SESSION II – URINALYSIS MICROSCOPICS

COURSE INSTRUCTIONS

1. Write your name on the designated area of each chapter exam. **Unidentified exams will not be graded.**

2. The exams are generally made up of four sections. Follow the instructions given in each section:
   
   - A. Multiple Choice
   - B. True or False
   - C. Matching
   - D. Short Answer

3. Complete all questions of the examination. Participants are reminded that this is an individual self-study course. Taking the examination is not meant to be a group effort. Credits are based on individual merit. Therefore, participants will be on the honor system when completing this examination. Group study can be done after the exams are graded and returned.

4. The Training and Evaluation Section has no regulated deadline. In order to avoid “urgent” requests for grading, we ask that you return completed exams in plenty of time to allow for grading to use for whatever other program deadlines you plan to use the credits. Return completed exams to:

   West Virginia Office of Laboratory Services
   Training & Evaluation Section
   167 11th Avenue
   South Charleston WV 25303

5. A final exam that covers the material of all three chapters is also enclosed and should be completed and returned with the other exams.

Thank you for your cooperation.
## CORRESPONDENCE COURSE OUTLINE

<table>
<thead>
<tr>
<th>Chapter</th>
<th>Topic</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Urinary System / Microscopy</td>
<td>1</td>
</tr>
<tr>
<td>2</td>
<td>Microscopic Examination of Urine Sediment</td>
<td>19</td>
</tr>
<tr>
<td>3</td>
<td>Quality Assurance Confirmatory Urine Screening Tests</td>
<td>39</td>
</tr>
<tr>
<td></td>
<td>Exams</td>
<td>61</td>
</tr>
</tbody>
</table>
Welcome to all participants in the Urinalysis correspondence Course. This is Session II of a two-part course. This session is designed to meet the needs of individuals performing moderate complexity urinalysis. It also serves as a review for all laboratorians.

**COURSE GOAL**

The goal of both courses is to provide participant with the information needed to improve the quality of urinalysis performed at each testing site.

**COURSE OBJECTIVES**

At the conclusion of Session II, participants can expect to know or accomplish the following:

- Know the anatomy & physiology of the kidney and relate to urinalysis
- Improve microscopy techniques
- Identify microscopic elements in urine sediment
- Assess quality of urinalysis procedures in your laboratory

The kidneys perform four major functions. They:

- excrete most of the unwanted end-products or wastes of body metabolism;
- control the concentration of most of the constituents of the body fluids;
- regulate blood acid-base balance; and,
- regulate water and electrolyte balance.

The mechanisms by which the kidney accomplishes these functions include:

- glomerular filtration
- tubular reabsorption
- tubular secretion
- renal blood flow

Through these mechanisms, the kidney selectively excretes or retains substances according to the specific body needs.
RENNAL ANATOMY & PHYSIOLOGY

The purpose of this section is to discuss the basic principles of urine formation and the mechanisms by which metabolic wastes are removed from the blood. A basic knowledge of the anatomy of the urinary system and urine formation is an important aid in understanding urinalysis.

The urinary system includes two kidneys, two ureters, one bladder and one urethra. In general, kidneys form the urine and the urine flows from the kidneys into the ureters and is passed to the bladder for storage. It is eliminated from the body through the urethra.

The kidney has two anatomical portions:

- 'Cortex' - The outer portion of the kidney which is made up of the glomerular portions of the nephron and the proximal convoluted tubules.

- 'Medulla' - The Central portion of the kidney consisting of the loop of Henle, distal convoluted tubules and collecting ducts.
The nephron is the functional unit of the kidney. Urine is formed in the nephron. Each kidney contains approximately 1 to 1.5 million nephrons. Each nephron is capable of forming urine by itself. Therefore, it is not necessary to discuss the entire kidney but merely the activities in the single nephron to explain the function of the kidney.

Each nephron is composed of the:
- glomerulus
- proximal and distal convoluted tubules
- ascending and descending loop of Henle

**Figure 1-3:** The structure of the nephron along with the renal circulatory system; modified from *Modern Urine Chemistry*, Miles, Inc., 1991.
THE GLOMERULUS

The glomerulus delivers blood to the nephron and is essentially a filtering system. It is a network of up to 50 parallel capillaries covered by epithelial cells and enclosed in a capsule commonly referred to as Bowman’s capsule.

Blood enters the glomerulus from the renal artery through the afferent arteriole and then leaves through the efferent arteriole. Pressure of the blood in the glomerulus causes fluid to filter into the Bowman’s capsule. The Bowman’s capsule forms a membrane that allows passage of substances with molecular weights less than 70,000. This filtered fluid is called glomerular filtrate. Glomerular filtrate is basically blood plasma without proteins and fats. It has a specific gravity of 1.008 - 1.010, an important piece of information to know if a urine specific gravity on a patient becomes fixed at 1.010 (isosthenuria) which indicates a loss of the concentrating ability of the kidney.

Approximately 180 liters of glomerular filtrate is produced daily, yet only one to two liters of urine is eliminated from the body daily. Therefore, much of the glomerular filtrate is reabsorbed into the blood. From here, the glomerular filtrate passes into the tubules where reabsorption, secretion and urine concentration occur.

![Diagram of the Glomerulus](image)

Figure 1 – 4: Functional structure of the glomerulus. Modified from Ringsrud and Linne, Urinalysis and Body fluids, Mosby, 1995.
TUBULES

The long tubule in which the glomerular filtrate is converted into urine is composed of several distinct segments. The uppermost portion, continuous with the glomerulus is the **proximal convoluted tubule**, followed by the descending and ascending loop of Henle. This is followed by the **distal convoluted tubule**. The distal convoluted tubules from several nephrons drain into the **collecting duct**. It is in these tubules that reabsorption, secretion and concentration occur. See Figure 1-3 for an illustration of the tubules and their structure within the nephron.

