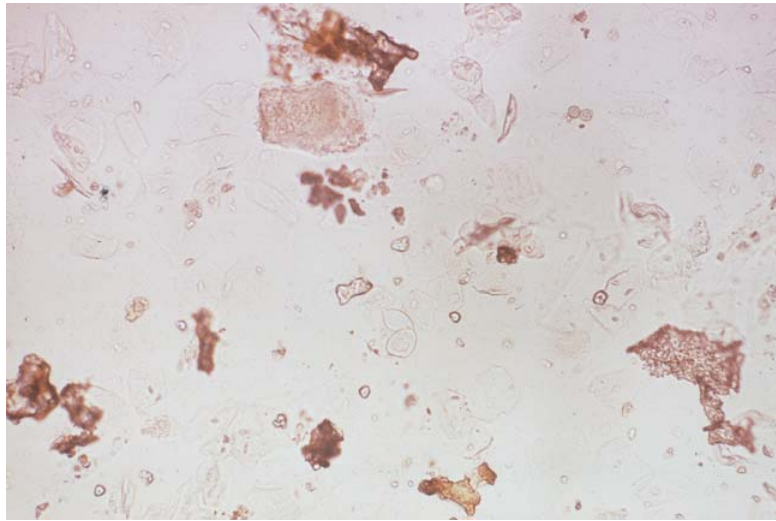
*Schistosoma hematobium* is a parasite indigenous to the urinary tract. It is of great importance on a worldwide basis, but isn't found in the United States. It is common in Africa, Egypt, Syria, Iraq, and Iran. This parasite and the two mentioned above will be discussed in greater detail in volume 4 of the 4T051B course.

### **Artifacts**

In the examination of urinary sediment, distinguishing between actual urinary constituents (normal and abnormal) and contaminants or artifacts is of primary importance. Let's review a few of the most common artifacts found in urine.

*Fecal contamination*

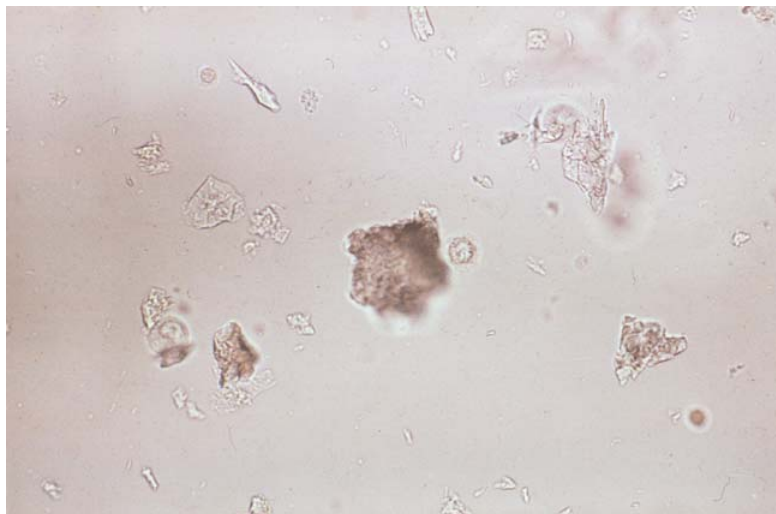
Fecal contamination is seen mostly in incontinent babies and elderly persons. It is generally identified by its brown coloration, assortment of bizarre shapes, large columnar cells, and wide range of sizes, as shown in figure 3-37.



**Figure 3-37. Fecal contamination.**

*Talc granules*

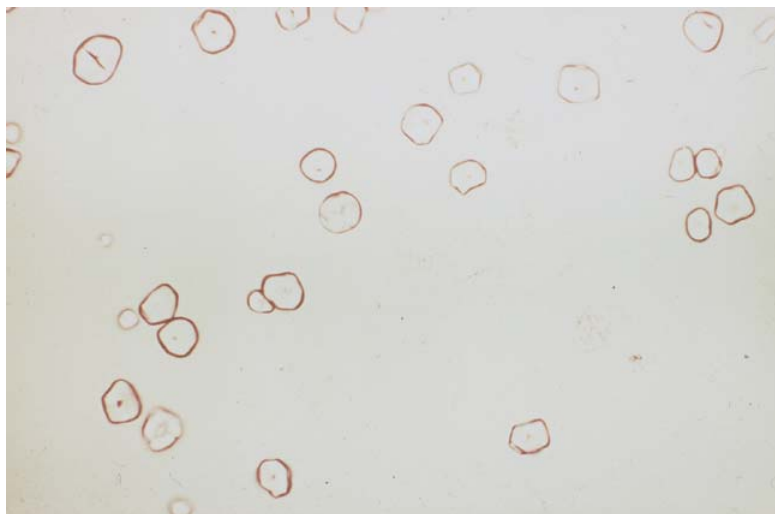
Talcum powder is commonly used to prevent chafing or a heat rash. It appears in urine as a colorless to pale brown, amorphous crystal. See figure 3-38.



**Figure 3-38. Talc granules.**

### *Starch granules*

Various body dusting powders and surgical gloves contain cornstarch or a starch derivative, therefore, starch granules are commonly found in urine specimens collected by catheterization. They have a relatively regular spherical shape, are large, and have a high refractive index. Refer to figure 3-39.



**Figure 3-39. Starch granules.**

### *Fiber*

Hair and cotton fibers frequently appear in urinary sediment. These animal and plant fibers have a high refractive index, possess anisotropic properties, and are usually much longer than urinary casts, as shown in figure 3-40.



**Figure 3-40. Fiber.**

## **415. Miscellaneous urine procedures and automation in urinalysis**

You'll close out this unit, and volume, with an introduction to miscellaneous urine procedures and automation in urinalysis. As you can probably gather from the previous lessons and units, urine is

comprised of varied and numerous chemical constituents. It would be difficult to research all of these constituents in this volume, thus, only an introduction is presented.

### 24-hour urine

Clinically it is often valuable to know the total amount of an analyte excreted in the urine during a 24-hour period. A random urine sample doesn't provide this information; therefore, a 24-hour specimen is collected. After the collection, the specimen is processed. This includes measuring the total volume of urinary output and aliquoting the specimen into proper containers for analysis. 24-hour urine specimens are collected in order to obtain a quantitative analyte value. Results are reported as mg/dl or in other units as indicated by the test method. Depending on the total volume of urine voided, the amount of the analyte excreted by the kidneys in a given day can be easily calculated using appropriate formulas. The table shows some of the tests or procedures ordered on 24-hour urine samples. Some of these procedures can be performed on random urine specimens (e.g., electrolytes: sodium, potassium, and chloride).

Procedure		
Creatinine	Electrolytes (Na, K, Cl)	Osmolality
Aldosterone	Estrogen	Oxalate
Amino acids	Homogentisic acid	Phosphorous
5-Aminolevulinic acid	Homovanillic acid	Porphyrins
Calcium	5-Hydroxyindole-acetic acid	Pregnanetriol
Catecholamines	Hydroxyproline	Total protein
Cystine	Nitrogen	Uric acid
Beta-hCG	17-Ketosteroids	Urobilinogen
Citrate	Magnesium	Vanillylmandelic acid
Copper	Mercury	Zinc
Cortisol (free)	Metanephrines	

As with all procedures or tests ordered, check your laboratory's OI for the type and amount of specimen required, whether it is performed in your laboratory or a reference laboratory, the principle or methodology used, and the reportable units. The majority of the analytes in the above table are performed in the same manner as a blood, plasma, or serum analysis. Most instrumentation performs the same procedure on urine (with little specimen preparation, manipulation, or treatment) as with serum.