PROXIMAL CONVOLUTED TUBULE
Reabsorption and Secretion

The **proximal convoluted tubule** is where 80% of the fluid and electrolytes filtered by the glomerulus are reabsorbed. Reabsorption is accomplished through two transport mechanisms called **active and passive transport**. **Active transport** produces the energy necessary for a substance to be absorbed against a gradient from a region of lower concentration to one of higher concentration. Active transport is responsible for the reabsorption of glucose, amino acids, uric acid, albumin, magnesium and phosphate ions. **Passive transport** is the movement of molecules across a membrane caused by differences in their concentration or electrical potential on opposite sides of a membrane. Substances move passively down a concentration gradient from a region of higher to a region of lower concentration. Most of the water in the glomerular filtrate is passively reabsorbed. Chloride, bicarbonate and potassium ions together with 40-50% of the urea present in the glomerular filtrate are passively reabsorbed with water at the proximal convoluted tubule.

The proximal tubule has a limit as to how much substance will be reabsorbed from the glomerular filtrate. This is referred to as the **renal threshold**. When the plasma concentration of a substance is greater than its renal threshold, it will remain in the glomerular filtrate and be excreted in the urine. For example, the renal threshold for glucose is about 160 to 180 mg/dl. When a patient with diabetes mellitus has a blood glucose concentration greater than 180 mg/dl, excess glucose will be eliminated in the urine.

The renal threshold differs with each substance. Knowledge of the renal threshold and the plasma concentration can be used to distinguish between filtration of excess solute and renal tubular damage. For example, glucose appearing in the urine of a person with normal blood glucose is the result of tubular damage and **not** diabetes mellitus.

**SECRETION**

In contrast to tubular reabsorption in which substances are removed from the glomerular filtrate and returned to the blood, tubular secretion involves the passage of substances from the blood to the glomerular filtrate as shown in the diagram below. (Figure 1-5)

Tubular secretion serves two functions:

- Eliminates waste products that are not filtered by the glomerulus.
- Regulates acid-base balance through the secretion of hydrogen ions.

In the proximal convoluted tubule, hydrogen ions, phosphate ions, organic acids, and certain drugs are secreted.

The regulation of acid-base balance to maintain a normal blood pH of 7.4 is dependent on secretion of hydrogen (H+) ions. In general, the reactions among hydrogen (H+), bicarbonate (HCO$_3^-$) and phosphate (HPO$_4^{2-}$) ions along with ammonia (NH$_3$) maintain blood pH. The resulting ammonium ion (NH$_4^+$) is excreted in the urine.
LOOP OF HENLE

The loop of Henle is the principal site where urine is concentrated as a mechanism for conserving body water. The descending portion of the loop is the concentration portion. It is permeable to water but not to solutes. As the loop passes farther into the medulla, the fluid soon to be urine concentrates. The mechanism by which water moves down the descending loop and into the bloodstream is called the counter current mechanism.

The ascending loop of Henle serves as a diluting segment because of its ability to actively secrete sodium and chloride, but prevent water loss.

DISTAL CONVOLUTED TUBULE

There are two main functions of the distal convoluted tubule:

- The final reabsorption of sodium (maintaining water and electrolyte balance)
- Removal of excess acid from the body (acid/base balance)

Sodium is actively reabsorbed with some bicarbonate. However, the mechanism for sodium reabsorption is through the sodium/potassium pump under the control of the hormone aldosterone. The sodium potassium pump is initiated by low plasma sodium and/or low blood pressure. Through a cascade of hormone stimulation involving renin and aldosterone, active absorption of sodium ions are exchanged for potassium which is excreted by the tubular cells. Overall, there is an increase in plasma sodium and water with a decrease in body potassium levels.

The pH of the final urine is affected by the distal convoluted tubules, especially by an excretion of hydrogen and ammonium ions in exchange for sodium. The blood pH is maintained at about 7.4 whereas urine commonly has a pH of 5 or 6.

COLLECTING DUCT

Finally, the fluid (soon to be urine) flows into the collecting duct which collects this fluid from several nephrons. It is the portion of the nephron where the final concentration of urine is done. According to S. K. Strasinger, “final concentration of the filtrate through the reabsorption of water begins in the late distal convoluted tubule and continues in the collecting duct. Reabsorption is dependent on the osmotic gradient in the medulla and a hormone produced in the pituitary gland called antidiuretic hormone (ADH) or vasopressin. The process is controlled by the presence or absence of ADH which renders the walls of the distal convoluted tubules and collecting duct permeable or impermeable to water. A high level of ADH increases permeability, resulting in increased reabsorption of water and a low volume, concentrated urine. Likewise, absence of ADH renders the wall impermeable to water resulting in a large volume of dilute urine. Production of ADH is determined by the state of body hydration.”

ADH CONTROL SUMMARIZED

\[ \text{Body hydration} = \uparrow, \quad \text{ADH} = \uparrow, \quad \text{Urine volume (diluted)} \]
\[ \text{Body hydration} = \downarrow, \quad \text{ADH} = \downarrow, \quad \text{Urine volume (concentrated)} \]

URETERS AND BLADDER

The fluid that leaves the collecting ducts and enters the ureter is now urine. The ureters connect the kidneys to the bladder. The urine is temporarily stored in the bladder and is eliminated from the body through the urethra.
RENAL DISEASES

Disease states as well as disorders directly affecting the kidney result in abnormal renal function and urinalysis tests. This section discusses the various kidney diseases and the significance of these diseases on urinalysis results.