### Urinary calculi or kidney stones

The formation of urinary calculi is a common occurrence within the population of the United States. Calcium oxalate calculi are the most prevalent, with onset between 30 and 40 years of age. Calcium oxalate calculi are more common in males than in females. Subsequent recurrences of calculi are frequent, but with appropriate identification of the calculi and their associated risk factors, their formation can be greatly reduced. Children are rarely affected with calcium oxalate calculi. Upper (renal) calculi are most commonly seen in Western industrialized countries, whereas lower (bladder) calculi are not. Other types of calculi include phosphate, cystine, and uric acid.

### *Passage of calculi*

The passage of urinary calculi through the ureters produces renal colic; this is characterized by severe pain in the back, radiating to the groin. If the calculi is passed through the urethra, hematuria with extreme pain is common. Calculi that obstruct the pelvis of the kidney or ureter can cause hydronephrosis. Infection is a common consequence from urinary calculi.

### ***Investigation of patients who form urinary calculi***

The following is a list of laboratory tests used to investigate the formation of urinary calculi in specific patients.

- *Urine examinations:* Routine urinalysis, qualitative test for cystine, and urine culture.
- *24-hour specimens:* Sodium, calcium, phosphorous, uric acid, oxalate, and creatinine clearance.
- *pH:* Urine pH determination on a fresh specimen is important in determining the kinds of crystals likely to be precipitated.
- *Serum chemistry:* Calcium, phosphorus, uric acid, and electrolytes.

### ***Procedures***

Procedures performed in histology, reference laboratories, or other departments in the MTF for investigating urinary calculi.

- *Calculi or stone analysis:* Gross, microscopic, and chemical examinations.
- *Radiological examination:* Asymptomatic stones are sometimes found. All stones are radiopaque except pure uric acid and the rare xanthine; cystine stones are opaque due to their sulfur content.

### ***Automation***

Urinalysis is not immune to the trend towards automation in the clinical laboratory. Urinalysis instrumentation ranges from semiautomated to completely automated.

#### ***Semiautomated***

Some manufacturers of reagent strips have designed semiautomated, bench-top instruments that can read reagent strips. The strip is placed on a conveyer belt-like platform. The platform moves the strip into a reading chamber. The strip is read by a reflectance spectrophotometer that analyzes the color intensity of the light reflected from the reagent area. The instrument prints the results in clinically meaningful units. Advantages of semiautomated instruments include standardizing incubation times and interpreting color intensity of the reaction on the reagent pad.

#### ***Automated***

Some urinalysis instrument workstations combine several automated subsystems to perform a complete urinalysis. Specific gravity is measured by a mass gravity meter, urine chemistries are measured by a standard reflectance spectrophotometer, and the microscopic analysis is simplified with an automated intelligent microscope system. No centrifugation is required and specimen handling is minimal. In the analysis, the urine specimen is poured into the instrument's entry port over a urine chemistry reagent strip. The reagent strip is then placed in the reflectance spectrophotometer reader platform. The urine chemistries are automatically timed, read, and collated by the internal computer. A portion of the specimen is diverted to the harmonic oscillator mass gravity meter for specific gravity determination. Another portion of the sample is stained and passed into a laminar flow chamber where the formed elements are detected and imaged by a video camera mounted to a microscope and a stroboscopic lamp that allows stop-motion images. Electronic centrifugation takes place as the computer recognizes and discards empty images. Images that contain sediment elements are sorted by size and presented to the operator on the screen for identification. Because the volume of the laminar flow chamber is known, the images are counted and related to a volume of urine with precision that exceeds that which is obtained by centrifuging the specimen and using a slide and coverslip.

### ***Pregnancy***

Pregnancy tests and principles are discussed in-depth in *Immunology*, volume 2, 4T051C, because of the antigen-antibody techniques utilized in these methods.

### Quality control

Urinalysis quality control, like other laboratory section QC, includes running high and low matrix controls, equipment and instrument maintenance, and method validation. Matrix controls are solutions that mimic urine and contain known concentrations of the analytes tested for with the reagent strip, test tablet, or chemistry analyzer. All the guidelines that are included in a good quality assurance program apply to the urinalysis section.

### Self-Test Questions

After you complete these questions, you may check your answers at the end of the unit.

#### 411. Urine specimen processing and physical characteristics of urine

1. Match the terms in column B with the statement in column A by placing the letter of the column B item beside the number of the column A item that most nearly describes it. Each element in column B may be used once, more than once, or not at all.

<i>Column A</i>		<i>Column B</i>	
___ (1)	Difficult to define or describe, but can indicate certain disease processes.	a.	Normal constituents.
___ (2)	Relates to the hydrogen ion ( $H^+$ ) concentration or activity of a solution compared to a given standard solution.	b.	Polyuria.
___ (3)	The ratio of density (weight per unit volume) of a solution compared to the density (weight per unit volume) of an equal volume of water at a constant temperature.	c.	Oliguria.
___ (4)	Derived from the food we have eaten or are the waste products of metabolism.	d.	Anuria.
___ (5)	Some shade of yellow: almost colorless, yellow-green, straw, pale yellow, bright yellow, yellow-orange, light amber, or dark amber.	e.	Color.
___ (6)	Defined as the particles dissolved in a solution.	f.	Appearance.
___ (7)	Results from the presence of urochrome, uroerythrin, and urobilin.	g.	Foam.
___ (8)	Total lack of urine output.	h.	Odor.
___ (9)	The ratio of the velocity of light in air to the velocity of light in solution.	i.	Specific gravity.
___ (10)	Valuable test when estimating the kidney's concentrating ability.	j.	Refractive index.
___ (11)	Occurs particularly in diabetes mellitus and diabetes insipidus.	k.	Osmolality.
___ (12)	Total urine volume is more than 2 liters in a 24-hour period.	l.	pH.
___ (13)	Described as clear, hazy, cloudy, and turbid.		
___ (14)	Urinary output of less than 500 ml in a 24-hour period.		
___ (15)	Urea, sodium chloride, phosphoric acid, sulfuric acid, uric acid, hippuric acid, creatinine, ammonium, potassium, calcium, oxalate, phosphate, amines, amino acids, proteins, enzymes, purines, leukomaines, urobilin, sugars, cholesterol, hormones, fatty acids, vitamins, and metals.		

#### 412. Urinalysis—routine chemical procedures

1. What is on the reaction or reagent pads of reagent strips?
2. Where can you find the incubation time sequence and corresponding color chart for the reagent strips?
3. What can the color change on the reagent pad represent?
4. What are the sugars that can be found in urine with the most common listed first?