ACUTE GLOMERULONEPHRITIS

Glomerulonephritis is an inflammatory process that affects the glomerulus. Acute implies a rapid onset of symptoms. It is frequently seen in children and young adults following respiratory infections caused by strains of group A streptococci. The streptococci are believed to form immune complexes with circulating antibodies that become deposited in the glomerular membrane which may result in glomerular membrane damage. Similar symptoms may also be seen following endocarditis, abscesses and pneumonia which also produce circulating bacterial antigens. Eradication of the infection in most cases manages this disease.

Primary urinalysis findings include hematuria, increased protein; oliguria, red blood cell casts, dysmorphic red blood cells, hyaline and granular casts, and while blood cells.

RAPIDLY PROGRESSIVE GLOMERULONEPHRITIS

This is a more serious form of glomerular disease with a poor prognosis often ending in renal failure. Symptoms are initiated by dysfunction of immune complexes in the glomerulus often as a complication of another form of glomerular nephritis or a disorder such as lupus. Permanent damage may be caused to the glomerular capsule. Initial urinalysis findings are similar to acute glomerulonephritis but become more abnormal with time.

ACUTE INTERSTITIAL NEPHRITIS

This is an inflammatory syndrome with a rapid onset of clinical symptoms associated with renal dysfunction. Causes of the syndrome include acute pyelonephritis, drug toxicity, graft rejection and immune disorders. Symptoms are usually reversed when the cause is removed.

Urinalysis findings include red blood cells, white blood cells and white blood cell casts without the presence of bacteria and mild to moderate proteinuria. The findings of eosinophils in the urine sediment are also diagnostic.

CHRONIC GLOMERULONEPHRITIS

Chronic glomerulonephritis is a variety of disorders that produce continual or permanent damage to the glomerulus. The onset begins with mild symptoms such as recurring hematuria (in young adults) or hypertension and gradually progresses to end stage renal disease.

The most common cause is an IgA nephropathy in which immune complexes are deposited on the glomeruli basement membrane. Patients usually have with an episode of macroscopic hematuria associated with strenuous exercise or an infection. Recovery of the macroscopic form is spontaneous; however, asymptomatic microhematuria may remain elevated. Examination of the urine in symptomatic chronic glomerulonephritis reveals the presence of blood, protein, and a variety of casts, including broad casts and a fixed specific gravity of 1.010 indicating loss of renal concentration ability.

MEMBRANOUS GLOMERULONEPHRITIS

This is a pronounced thickening of the glomerular capillaries with IgG deposits on the glomerular basement membrane. Autoimmune diseases deposit the immune complexes on the membrane along with increased production of epithelial cells on the membrane. Laboratory findings include microscopic hematuria and elevated urine protein excretion. Demonstration of systemic lupus erythematosus or hepatitis through blood tests can aid in diagnosis.
**MESANGIOCAPILLARY GLOMERULONEPHRITIS**

This form of glomerulonephritis is characterized by two different alterations in the cellularity of the glomerulus and peripheral capillaries. Type 1 displays increased cellularity in the subendothelial cells of Bowman’s Capsule causing thickening of the capillary walls. Type II displays extremely dense deposits in the glomerular basement membrane. Many patients are children.

The laboratory findings include hematuria, proteinuria, and decreased serum complement levels.

**NEPHROTIC SYNDROME**

The nephritic syndrome is characterized by the appearance of massive proteinuria, edema, high levels of serum lipids, and low levels of serum albumin. Circulatory disorders that affect the pressure and flow of blood to the kidney are one of the most frequent causes of the nephrotic syndrome, and it may occur as a complication in cases of glomerulonephritis. Increased permeability of the glomerular membrane caused by changes in the electrical charges in the glomerular membrane permit the passage of high molecular weight proteins and lipids into the glomerular filtrate. Absorption of the lipid-containing proteins by the renal tubular cells followed by cellular sloughing produces the characteristic oval fat bodies seen in the sediment examination. Fatty casts are also seen.

**MINIMAL CHANGE DISEASE**

Minimal change disease produces little cellular change in the glomerulus. Patients are frequently children with edema, proteinuria, and transient hematuria. Although the etiology is unknown at this time, allergic reactions, recent immunizations, and possession of the HLA-B12 antigen have been associated. The prognosis is generally good.

**PYELONEPHRITIS**

Acute pyelonephritis is most often seen in women following an untreated case of cystitis or lower urinary tract infection, and does not cause permanent damage to the renal tubules. Recurrent infections caused by structural abnormality or obstruction of the urinary tract that allow bacterial to remain in the kidney, can result in tubular damage.

Urinalysis findings in both acute and chronic pyelonephritis are similar and include white blood cell casts, bacteria, positive nitrite reaction, and possible proteinuria or hematuria.

With the exception of the presence of white blood cell casts that are indicative of tubular involvement, similar results will be found with infections of the lower urinary tract such as cystitis.

**CYSTITIS**

Cystitis is an inflammatory condition of the urinary bladder and ureters, characterized by pain, and frequency of urination. It may be caused by a bacterial infection, calculus, or tumor. Urinalysis results show macroscopic hematuria. Microscopic findings include numerous white blood cells (predominantly neutrophils), red blood cells, transitional epithelial cells, and bacteria. There is a noted absence of casts.

**VIRAL URINARY TRACT INFECTIONS**

Rarely the urinary tract may have an infection that is of viral origin. The urinalysis reveals macroscopic hematuria and occasional proteinuria. The microscopic urinalysis shows enlarged mononuclear white blood cells (lymphocytes & monocytes) some with intracellular and/or cytoplasmic inclusions. Red blood cells are also present. As in bacterial cystitis, there is a noted absence of casts.
THE MICROSCOPE

The concluding portion of the urinalysis procedure is the microscopic examination of the urine sediment. Its purpose is to identify insoluble materials that may be in the urine due to renal or urinary tract disease. The urine sediment consists of solid material which includes casts, cells, crystals and amorphous deposits of chemicals.

The examination of urine sediment requires well-trained, skilled and knowledgeable personnel. According to the CLIA-88 federal laboratory regulations, the microscopic analysis of urine sediment is a "moderately complex" test. Personnel examining the urine sediment must follow a procedure that ensures consistency in all aspects of specimen preparation, examination, identification and reporting. They must be skilled in the use of the microscope.

This section is a general review of the microscope. It is not intended to substitute for the manufacturer’s manual on the microscope(s) used at your facility. During this course, we challenge you to read your microscope manual and become familiar with its contents.
## MICROSCOPE PARTS AND FUNCTIONS

<table>
<thead>
<tr>
<th>Part</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>Arm</td>
<td>Connects the two lens systems to the base and supports the stage, condenser, and focus adjustment knobs.</td>
</tr>
<tr>
<td>Base</td>
<td>The stand on which the microscope rests; houses the light source and neutral density filter.</td>
</tr>
<tr>
<td>Brightfield</td>
<td>Concentrates light on a specimen, so that specimen contents alter the light, and project their dark image against a light background onto the objective.</td>
</tr>
<tr>
<td>Coarse adjustment knob</td>
<td>Used for rapid focusing of the specimen.</td>
</tr>
<tr>
<td>Condenser</td>
<td>The lens system beneath the microscope state positioned to concentrate light correctly on the specimen and direct light rays into the objective. When the condenser is used at a lowered position, the resolving power is reduced.</td>
</tr>
<tr>
<td>Condenser knob</td>
<td>Located at the side of the microscope and below the stage, it moves the condenser up and down. During most microscopic examinations the condenser should be at its highest point.</td>
</tr>
<tr>
<td>Eyepiece</td>
<td>Contains the ocular lenses. The interpupillary distance between the two eyepieces of a binocular microscope is adjusted by moving the eyepieces together or apart.</td>
</tr>
<tr>
<td>Field diaphragm</td>
<td>An aperture diaphragm which restricts the area of illumination.</td>
</tr>
<tr>
<td>Fine adjustment knob</td>
<td>Focuses the lens in small increments.</td>
</tr>
<tr>
<td>Focusing knobs</td>
<td>Used to change the distance between the specimen and the objective lens. This is accomplished by moving the stage. The coarse adjustment knob moves the stage quickly and for a greater distance. The fine adjustment knob moves the state only slightly for a sharper focus.</td>
</tr>
<tr>
<td>Iris diaphragm</td>
<td>An aperture located in the condenser. It has a lever that controls the amount of light passing through it into the condenser.</td>
</tr>
<tr>
<td>Nosepiece</td>
<td>Revolving plate that holds the objective lenses. The nosepiece easily rotates until the objective lens is in line with the light path.</td>
</tr>
<tr>
<td>Objective</td>
<td>The lens system nearest the specimen used to magnify the specimen. It directs the image forming rays of the specimen to the oculars.</td>
</tr>
<tr>
<td>Stage</td>
<td>Flat platform on which the slide is placed for viewing. The slide is held in place by clips attached to the top of the stage. The stage is moved from side to side and front to back by two control knobs located.</td>
</tr>
</tbody>
</table>
MICROSCOPE MAINTENANCE

The microscopic urinalysis begins with a properly maintained microscope. In order to keep your microscope working properly, it is important to invest the time and money in both daily care and professional servicing. Attention to daily care and prompt evaluation of problems can prolong the life of your microscope and prevent expensive repairs.

DAILY PERFORMANCE CHECK

Each day, before using your microscope you should:

- Check for cleanliness, oil residue, and scratches on the objectives.
- Verify that both coarse and fine focus adjustment knobs move freely.
- Assure the bulb is functional and that a spare bulb is available.
- Check the electrical cord to assure it is undamaged.
- Verify that the immersion oil is clear (free of fungal growth or debris).
- Assure the light source is aligned to result in optimal illumination.

At the completion of each day, you should:

- Rotate the objectives to assure that the low power (10X objective) is in the working position.
- Remove the slide from the slide holder and clean the stage using lens safe tissues and a mild detergent.
- Assure that the objectives and the condenser are clean by wiping with lens paper moistened in lens cleaning solution.
- Turn off the lamp.
- Cover your microscope with dust cover.

CLEANING THE MICROSCOPE

In addition to the daily maintenance of the microscope, it is necessary to thoroughly clean the microscope periodically. When cleaning the scope, it is a good time to examine it to ensure that professional service and repairs are not needed. In order to thoroughly clean the microscope you will need:

- Cotton tipped swabs
- Lens safe paper
- Lens cleaner
- A mild detergent

Clean each of the eye pieces of your microscope using a cotton tipped swab that has been slightly moistened in lens cleaning solution (Do not use Xylene or other solvents.). Polish the lens with lens safe paper and hold it up to the light to assure the lens has not become scratched and that it is thoroughly clean. Shake the eye piece slightly to assure that it does not rattle. If rattling occurs, the lens may need to be tightened. Do not attempt to clean the inside of the eyepiece or the eye tubes of your microscope.