5. What determines how high blood glucose levels will rise before glycosuria occurs?
6. How do you define renal threshold?
7. Screening urine specimens for glucose is the best method for detecting what disorder?
8. What other pathological conditions can cause glycosuria?
9. What are some of the normal conditions that can cause glycosuria?
10. What is a brief definition of reducing substances and name a few?
11. What is a more specific test than the Benedict' reaction for glucose and why was it required?
12. What can cause false-negative or decreased results with this test?
13. Why is it important to detect bilirubin in the urine?
14. What reagent is on the reagent strip and how is bilirubin detected?
15. Why must the urine specimen be absolutely fresh for bilirubin testing?
16. What can cause false-positive and false-negative results, respectively?
17. When are ketone bodies found in the blood and urine?
18. What are the three ketone bodies found in urine?
19. What effect do high ketone levels have on the human body?
20. How does the reagent strip detect ketone bodies and which ketone body does it detect?

21. A urine specimen specific gravity above 1.025 is a characteristic of which clinical conditions?
22. What is the principle of the specific gravity indirect test method?
23. What is the confirmatory test for specific gravity?
24. Hematuria indicates what diseases or disorders?
25. Hemoglobinuria can be associated with what conditions?
26. In what conditions is myoglobinuria seen?
27. How are hematuria, hemoglobinuria, and myoglobinuria distinguished?
28. What reagents are in the reagent pad for hemoglobin and what is the basis for detection?
29. What is the normal pH range of urine and what is the mean pH?
30. What reagents does the pad contain for measuring pH?
31. What is probably the most significant pathological condition found in routine urinalysis and what causes this condition?
32. What protein replaces albumin as the predominant protein found in the urine of patients with multiple myeloma?
33. What conditions can cause normal or healthy persons to excrete abnormal quantities of protein in the urine?
34. What is the principle of the reagent strip for protein and what is the basis of detection?
35. Depending on the patient population, what may be required for protein testing?



36. What is the most common precipitation procedure used for protein testing and how is the actual type of protein present identified?
37. Under what conditions is urobilinogen completely absent from urine and feces?
38. How is urobilinogen and other bile pigments detected?
39. Which test is a rapid screening method to detect the presence of bacteria in urine?
40. What does the nitrite test depend upon?
41. What can the leukocyte esterase test, in conjunction with the nitrite test, successfully detect?
42. The reagent strip for the leukocyte esterase tests is based on what test methodology?

**413. Urinalysis—microscopic procedures**

1. Under what circumstances is a microscopic examination usually performed?
2. What is the specimen of choice for urine microscopic examinations and why?
3. What items or areas of urine specimen processing should be standardized?
4. What are the stains used for identifying elements of urinary sediment?
5. Read the statements below and determine the correct term for each statement. Locate and circle the term in the block of letters. Terms may be listed forwards or backwards, horizontally, vertically, or diagonally.

S	Q	U	A	M	O	U	S	Q	W	E	R	T	U	T
D	F	G	H	P	V	Q	X	Z	C	B	N	Y	O	R
R	S	D	C	T	A	Y	H	J	L	K	M	E	P	A
E	U	I	Y	T	L	M	S	X	C	P	O	A	G	N
N	F	C	P	O	F	A	T	T	Y	C	A	S	T	S
A	R	T	Y	U	A	X	B	N	W	X	I	T	A	I
L	K	G	L	I	T	T	E	R	C	E	L	L	M	T
H	R	V	Q	P	B	Q	Z	W	G	B	C	F	M	I
Z	A	Q	W	D	O	F	H	I	J	B	R	Y	H	O
M	U	C	U	S	D	Z	Y	R	P	Z	C	V	O	N
Q	R	T	Y	J	I	F	P	E	D	C	B	L	R	A
X	Z	L	N	M	E	V	E	D	Y	S	D	E	S	L
K	J	G	L	V	S	D	R	B	M	Q	C	U	F	H
S	T	R	Q	B	K	Z	T	L	X	R	R	M	A	N
W	H	I	T	E	B	L	O	O	D	C	E	L	L	S
B	Y	C	D	Z	L	G	N	O	W	R	N	U	L	P
A	P	U	B	S	L	R	I	D	I	K	A	J	P	E
I	O	K	U	D	L	V	C	C	S	M	T	V	R	R
R	T	D	B	B	E	G	Y	E	U	P	E	Q	O	M
E	O	A	B	K	C	N	M	L	C	R	D	W	T	A
T	N	C	L	V	T	U	G	L	V	N	C	D	E	T
C	I	H	E	I	S	Q	X	S	Y	R	E	V	I	O
A	C	X	S	N	O	B	K	L	G	U	L	P	N	Z
B	Z	D	M	P	H	V	M	W	S	A	L	A	D	O
L	I	P	I	D	G	L	O	B	U	L	E	S	U	A

Statements	
1. Largest of the epithelial cells found in urine.	11. Absence of motility due to urine toxicity.
2. Line the renal pelvis and calyces, ureter, and urinary bladder.	12. Can be either a bacillary or coccal form.
3. Line the tubules within the nephron.	13. May be confused with fat globules or red blood cells.
4. Renal tubular epithelial cells filled with absorbed globules of lipid.	14. Common element found more frequently in women than in men.
5. Can be free-floating.	15. Appear to be twinkling in fresh urine.
6. Casts that contain free fat or oval fat bodies.	16. Acronym for polymorphonuclear neutrophils.
7. Increased numbers ordinarily indicates inflammatory conditions.	17. Ghost cells result in this type of urine.
8. Appear as smooth biconcave disks.	18. Crenated cells are found in this type of urine.
9. Found in hypotonic or dilute urine.	19. Immunological studies show that this is part of the mucus.
10. Found in hypertonic or concentrated urine.	20. Sometimes confused with red blood cells, but vary greatly in size.

#### 414. Identifying urinary crystals and casts

- Match the terms in column B with the statement in column A by placing the letter of the column B item beside the number of the column A item that most nearly describes it. Each element in column B may be used once, more than once, or not at all. **NOTE:** On this page you will find column A items 1-18; turn the page to complete items 19-36. Column B also is provided for you in its entirety.

##### Column A

- \_\_\_ 1. The second most frequently seen crystal.
- \_\_\_ 2. Present in alkaline urine; vary in size, and usually have a prismatic form that is described as a coffin lid.
- \_\_\_ 3. Spheroids with a dense central area or nidus surrounded by concentric bands; spokes radiate from the nidus.
- \_\_\_ 4. Large, flat, transparent rectangular plates with irregular sides that often show a missing corner or staircase pattern.
- \_\_\_ 5. May be in the shape of rods, cubes, rosettes, six-sided plates, rhombi, stars, spears, needles, barrels, and whetstone.
- \_\_\_ 6. Typically translucent, colorless, cigar-shaped, and somewhat difficult to see because of their low refractive index.
- \_\_\_ 7. Granules vary in size and shape; seen in renal diseases where extensive cellular damage and death have occurred.
- \_\_\_ 8. Presence of large numbers in urinary sediment indicates severe, chronic renal disease.
- \_\_\_ 9. The most diagnostically significant of all the formed elements found in urinary sediment.
- \_\_\_ 10. Fine, brownish tan granules that usually precipitate in neutral or acid urine that has been refrigerated.
- \_\_\_ 11. Two basic shapes: octahedral and dumbbell; octahedral shape appears as an envelope.
- \_\_\_ 12. Formation due to alterations in urine flow, pH, ionic strength, concentration, and salinity of the glomerular filtrate.
- \_\_\_ 13. Flat, thin, six-sided (hexagonal) plates, that are clear and colorless; seen in patients with inherited metabolic disorders.
- \_\_\_ 14. Classified as *acellular* or *cellular*.
- \_\_\_ 15. Should only be considered pathogenic when found in fresh, warm urine.
- \_\_\_ 16. They often appear as fan-shaped bundles or sheaves of wheat.
- \_\_\_ 17. Described as crab-like, thorn-apple, and scorpion-like.
- \_\_\_ 18. Found in the urine of patients with renal diseases, the nephrotic syndrome, and lymphatic obstruction.

##### Column B

- a. Uric acid.
- b. Amorphous urates.
- c. Calcium oxalate.
- d. Amorphous phosphates.
- e. Triple phosphate.
- f. Calcium carbonate.
- g. Ammonium urate.
- h. Tryosine.
- i. Leucine.
- j. Cystine.
- k. Cholesterol.
- l. Sulfadiazine.
- m. Casts.
- n. Hyaline.
- o. Finely granular.
- p. Coarsely granular.
- q. Waxy.
- r. Red blood cell.
- s. Hemoglobin.
- t. White blood cell.
- u. Epithelial.
- v. *Trichomonas vaginalis*.
- w. *Enterobius vermicularis*.
- x. Fecal contamination.
- y. Starch granules.
- z. Fiber.



4. What is the passage of calculi characterized by?
5. What are some of the disorders caused by renal calculi?
6. How do semiautomated instruments read the reagent strip?
7. In automated instrument methodology how are specific gravity and urine chemistries measured?
8. What does urinalysis quality control include?

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### Answers to Self-Test Questions

**409**

1. Various enzyme and transport systems coexist within the urinary system; some are related while others are physically and physiologically quite separate; and the kidney has many functions that may or may not be affected in a given pathologic process or disease.
2. What passes into and out of the kidney.
3. The nephron functions of glomerular filtration, the tubules' reabsorptive capacity for water and electrolytes as manifested by their urine concentration abilities, and the kidney's ability to excrete endogenous and exogenous compounds.
4. The clearance of some substances from the kidneys.
5. The plasma concentration and excretory rate, which, in turn, involve the GFR and renal plasma flow (RPF).
6. Inulin (a naturally occurring polysaccharide); *p*-aminohippuric acid (PAH).
7. Because the necessary blood and urine assays for inulin and PAH are too difficult and too time-consuming.
8. Serum or plasma and urine creatinine.
9. Protein nitrogen (protein substances containing nitrogen) and nonprotein nitrogen (NPN) substances.
10. Urea, creatine, creatinine, uric acid, ammonia, and amino acids.
11. The use of proteins (in the form of amino acids) by the body's cells produce waste materials that contain the element nitrogen; chiefly urea—which occurs primarily in the liver through hepatic enzymes.
12. If waste products (toxic chemicals) are allowed to build up in the blood stream, all of the body's cells and systems are literally poisoned which leads to the death of the cell, organ, system, and the body.
13. Prerenal, renal, and postrenal urea azotemia.
14. Indirect; based on preliminary hydrolysis of urea with urease followed by some process that quantitates the ammonium ion; and direct methods, the condensation of diacetyl with urea to form the chromogen diazine.
15. Creatine is synthesized in the kidneys, liver, and pancreas by two enzymatically mediated reactions.
16. Dietary (exogenous) intake of creatinine causes only minor variations in daily creatinine excretion and it parallels endogenous production.
17. Because of the release of noncreatinine chromogens from the cells.
18. Jaffé reaction; an acid blank.

19. Creatininase (creatinine iminohydrolase or deiminase) and creatinine hydrolase (creatinine amidohydrolase).
20. Isotope-dilution mass spectrometry.

**410**

1. The major product of the catabolism of purine nucleosides; production occurs primarily in liver and intestinal mucosa.
2. Increased uric acid synthesis, decreased renal excretion of uric acid, and miscellaneous causes.
3. Disorder of purine metabolism or renal excretion; when monosodium urate precipitates, from supersaturated body fluids, deposit in and around joints, bursae, periarticular cartilage, bone, and subcutaneous tissue.
4. (1) Gouty nephropathy with urate deposition in renal parenchyma; (2) acute intratubular deposition of urate crystals; and (3) urate nephrolithiasis.
5. In order to prevent urate-induced renal damage.
6. Severe liver disease with decreased synthesis of purines, hereditary deficiency of xanthine oxidase, administration of uricosuric drugs, and a defect in renal tubular reabsorption of uric acid (Fanconi's syndrome).
7. Phosphotungstic acid methods (spectrometric) and uricase methods (enzymatic).
8. Gas chromatography/mass spectrometry (GC/MS).
9. Gastrointestinal tract; amino acid metabolism.
10. Toxic effects on the central nervous system; inherited enzyme deficiencies of the urea cycle?
11. Because ammonia procedures must be performed immediately after the venipuncture; EDTA and heparin (without ammonia salts).
12. To prevent in vitro metabolism of nitrogenous compounds and the degradation of glutamine.
13. Smoking, laboratory atmosphere contamination, poor venipuncture, and metabolism of nitrogenous constituents.
14. Organic compounds containing an amino ( $-\text{NH}_2$ ) and a carboxyl ( $-\text{COOH}$ ) group; they are the building blocks for proteins.
15. Primary disease is due to an inherited enzyme defect, also called an inborn error of metabolism.
16. Phenylketonuria is the most severe manifestation of hyperphenylalaninemia due to phenylalanine 4-monooxygenase deficiency, with accumulation and excretion of phenylalanine, phenylpyruvic acid, and related compounds and inherited as an autosomal recessive trait.
17. It is characterized by severe mental retardation, tumors, seizures, hypopigmentation of hair and skin, eczema, and a mousy odor.
18. By early restriction of dietary phenylalanine.
19. Guthrie test; bacterial spores (*Bacillus subtilis*) and a phenylalanine competitive growth inhibitor are incorporated into an agar medium, blood or urine soaked filter paper is laid on the agar surface, and the plate is incubated and observed for bacterial growth.
20. Thin-layer chromatography, ion-exchange or high-performance liquid chromatography, and gas chromatography/mass spectrometry.

**411**

1. (1) h.  
(2) l.  
(3) i.  
(4) a.  
(5) e.  
(6) k.  
(7) e.  
(8) d.

- (9) j.
- (10) k.
- (11) b.
- (12) b.
- (13) f.
- (14) c.
- (15) a.