Remove the objectives one at a time from the microscope by unscrewing them from the nosepiece. Clean the 10X and 40X objectives using a cotton tipped swab which has been slightly moistened with lens cleaning solution. Discard the swab as it collects dirt. It may take a series of swabs to complete thorough cleaning. Polish the objective with lens paper. Remove one eyepiece from the microscope and invert it. Holding the objective 3 inches from the lens of the objective, use the eyepiece to magnify the objective lens and check for scratches and fingerprints. Blot excess oil from the oil immersion objective and examine the objective using the eyepiece. It should be free of scratches and cracks.

Wipe the microscope stage and body with a mild detergent on a soft cloth. Assure that dust and oil are removed.

Clean the condenser lens and any auxiliary lenses using cotton-tipped swabs which have been slightly moistened in lens cleaning solution. Polish all lenses with lens paper.
CHANGING THE BULB

When changing the bulb on your microscope, the proper bulb should be used. Check the model number for an exact match or check with the manufacturer of your microscope before making any substitution. It is possible that bulbs which appear the same may differ in the placement of internal filaments which would affect the performance of the light source.

When changing the bulb, always unplug the microscope. After the bulb has cooled, use a cloth or gauze to grasp the old bulb and remove it from the microscope. Use a cloth or gauze to remove the new bulb from the package and insert it into the microscope. It is important that if you do touch the new bulb, use lens paper to carefully polish your fingerprints from the bulb before installing it into the microscope. Oil from your fingers will “cook” onto the bulb resulting in a shortened bulb life and possibly in uneven illumination over a period of time.

MICROSCOPIC TECHNIQUES

Most often urine sediment is examined as a wet mount of a preparation of concentrated stained sediment using a brightfield microscope. Because it is a wet preparation, oil immersion (100X) is not used. The preparation is examined under low power (10X objective) and high dry power (40X objective).

10X Objective

The 10X objective is used primarily to view casts and crystals, scan the slide, and locate elements or areas for more detailed examination. It is important to begin with the 10X objective when a specimen is examined under the microscope. If you do not scan the sample on 10X, important diagnostic information could easily be overlooked.

40X Objective

The 40X objective on most microscopes is a “dry” objective. Immersion oil is not used to view the sample. For this reason it is often called the “high-dry” objective. This objective is used to examine smaller elements that have been located and placed into the center of the field using the 10X objective. This objective is most useful for identification of red and white blood cells, epithelial cells, bacteria, yeast and parasites.

ADJUSTING THE LIGHT

Adjustment of the brightfield microscope for urine sediment can be difficult, but is essential. The light must be sufficiently reduced to give contrast to various unstained structures and the background liquid. The light is adjusted by closing the aperture iris diaphragm to increase contrast and by lowering the condenser slightly (1 to 2 mm). The condenser should not be lowered too much. Contrast is achieved by opening or closing the condenser. Translucent elements are easily overlooked using brightfield microscopy. Especially difficult to see are hyaline casts. Brightfield microscopy can be enhanced by the use of various stains available for urine sediment. Staining can be helpful in the identification of cells and casts. The most commonly used urine sediment stains are supravital stains called Sternheimer - Malbin and Toluidine Blue (0.5%). Sudan stains are available to identify fat. There is a disadvantage to stains because they can precipitate causing fine crystals or granules that can be mistaken for true crystals or amorphous materials. To stain or not to stain depends upon the experience of the testing personnel.
SCANNING SPECIMENS

Specimens should be examined carefully and systematically to avoid overlooking an important element or detail. Always begin with the 10X objective and scan the entire slide to look for elements which may require a closer look using the 40X objective. When scanning the slide, begin at one edge of the cover-slip and scan using a back-and-forth motion. Casts have a tendency to collect near the edges of the coverslip. Therefore, low power scanning of a minimum of 10 fields that include the perimeter is recommended. Calculate the average number per field.

POLARIZING MICROSCOPY

The polarizing microscopy technique is an important tool for identifying and differentiating urine sediment crystals, fat, starch and fibers. Polarized light is used to study structures that polarize light. Objects that polarize light are by definition birefringent. When viewed with crossed polarizing filters, they appear white against a black background. If a compensating filter is added, the birefringent body is seen against a magenta background. Certain crystals are identified by their pattern. Birefringent objects found in the urine sediment include crystals, fat (as cholesterol droplets), starch and fibers (which may be confused with casts). Both fat and starch show a characteristic Maltese cross pattern (a lighted cross against a dark background) when polarized. Protein material such as casts, cells and bacteria is not birefringent. Polarizing light can also differentiate amorphous materials.

MICROSCOPY TROUBLESHOOTING

When unable to obtain full performance from the microscope, consult the charts below for troubleshooting points.