**412**

1. The reagents required for the detection, reaction, and estimation of the analyte; and usually a buffer and other nonreactive components or chemicals.
2. Manufacturer's instructions in the product insert or on the reagent bottle.
3. A numerical value, positive or negative result, or a plus (+) grading scale (i.e., 1+, 2+, 3+, or 4+).
4. Glucose (most common), lactose, fructose, galactose, and pentose (arabinose and xylose).
5. Renal threshold of each individual.
6. Renal threshold is the term used for any glucose level that exceeds the concentration that cannot be reabsorbed by the renal tubules and is excreted in the urine.
7. Diabetes mellitus.
8. Kidney disease, pancreatic disease, endocrine disorders, liver disease, and damage to the central nervous system.
9. Stress situations and pregnancy, after exercise, and/or associated with anesthesia.
10. Substances can reduce a heavy metal from a higher to a lower oxidation state, for example copper (II) to copper (I); glucose, creatine, uric acid, homogentisic acid, ascorbic acid, chloroform, formaldehyde, lactose, fructose, galactose, and pentose (arabinose and xylose).
11. Glucose oxidase method, an enzymatic assay; false-positive glucose results due the presence of other reducing substances.
12. Large amounts of ascorbic acid from vitamin C or drugs (tetracyclines), presence of ketones, and refrigerated specimens.
13. It is an important early indicator of liver dysfunction and/or biliary obstruction.
14. Stabilized 2,4-dichloroaniline diazonium salt; 2,4-dichloroaniline diazonium salt that reacts with bilirubin in a strongly acid medium to form azobilirubin.
15. Because bilirubin rapidly breaks down when exposed to light and due to the oxidation of bilirubin to biliverdin.
16. Highly pigmented urine; large amounts of ascorbic acid or nitrites.
17. When fat is used as a major source of energy in place of carbohydrates.
18. Acetoacetic acid,  $\beta$ -hydroxybutyric acid, and acetone.
19. Depletes the body of its anions and thus produces acidosis.
20. Sodium nitroprusside reacts with acetoacetic acid to form a purple color; only acetoacetic acid.
21. Congestive heart failure (CHF), severe liver damage, dehydration, or severe water loss from vomiting or diarrhea.
22. A change in ionic concentration of a solution initiates a change in the dissociation constant (pK) of pretreated polyelectrolytes (polymethylvinyl) and this ionic change is detected by bromthymol blue (pH indicator).
23. Refractometer or urinometer.
24. Bladder and kidney tumors; trauma to the kidneys; glomerulonephritis; pyelonephritis; renal calculi (kidney stones); and bleeding disorders.
25. Trauma; intravascular hemolysis due to transfusion reactions, hemolytic anemias, or paroxysmal nocturnal hemoglobinuria; severe infections such as malaria, etc.; severe burns; poisoning with strong acids or mushrooms; or trauma from neoplastic diseases.

26. Traumatic muscle injury acquired from automobile accidents, beatings, or crush injuries; excessive unaccustomed exercise; exposure to toxic drugs or substances; and in rare hereditary disorders.
27. A combination of gross observations and chemical procedures.
28. The peroxidase-like activity of hemoglobin, which catalyzes the reaction of diisopropylbenzene dihydroperoxide and 3,3',5,5'-tetramethylbenzidine to form a color change.
29. 4.5 to 8.0 with a mean between 5.0 and 6.0.
30. Methyl red and bromthymol blue indicators.
31. Proteinuria; glomerular damage, tubular damage, or overflow from excessive production of low-molecular weight proteins.
32. Bence Jones protein.
33. Strenuous physical exercise, pregnancy, exposure to extreme cold, and psychological stress.
34. Colorimetric methodology based on the concept of the protein error of indicators.
35. Both reagent strip and precipitation methods.
36. Sulfosalicylic acid method; electrophoresis, immunoelectrophoresis, immunodiffusion, or ultracentrifugation techniques.
37. Biliary blockage.
38. Ehrlich reaction; in which urobilinogen reacts with para-dimethylaminobenzaldehyde in a strongly acid medium to form a pink-red color.
39. Nitrite.
40. Upon the conversion of dietary nitrate to nitrite by the action of bacteria in the urine.
41. Asymptomatic UTIs.
42. The principle that leukocyte esterase catalyzes the hydrolysis of the derivatized pyrrole amino acid ester to liberate 3-hydroxy-5-phenyl pyrrole and then this pyrrole reacts with a diazonium salt to produce a purple end product.

**413**

1. When requested by the physician; when determined by laboratory protocol (e.g., immunosuppressed, urology-nephrology, diabetic, or pregnant patients); and when any abnormal physicochemical or reagent strip result is obtained.
2. The first-morning specimen because it is the most concentrated.
3. Volume of urine centrifuged, time and speed of centrifugation, concentration and volume of sediment examined, and reporting results.
4. Sternheimer-Malbin, 0.5% Toluidine blue, Oil red O, Sudan III, Gram stain, Papanicolaou Hansel, Wright-Giemsa, Papanicolaou, and Prussian blue.



5.

<sup>1</sup> S	Q	U	A	M	<sup>4</sup> O	U	S							<sup>2</sup> T
					V							<sup>13</sup> Y		R
<sup>3</sup> R					A							E		A
E					L							A		N
N					<sup>6</sup> F	A	T	T	Y	C	A	S	<sup>19</sup> T	S
A					A							T	A	I
L		<sup>15</sup> G	L	I	T	T	E	R	C	E	L	L	M	T
					B								M	I
					O								H	O
<sup>14</sup> M	U	C	U	S	D		<sup>18</sup> H	Y	<sup>8</sup> R				O	N
					I			P	E				R	A
					E			E	D				S	L
					S			R	B		<sup>10</sup> C		F	
								T	L		R		A	
<sup>7</sup> W	<sup>17</sup> H	I	T	E	B	L	O	O	D	C	E	L	L	<sup>11</sup> S
	Y						N	O			N		L	P
A	P		<sup>20</sup> B		L		I	D			A		P	E
I	O		U		L		C	C			T		R	R
R	T		B		E			E			E		O	M
E	O		B		C			L			D		T	A
T	N		L		T			L			C		E	T
C	I		E		S			S			E		I	O
A	C		S	N	O						L		N	Z
<sup>12</sup> B			M		H						L			O
<sup>5</sup> L	I	<sup>16</sup> P	I	D	<sup>9</sup> G	L	O	B	U	L	E	S		A

414

1. (1) c.
- (2) e.
- (3) i.
- (4) k.
- (5) a.
- (6) n.
- (7) p.
- (8) q.
- (9) r.
- (10) b.
- (11) c.
- (12) m.

- (13) j.
- (14) m.
- (15) a.
- (16) l.
- (17) g.
- (18) k.
- (19) d.
- (20) q.
- (21) c.
- (22) s.
- (23) h.
- (24) o.
- (25) a.
- (26) z.
- (27) t.
- (28) i.
- (29) n.
- (30) f.
- (31) u.
- (32) w.
- (33) v.
- (34) y.
- (35) u.
- (36) x.

**415**

1. Measuring the total volume of urinary output and aliquoting the specimen into proper containers for analysis.
2. Clinically it is often valuable to know the total amount of an analyte, excreted in the urine during a 24-hour period and in order to obtain a quantitative analyte value.
3. Calcium oxalate; between 30 and 40 years of age.
4. Renal colic; severe pain in the back, radiating to the groin.
5. Hematuria with extreme pain is common, obstruction of the kidney pelvis or ureter can cause hydronephrosis, and infection.
6. By a reflectance spectrophotometer that analyzes the color intensity of the light reflected from the reagent area.
7. Mass gravity meter; urine chemistries are measured by a standard reflectance spectrophotometer.
8. Running high and low matrix controls, equipment and instrument maintenance, and method validation.

## Unit Review Exercises

**Note to Student:** Consider all choices carefully, select the *best* answer to each question, and *circle* the corresponding letter. When you have completed all unit review exercises, transfer your answers to ECI (AFIADL) Form 34, Field Scoring Answer Sheet.

**Do not return your answer sheet to AFIADL.**

46. (409) The *best* statement describing the abilities of *most* renal function tests is they can
  - a. differentiate between localized and generalized damage.
  - b. differentiate between temporary and permanent malfunction.
  - c. reveal primary and secondary deformity and what goes on inside the kidney.
  - d. reveal only whether or not dysfunction is present and a rough estimate of its severity.
47. (409) The theoretical concept defined as the volume of plasma from which a measured amount of substance can be completely eliminated into the urine per unit of time is the
  - a. clearance.
  - b. urine concentration.
  - c. plasma concentration.
  - d. glomerular filtration rate.
48. (409) Creatinine clearance is used for assessment of glomerular filtration rate because blood and urine assays for inulin and *p*-aminohippuric acid are
  - a. not well correlated to each other.
  - b. too difficult to collect as a 24-hour sample.
  - c. too difficult and time consuming to be practical in clinical laboratories.
  - d. not as sensitive as creatinine clearance procedures performed enzymatically.
49. (409) Which one of the following is the major constituent of nonprotein nitrogen substances?
  - a. Urea.
  - b. Uric acid.
  - c. Ammonia.
  - d. Amino acids.
50. (409) Which of the following classifications of urea azotemia is caused by carcinoma of the bladder or ureters and kidney stones?
  - a. Renal.
  - b. Prerenal.
  - c. Postrenal.
  - d. Nonrenal.
51. (409) Which of the following anticoagulants should not be used for plasma urea nitrogen procedures?
  - a. Sodium heparin.
  - b. Lithium heparin.
  - c. Potassium heparin.
  - d. Ammonium heparin.
52. (409) Indirect methods for urea are based on preliminary hydrolysis of urea with urease followed by some process that quantitates the
  - a. picrate ion.
  - b. ammonium ion.
  - c. diazine chromogen.
  - d. potassium iodide chromogen.

53. (409) Why is an individual's creatinine excretion rate relatively constant and what is its clearance an indicator of?
- a. Creatinine produced is not proportional to muscle mass; RPF.
  - b. Creatinine produced is not proportional to muscle mass; GFR.
  - c. Dietary intake of creatinine causes only minor variations; RPF.
  - d. Dietary intake of creatinine causes only minor variations; GFR.
54. (410) A disorder of purine metabolism which occurs when monosodium urates are deposited in and around joints, bursae, and subcutaneous tissues is known as
- a. gout.
  - b. toxemia.
  - c. hypouricemia.
  - d. Lesh-Nyhan syndrome.
55. (410) Which of the following uric acid methods is more specific and why?
- a. Uricase method; has a single or initial step of urate oxidation catalyzed by the enzyme uricase.
  - b. Uricase method; has the availability of high-quality, low-cost preparations of the uricase bacterial enzyme.
  - c. PTA method; because it uses spectrophotometrically wavelengths of 650 to 700 nm to read the tungsten blue reaction.
  - d. PTA method; because it relies on the development of a blue reaction (tungsten blue) as PTA is reduced by urate in an alkaline medium.
56. (410) Where does ammonia conversion to urea take place and why is it so important?
- a. Liver; because of its renal toxicity.
  - b. Intestines; because of its renal toxicity.
  - c. Liver; because of its central nervous system toxicity.
  - d. Intestines; because of its central nervous system toxicity.
57. (410) Falsely elevated ammonia results may be due to all of the following *except*
- a. poor venipuncture.
  - b. smoking by the technician.
  - c. contamination of specimen by the laboratory atmosphere.
  - d. placing the specimen immediately on ice after venipuncture.
58. (410) Normal amino acid metabolism starts in the
- a. liver.
  - b. blood.
  - c. kidneys.
  - d. intestines.
59. (410) Primary aminoaciduria and aminoacidemia is due to
- a. an inherited enzyme defect.
  - b. protein-energy malnutrition.
  - c. generalized renal tubular dysfunction.
  - d. protein intake above normal requirements.
60. (410) What test is used for neonatal screening for PKU and what microorganism does it usually incorporate into the agar medium?
- a. Guthrie test; *Bacillus cereus*.
  - b. Guthrie test; *Bacillus subtilis*.
  - c. Berthelot's test; *Bacillus cereus*.
  - d. Berthelot's test; *Bacillus subtilis*.

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61. (411) Which of the following urine volume terms is associated with a decreased urinary output of less than 500 ml in a 24-hour period?
- a. Anuria.
  - b. Oliguria.
  - c. Polyuria.
  - d. Hypouria.
62. (411) The chief or major pigment found in normal urine is
- a. urobilin.
  - b. urochrome.
  - c. uroerythrin.
  - d. urobilinogen.
63. (411) "Visible particulate matter is present and newspaper print is blurry if viewed through the specimen" is a description of the appearance term
- a. hazy.
  - b. clear.
  - c. cloudy.
  - d. turbid.
64. (411) Specific gravity is the
- a. hydrogen activity of a solution.
  - b. velocity of light in air to the velocity of light in solution.
  - c. measurement of the number of solute particles per unit amount of solvent.
  - d. weight of a substance compared with that of an equal volume of another substance taken as the standard.
65. (411) Which of the following physical characteristic measurements is not valuable in estimating the kidney's concentrating ability?
- a. Osmolality.
  - b. pH.
  - c. Specific gravity.
  - d. Refractive index.
66. (412) A reducing substance, *most* commonly found in urine, and is the *most* clinically significant is
- a. pentose.
  - b. lactose.
  - c. glucose.
  - d. galactose.
67. (412) Although the renal threshold varies from individual to individual, at what levels is the renal threshold for glucose exceeded and what is the result?
- a. 120 to 160 mg/dl; oliguria.
  - b. 160 to 200 mg/dl; oliguria.
  - c. 120 to 160 mg/dl; glycosuria.
  - d. 160 to 200 mg/dl; glycosuria.
68. (412) Which of the following glucose procedures is a more specific test for glucose and what methodology does it employ?
- a. Glucose oxidase; enzymatic.
  - b. Benedict's reaction; enzymatic.
  - c. Glucose oxidase; heavy metal reduction.
  - d. Benedict's reaction; heavy metal reduction.