<table>
<thead>
<tr>
<th>PROBLEM</th>
<th>CAUSE</th>
<th>REMEDY</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>I. Optical System</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>With illuminator on, the field of view is dark.</td>
<td>Field iris diaphragm is not opened sufficiently.</td>
<td>Open diaphragm to proper diameter.</td>
</tr>
<tr>
<td></td>
<td>Condenser is lowered too much.</td>
<td>Adjust condenser height.</td>
</tr>
<tr>
<td></td>
<td>Light path selector lever is pulled out to C position (triocular).</td>
<td>Push in lever up to CV or V position.</td>
</tr>
<tr>
<td>Field of view is cut off or illuminated irregularly.</td>
<td>Light path selector lever is stopped midway (triocular).</td>
<td>Click it into proper position according to your purpose.</td>
</tr>
<tr>
<td>---------------------------------------------------</td>
<td>-------------------------------------------------</td>
<td>-------------------------------------------------</td>
</tr>
<tr>
<td>Nosepiece is not clicked into place.</td>
<td>Slightly rotate nosepiece until it clicks into place.</td>
<td></td>
</tr>
<tr>
<td>Nosepiece is not correctly mounted.</td>
<td>Insert nosepiece dovetail into microscope frame all the way, then lock.</td>
<td></td>
</tr>
<tr>
<td>The power of the objective used exceeds the illumination capacity of condenser.</td>
<td>Choose correct condenser for objective and your purpose.</td>
<td></td>
</tr>
<tr>
<td>Condenser is not centered.</td>
<td>Center condenser.</td>
<td></td>
</tr>
<tr>
<td>Field iris diaphragm is stopped down excessively.</td>
<td>Open diaphragm to proper diameter.</td>
<td></td>
</tr>
<tr>
<td>Dust or dirt is visible in the field of view.</td>
<td>Dust, etc., on light exit lens.</td>
<td></td>
</tr>
<tr>
<td>Dust on condenser top lens.</td>
<td>Remove dust, and clean optical glass parts.</td>
<td></td>
</tr>
<tr>
<td>Dirty specimen/slide/cover glass.</td>
<td>Dust on eyepieces.</td>
<td></td>
</tr>
<tr>
<td>Excessive image contrast</td>
<td>Condenser is lowered too much.</td>
<td></td>
</tr>
<tr>
<td>Aperture iris diaphragm is stopped down too much.</td>
<td>Adjust condenser height.</td>
<td></td>
</tr>
<tr>
<td>Resolution problems: <em>image not sharp; lack of contrast; image details lack definition.</em></td>
<td>Improper objectives in use.</td>
<td></td>
</tr>
<tr>
<td>Nosepiece is incorrectly mounted.</td>
<td>Use objectives made for the microscope &amp; its component parts.</td>
<td></td>
</tr>
<tr>
<td>Objective incorrectly positioned in light path.</td>
<td>Insert nosepiece dovetail into body all the way &amp; lock into place.</td>
<td></td>
</tr>
<tr>
<td>Objective correction collar not adjusted right.</td>
<td>Click nosepiece into place.</td>
<td></td>
</tr>
<tr>
<td>Field of view is partially out of focus, or image is partly out of focus.</td>
<td>Nosepiece is not correctly mounted.</td>
<td></td>
</tr>
<tr>
<td>Objective is not correctly positioned in light path.</td>
<td>Insert nosepiece dovetail into microscope frame all the way &amp; lock in place.</td>
<td></td>
</tr>
<tr>
<td>Specimen is not correctly positioned on stage.</td>
<td>Slightly rotate nosepiece until it clicks into place.</td>
<td></td>
</tr>
<tr>
<td>Specimen image is partially out of focus.</td>
<td>Place specimen slide correctly on stage, and secure with stage, and secure with stage clips.</td>
<td></td>
</tr>
<tr>
<td>Can focus slide on low power; unable to focus on higher power objectives</td>
<td>Specimen slide upside down.</td>
<td></td>
</tr>
<tr>
<td>Objective not screwed into nosepiece fully.</td>
<td>Turn slide right side up.</td>
<td></td>
</tr>
<tr>
<td>Securely tighten objective into nosepiece.</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Field of view becomes only slightly brighter by increasing voltage.</td>
<td>Condenser is not centered.</td>
<td></td>
</tr>
<tr>
<td>Condenser is too low.</td>
<td>Adjust condenser height.</td>
<td></td>
</tr>
<tr>
<td>Too many or wrong kind of filters in light path.</td>
<td>Remove filters from light path.</td>
<td></td>
</tr>
<tr>
<td>PROBLEM</td>
<td>CAUSE</td>
<td>REMEDY</td>
</tr>
<tr>
<td>-----------------------------------------------------------------------</td>
<td>----------------------------------------------------------------------</td>
<td>------------------------------------------------------------------------</td>
</tr>
<tr>
<td><strong>II. Electrical System</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Illuminator is too bright (or too dark) even when adjusting control lever.</td>
<td>Line voltage selector switch not matched with local facility voltage.</td>
<td>Match selector switch to facility voltage.</td>
</tr>
<tr>
<td>Illuminator voltage cannot be raised.</td>
<td>Line voltage selector switch not matched with local facility voltage.</td>
<td>Match selector switch to facility voltage.</td>
</tr>
<tr>
<td>Lamp goes off and on.</td>
<td>Bulb filament is about to burn out.</td>
<td>Replace bulb.</td>
</tr>
<tr>
<td></td>
<td>Loose electrical connections.</td>
<td>Check all connections.</td>
</tr>
<tr>
<td>Bulb burns out frequently.</td>
<td>Line voltage selector switch not matched with local facility voltage.</td>
<td>Match selector switch to facility voltage.</td>
</tr>
<tr>
<td></td>
<td>Bulb is not the standard one for microscope.</td>
<td>Use only standard bulb.</td>
</tr>
<tr>
<td><strong>III. Coarse and Fine Adjustments</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Coarse adjustment knob is too tight.</td>
<td>Tension adjustment ring is too tight.</td>
<td>Loosen ring properly.</td>
</tr>
<tr>
<td></td>
<td>User trying to raise stage above focusing limit set by prefocusing lever.</td>
<td>Unlock lever, and adjust stage focusing level.</td>
</tr>
<tr>
<td>Stage drops or goes out of focus due to slipping fine adjustment knob.</td>
<td>Tension adjustment ring is too loose.</td>
<td>Tighten ring properly.</td>
</tr>
<tr>
<td>Stage cannot be raised to upper focusing limit.</td>
<td>Prefocusing lever engaged lower than focusing limit.</td>
<td>Unlock lever and adjust.</td>
</tr>
<tr>
<td>Stage cannot be lowered to lower limit.</td>
<td>Stage mount too low.</td>
<td>Raise stage mount.</td>
</tr>
<tr>
<td>Objective front lens hits specimen before achieving focus.</td>
<td>Specimen placed on stage upside down.</td>
<td>Reverse specimen slide.</td>
</tr>
<tr>
<td></td>
<td>Stage mounted too high objectives out of focus.</td>
<td>Lower stage mount and realign focus.</td>
</tr>
<tr>
<td></td>
<td>Objectives not parfocal.</td>
<td>Parfocal objectives.</td>
</tr>
<tr>
<td></td>
<td>Objective not screwed in nosepiece securely.</td>
<td>Securely tighten objective in nosepiece.</td>
</tr>
<tr>
<td><strong>IV. Observation tubes</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Incomplete binocular vision.</td>
<td>Interpupillary distance is not correctly adjusted.</td>
<td>Correct the interpupillary distance.</td>
</tr>
<tr>
<td></td>
<td>Diopter adjustment is incomplete.</td>
<td>Complete the diopter adjustment.</td>
</tr>
<tr>
<td></td>
<td>Right and left eyepieces are not matched.</td>
<td>Use only matched pair of eyepieces.</td>
</tr>
<tr>
<td></td>
<td>User unaccustomed to binocular vision.</td>
<td>Before looking into binocular observation tube look off into distance.</td>
</tr>
<tr>
<td><strong>V. Stage</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Image easily goes out of focus when you touch the stage.</td>
<td>Stage is not correctly locked in place.</td>
<td>Clamp stage securely.</td>
</tr>
<tr>
<td>Specimen stops midway on the side-to-side traverse.</td>
<td>Specimen or slide is not positioned correctly.</td>
<td>Adjust specimen or slide position correctly.</td>
</tr>
</tbody>
</table>
## GLOSSARY OF TERMS