69. (412) Which of the following interferents *does not* cause a false-negative or falsely lower result with the reagent strip?
- Ketones.
  - Fructose.
  - Ascorbic acid.
  - Refrigerated specimen.
70. (412) Which of the following chemical(s) used in the reagent strip reacts with bilirubin to form azobilirubin?
- Sodium nitroprusside.
  - Tetrabromphenol blue.
  - Stabilized 2,4-dichloroaniline diazonium salt.
  - Diisopropylbenzene dihydroperoxide, 3,3',5,5'-tetramethylbenzidine.
71. (412) What reagent is combined with sodium nitroprusside to enhance color differentiation in the confirmatory test for ketone bodies?
- Lactose.
  - Pentose.
  - Methyl red.
  - Bromthymol blue.
72. (412) Properly functioning kidneys are able to alter the specific gravity of urine in a range from
- 1.000 to 1.025.
  - 1.000 to 1.030.
  - 1.003 to 1.035.
  - 1.003 to 1.040.
73. (412) Which of the following conditions may be a result of a transfusion reaction, severe burns, or poisoning with strong acids or mushrooms?
- Hematuria.
  - Bilirubinuria.
  - Myoglobinuria.
  - Hemoglobinuria.
74. (412) What is the normal pH range of freshly-voided urine and what are the indicators used in the reagent strip?
- 3.5 to 7.0; methyl red and bromthymol blue.
  - 3.5 to 7.0; neutral red tetrabromphenol blue.
  - 4.5 to 8.0; methyl red and bromthymol blue.
  - 4.5 to 8.0; neutral red tetrabromphenol blue.
75. (412) Which one of the following conditions is probably the *most* significant pathological condition found in routine urinalysis?
- Ketonuria.
  - Hematuria.
  - Proteinuria.
  - Bilirubinuria.
76. (412) Which of the following proteins is the primary protein fraction normally excreted in urine?
- Albumin.
  - Globulin.
  - Bence Jones.
  - Tamm-Horsfall.

- 
- 
77. (412) Bence Jones protein may be found in all of the following *except*
- multiple myeloma.
  - macroglobulinemia.
  - malignant lymphoma.
  - upper urinary tract infections.
78. (412) The reagent strip method for detecting protein in urine is based on the
- protein error of indicators with bromthymol blue as the indicator.
  - protein error of indicators with tetrabromphenol blue as the indicator.
  - peroxidase-like activity of protein with bromthymol blue as the indicator.
  - peroxidase-like activity of protein with tetrabromphenol blue as the indicator.
79. (412) In the confirmatory test for urinary protein, the *most* commonly used precipitation procedure is the
- sodium sulfate method.
  - sulfosalicylic acid method.
  - trichloroacetic acid method.
  - trichloroacetic acid with sodium sulfate method.
80. (412) In what condition is urobilinogen completely absent in the urine?
- Liver disease.
  - Biliary blockage.
  - Hemolytic disorders.
  - All of the above.
81. (412) Why *must* a urine sample be fresh and protected from light before urobilinogen testing?
- Urobilinogen is converted to urobilin upon standing.
  - Urobilinogen is converted to bilirubin upon standing.
  - Bilirubin is converted to urobilin during refrigeration.
  - Bilirubin is converted to urobilinogen during refrigeration.
82. (412) Which one of the following reagent strip tests are very useful in detecting asymptomatic urinary tract infections?
- Nitrite and blood.
  - Protein and blood.
  - Nitrite and leukocyte esterase.
  - Protein and leukocyte esterase.
83. (413) Which of the following stains are common supravital stains used for identifying most cells and casts in urinary sediment?
- Oil red O and Sudan III.
  - Gram stain and Papanicolaou.
  - Hansel, Wright-Giemsa, and Prussian blue.
  - Sternheimer-Malbin and 0.5% Toluidine blue.
84. (413) What epithelial cells in large numbers may indicate inflammatory conditions, catheterization, or a pathological process such as malignancy and how are they identified?
- Squamous epithelial cells; by abundant, flat cytoplasm with small nuclei.
  - Transitional epithelial cells; by abundant flat cytoplasm with small nuclei.
  - Squamous epithelial cells; which are round to oval and have a large, centrally located nucleus.
  - Transitional epithelial cells; which are round to oval and have a large, centrally located nucleus.

85. (413) Which of the following urinary elements contains lipid globules and may be seen in patients with severe renal dysfunction, heavy metal poisoning, glomerular injury, or diabetes mellitus?
- a. Fatty cast.
  - b. Glitter cells.
  - c. Red blood cell cast.
  - d. Renal epithelial cells.
86. (413) What red blood cell forms may be seen in hypertonic or concentrated (high specific gravity) urine?
- a. Swollen and ghost.
  - b. Crenated and ghost.
  - c. Swollen and prickly.
  - d. Crenated and prickly.
87. (413) The presence of bacteria is considered important and clinically significant if found with
- a. red blood cells and protein.
  - b. red blood cells and glucose.
  - c. white blood cells and protein.
  - d. white blood cells and glucose.
88. (413) Yeast may be confused with fat globules and
- a. red blood cells; however yeast are larger in size.
  - b. red blood cells; however yeast are smaller in size.
  - c. white blood cells; however yeast are larger in size.
  - d. white blood cells; however yeast are smaller in size.
89. (414) Which of the following urinary crystals is probably the most common crystal encountered in urinary sediment and in what type of urine is it found?
- a. Uric acid; acid.
  - b. Uric acid; alkaline.
  - c. Triple phosphate; acid.
  - d. Triple phosphate; alkaline.
90. (414) Which of the following urinary crystals appears as an “envelope”?
- a. Uric acid.
  - b. Calcium oxalate.
  - c. Triple phosphate.
  - d. Ammonium urate.
91. (414) Which of the following urinary crystals are normally found in alkaline urine?
- a. Triple phosphate and calcium oxalate.
  - b. Amorphous urates and calcium oxalate.
  - c. Triple phosphate and calcium carbonate.
  - d. Amorphous urates and calcium carbonate.
92. (414) Which of the following urinary crystals appears as a “coffin lid”?
- a. Calcium oxalate.
  - b. Triple phosphate.
  - c. Ammonium urate.
  - d. Calcium carbonate.



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93. (414) What abnormal crystal has a silky needle-shaped appearance and is associated with tissue degeneration or necrosis?
- Leucine.
  - Cystine.
  - Tyrosine.
  - Cholesterol.
94. (414) Which of the following crystals is usually flat, thin plates that can fuse into a rosette configuration and can be seen in the urine of patients with what type of condition?
- Cystine; the nephrotic syndrome.
  - Cholesterol; the nephrotic syndrome.
  - Cystine; inherited metabolic disorder.
  - Cholesterol; inherited metabolic disorder.
95. (414) Which of the following casts is considered the prototype for all the other casts?
- Waxy.
  - Hyaline.
  - Finely granular.
  - Coarsely granular.
96. (414) Which of the following characteristics describe a finely granular cast?
- Small, irregular, and easy to distinguish granules.
  - Large, irregular, and easy to distinguish granules.
  - Small, regular, and difficult to distinguish granules.
  - Large, regular, and difficult to distinguish granules.
97. (414) How are waxy casts characterized and with what condition are they associated?
- Smooth surface and blunt end; oliguria.
  - Notched surface and blunt end; oliguria.
  - Smooth surface and round end; polyuria.
  - Notched surface and round end; polyuria.
98. (414) Most texts agree that the most diagnostically significant of all the formed elements found in urinary sediment is the
- waxy cast.
  - hemoglobin cast.
  - red blood cell cast.
  - white blood cell cast.
99. (414) In the United States, what parasite is most frequently encountered in urinary sediment?
- Entamoeba coli*.
  - Trichomonas vaginalis*.
  - Enterobius vermicularis*.
  - Schistosoma hematobium*.
100. (415) Why are 24-hour urine samples collected?
- Random samples cannot be used for chemical analyses.
  - It is important to know the exact amount of urine produced.
  - It is often valuable to know the total amount of an analyte excreted during a day.
  - A random urine specimen doesn't provide enough sample for the testing of an analyte.

**When you complete this course, please complete the student survey on the Internet at this URL:  
[http://www.maxwell.af.mil/au/afiadl/operation/survey\\_fr.htm](http://www.maxwell.af.mil/au/afiadl/operation/survey_fr.htm).**

## **Student Notes**

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## National Committee for Clinical Laboratory Standards— Guidelines

<i>Title</i>	<u>Number</u>	<u>Date</u>
<i>Urinalysis and Collection, Transportation, and Preservation of Urine Specimens; Approved Guideline</i>	GP16–A2	November 2001

## Student Notes

## Glossary

### Terms

<b>Accuracy</b>	An agreement between the best estimate of a quantity and its true value.
<b>Ameboid</b>	Movement in the style of an ameba, by pseudopodia (false foot).
<b>Asymptomatic</b>	Showing or causing no symptoms.
<b>Bacillary</b>	Pertaining to rod-like forms.
<b>Bacteriuria</b>	The presence of bacteria in urine.
<b>Biliary</b>	Pertaining to the bile, and bile ducts.
<b>Catalyst</b>	Any substance that brings about catalysis; also called accelerant.
<b>Catheter</b>	A tubular, flexible, surgical instrument for withdrawing fluids from (or introducing fluids into) a cavity of the body, especially one for introduction into the bladder through the urethra for the withdrawal of urine.
<b>Chemotherapy</b>	The treatment of disease by chemical agents; first applied to use of chemicals that affect the causative organism unfavorably but do not harm the patient.
<b>Chromatography</b>	A method of chemical analysis in which the solution to be analyzed is poured into a vertical glass tube containing an adsorbent (or stationary phase), the different solutes moving through the stationary phase at different velocities according to their degree of attraction to it, and producing bands of color at different levels of the adsorption column.
<b>Chromogen</b>	Any substance that may give origin to a coloring matter.
<b>Chromogenic</b>	Producing a pigment or coloring matter.
<b>Colorimetric</b>	A chemical procedure in a reagent reacts with a particular chemical constituent to produce a measurable color.
<b>Colorimetry</b>	The use of color density measurement in the assaying of a given constituent.
<b>Cystitis</b>	Inflammation of the urinary bladder hypersensitivity, characterized by a large number of mononuclear leukocytes and eosinophils in the bladder mucosa and musculature, and in the urinary sediment.
<b>Cytology</b>	The science that deals with the function and structure of body cells.
<b>Cytoplasmic</b>	Pertaining to or contained in cytoplasm.
<b>Dysuria</b>	Painful or difficult urination.
<b>Endogenous</b>	Produced within a cell or within an organism; growing from within.
<b>Etiologic</b>	Pertaining to the cause of a disease.

<b>Exogenous</b>	Growing by additions to the outside; developed or originating outside the organism.
<b>Genitourinary</b>	Pertaining to the genital and urinary organs; urogenital; urinosexual.
<b>Glomerulonephritis</b>	Inflammation of the capillary loops in the glomeruli of the kidney.
<b>Glomerulus</b>	A tuft or cluster; used in anatomical nomenclature as a general term to designate such a structure.
<b>Hemolysis</b>	The liberation of hemoglobin.
<b>Hepatotoxicity</b>	The quality or property of exerting a destructive or poisonous effect upon liver cells.
<b>Hypertonic</b>	A biological term denoting a solution which when bathing body cells causes a net flow of water across the semi-permeable cell membrane out of the cell.
<b>Intracellular</b>	Situated or occurring within a cell or cells.
<b>Lyse</b>	To cause or produce disintegration of a compound, substance, or cell.
<b>Mucoprotein</b>	A compound present in all connective and supporting tissues, containing mucopolysaccharide as prosthetic groups; they are relatively resistant to denaturation.
<b>Necrosis</b>	Death of tissue, usually as individual cells, groups of cells, or in small localized areas.
<b>Neoplasm</b>	A new tissue growth that is out of control.
<b>Nephropathy</b>	Disease of the kidney.
<b>Neutrophil</b>	A granular leukocyte having a nucleus with three to five lobes connected by slender threads of chromatin, and cytoplasm containing fine inconspicuous granules; called also polymorphonuclear, polynuclear, or neutrophilic leukocytes.
<b>Phagocyte</b>	Any cell that ingests microorganisms or other cells and foreign particles.
<b>Photometer</b>	A device for measuring the intensity of light.
<b>Pleomorphic</b>	Occurring in various distinct forms.
<b>Prostate</b>	A gland in the male which surrounds the neck of the bladder and the urethra.
<b>Protease</b>	A general term for a proteolytic enzyme.
<b>Pyuria</b>	The presence of pus in the urine.
<b>Quality assurance (improvement)</b>	A well-defined, organized program designed to enhance patient care through the ongoing objective assessment of important aspects of patient care and the correction of identified problems.
<b>Quality control (QC)</b>	A system for maintaining proper standards in manufactured goods, especially by regular inspection of the product.

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<b>Refraction</b>	The deviation of light in passing obliquely from one medium to another of different density.
<b>Sphincter</b>	A ring-like band of muscle fibers that constricts a passage or closes a natural orifice.
<b>Standard</b>	Something considered by an authority or by general consent as a basis of comparison or a material against which other materials can be compared.
<b>Supernatant</b>	Situated above or on top of something.
<b>Supravital</b>	Denoting a staining method in which the dye is added to a medium of cells already removed from the living organism.
<b>Turbidimetric</b>	A procedure in which the turbidity of a test solution is measured.
<b>Urease</b>	A colorless, crystalline globulin first extracted by Takeuchi from soy bean. It is also found in mucous urine passed during inflammation of the bladder. It is an enzyme elaborated by various microorganisms, and is capable of causing the change of urea into benzoic acid and glycol.
<b>Urethritis</b>	Inflammation of the urethra.
<b>Viable</b>	Capable of living.

## Abbreviations and Acronyms

<b>ADH</b>	Antidiuretic hormone
<b>ASCP</b>	American Society of Clinical Pathologists
<b>EM</b>	Electron microscopy
<b>FITC</b>	Flourescein isothiocyanate
<b>FOUO</b>	For official use only
<b>GBM</b>	Glomerular basement membrane
<b>GFR</b>	Glomerular filtration rate
<b>HPF</b>	High-power field
<b>ICM</b>	Interference-contrast microscopy
<b>LPF</b>	Low-power field
<b>MTFs</b>	Medical treatment facilities
<b>NA</b>	Numerical aperture
<b>NCCLS</b>	National Committee for Clinical Laboratory Standards
<b>OI</b>	Operating instructions

<b>PKU</b>	Phenylketonuria
<b>PMN</b>	Polymorphonuclear neutrophil
<b>PPE</b>	Personal protective equipment
<b>RCF</b>	Relative centrifugal force
<b>RI</b>	Refractive index
<b>RPF</b>	Renal plasma flow
<b>SEM</b>	Scanning electron microscopy
<b>SSN</b>	Social Security Number
<b>TBM</b>	Tubular basement membrane
<b>TEM</b>	Transmission electron microscopy
<b>THM</b>	Tamm-Horsfall mucoprotein
<b>TNTC</b>	Too numerous to count
<b>UTI</b>	Urinary tract infection



## **Student Notes**

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