<table>
<thead>
<tr>
<th>Term</th>
<th>Definition</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bacillus</td>
<td>Any rod shaped bacterium.</td>
</tr>
<tr>
<td>Brightfield</td>
<td>A microscopy technique in which light passes directly through specimen and into the objective, making specimen image appear dark against a bright background.</td>
</tr>
<tr>
<td>Brownian Motion</td>
<td>The random, dancing, zigzag movements of minute, microscopic particles suspended in liquid. This motion is due to collisions of the particles with the individual random-moving molecules of the solvent.</td>
</tr>
<tr>
<td>Coccus/Cocci</td>
<td>A bacterial cell with a spherical shape.</td>
</tr>
<tr>
<td>Compound microscope</td>
<td>A microscope made up of two lens systems, eyepiece and objective.</td>
</tr>
<tr>
<td>Contrast</td>
<td>A relative difference between the brightest and darkest parts of the specimen; crispness. It is controlled by the aperture diaphragm. Too little contrast results in lack of definition; too much contrast reduces resolution.</td>
</tr>
<tr>
<td>Cover glass</td>
<td>An ultra thin glass made to cover the specimen on the slide. It is a part of the image forming system. The cover glass has optical properties which are taken into account in computing and designing objective. Manufacturers specify the thickness of the cover glass for general use on the microscope.</td>
</tr>
<tr>
<td>Definition</td>
<td>The brilliance, clarity, distinctness, and sharpness with which the microscope magnifies and reproduces specimen detail.</td>
</tr>
<tr>
<td>Depth of field</td>
<td>Distance just above and below the focal plane (area being examined) that can be focused clearly.</td>
</tr>
<tr>
<td>Dry objectives</td>
<td>Microscope objectives designed to be used dry, i.e. without oil.</td>
</tr>
<tr>
<td>Focal Length</td>
<td>The distance of the focus from the surface of a lens.</td>
</tr>
<tr>
<td>Field of view</td>
<td>The visible area through an in-focus lens.</td>
</tr>
<tr>
<td>Filters</td>
<td>Used to control the intensity or colors of illumination.</td>
</tr>
<tr>
<td>Neutral Density Filters</td>
<td>A filter at the light source to control the intensity (brightness) of illumination.</td>
</tr>
<tr>
<td>Total magnification</td>
<td>Magnification of eyepiece x magnification of objective. Example: eyepiece = 10X and objective = 40X Total magnification = (10X) x (40X) = 400X</td>
</tr>
<tr>
<td>Microscopy</td>
<td>The science of the uses and applications of microscopes. Two objectives of microscopy are forming a magnified image with as few optical defects as possible, and achieving good resolution and contrast. Contrast is based on the differential absorption of light between the specimen under study and its background; resolution is the ability to reveal and separate fine detail.</td>
</tr>
<tr>
<td>Motility</td>
<td>Having spontaneous but not conscious movement, contractility, ability of an organism to move in the medium, usually associated with the presence of flagella, cilia, or pseudopodia.</td>
</tr>
<tr>
<td>Term</td>
<td>Definition</td>
</tr>
<tr>
<td>-------------------------------------------</td>
<td>------------------------------------------------------------------------------------------------------------------------------------------</td>
</tr>
</tbody>
</table>
| Numerical aperture (NA)                   | A number, usually engraved on the objectives and condensers, expressing the size of the cone of light delivered by the condenser or collected by the objective. NA is defined by the formula: $\text{NA} = n \sin \theta$

where $\theta$ = the half-angle of the light cone entering the objective; and, $n$ = refractive index of medium between specimen and objective:

- air: $n = 1.000$
- distilled water: $n = 1.333$
- oil and glass: $n = 1.515$

The higher the NA, the greater the resolving power; however, the NA of the condenser must be ≥ the NA of the objective to achieve the full resolving power of the objective. |
| Parfocal                                  | The objectives are constructed so that only slight refocusing with the fine adjustment knob is needed after rotating to another objective. |
| Refractive index                          | Ratio of the speed of light in the first medium to the speed of light in the second medium.                                             |
| Resolution                                | The ability of a microscope to reveal fine detail in a specimen. The better the resolving power of a microscope the closer two objects can be and still be distinguished as two objects. |
| Stage                                     | The platform on which the microscope slide is placed.                                                                                  |
| Focal Plane                               | The up/down level to which the microscope slide is placed.                                                                              |
| Image                                     | A picture or conception:

- **Real** - one formed by the collection rays in which the object is pictured as being inverted. Its presence can be viewed only by insertion of a receiving screen, etc.
- **Virtual** - formed by a converging lens. The image seems to be situated on the same side of the lens as the object. |
| Immersion oil                             | An oil with the same refractive index as glass, 1.515; used between the cover glass and an oil immersion objective to prevent scattering of light in air. |
| Interpupillary distance                   | The distance between the eyes. The eyepieces of a binocular scope must be adjusted so that left and right images merge into one. |
| Iris diaphragm                            | Adjustable assembly of thin metal leaves for varying the size of openings that determine the cross section of the light ray bundle entering the condenser and the objectives. Both the field and aperture diaphragms are iris diaphragms. |
| Koehler illumination                      | A method of optical illumination providing bright, evenly dispersed, glare-free light with good contrast and resolution. The light beam is focused on the back focal plane of the condenser, the field diaphragm is focused in the field of view, and then the light is focused again at the backless of the objective. |
| Lens                                      | A piece of glass or other transparent substance shaped to gather or scatter light rays, and used in the microscope and other instruments to magnify, increasing the visual acuity of the human eye. |
| Magnification                             | The number of times larger the image appears as seen through the microscope, than it appears to the eye at a distance of 10 inches (25.3 cm). The ratio, in diameters, usually is expressed as “power”, “times”, or “X”. |
| Working distance                          | Distance between coverslip of a slide and the tip of an objective. The low power objective has the greatest working distance. The oil immersion objective has a very small working distance. |
CHAPTER OBJECTIVES

- Recognize the formed elements in the urine sediment, which include:
  - Red blood cells
  - White blood cells
  - Epithelial cells
  - casts
  - Crystals
  - Amorphous material
  - Artifacts
- Understand the clinical significance and origin of the various formed elements in urine sediment
- Describe the standardized examination of urine sediment

URINE SEDIMENT STANDARDIZATION

The urine sediment consists of any formed elements suspended in the urine. These elements include cells, casts, crystals, amorphous materials, and artifacts. The physical appearance and reagent strip test results are clues in the identification of the sediment constituents. The visual, chemical, and microscopic examination of sediment must be correlated to ensure accuracy of the urinalysis results.

Traditionally, a urine sediment examination has been part of routine urinalysis. However, recent studies have shown that the microscopic examination may not be necessary on every urine specimen tested.

NCCLS states that “the decision to perform a microscopic examination must be made by each individual laboratory based on its patient population.” Urine microscopics should be performed when requested by the physician, when determined by laboratory protocol, or when any visual or chemical result is abnormal. Before adopting this policy, the laboratory must have good standardized techniques and a quality control program in place.

Conditions to skip the microscopic examination:
- yellow or straw in color, with a clear appearance
- reagent strip tests are negative or normal
Conditions to perform the microscopic examination:

- any color other than yellow
- hazy, cloudy, or turbid
- protein greater than trace
- glucose, blood, nitrite, or leukocyte is positive
- if the clinician requests it

**SPECIMEN REQUIREMENTS**

The first morning midstream clean catch urine specimen is the specimen of choice for urine microscopics. It is a good representative sample of the patient’s condition and is less likely to be contaminated from external sources.

Random specimens are acceptable but need to be interpreted carefully with respect to contamination. Ideally, 25-50 ml should be collected into a clean 100 ml screw cap container. At least 12 ml is required for urine sediment concentration. Specimens should be centrifuged and examined within 2 hours of collection held at room temperature. Refrigeration of the urine retards urine decomposition but causes amorphous material to deposit which could obscure pathologic constituents.

**URINE SEDIMENTATION PROCEDURE**

To ensure consistent urine microscopic results, it is necessary to standardize the procedure. Standardized urine sediment systems with specially designed centrifuge tubes and pipettes are available. Systems differ in final urine sediment volume, but are alike in providing a capped centrifuge tube, transfer pipette, stain (optional), and specially designed slides. If the traditional glass microscope slides and cover slips are used, a uniform volume of concentrated sediment should be placed on the slide and covered with a standard #1 coverslip (22 x 22 mm).

Whatever system is used, the following specific factors must be standardized:

- **Urine volume.** Twelve ml is generally recommended for centrifugation and is the volume employed by standardized systems. If a different volume is used, the final concentration of urine examined must be applied to numerical counts.

- **Time of centrifugation.** Five minutes is recommended.

- **Speed of centrifugation.** NCCLS recommends a relative centrifugal force (RCF) of 400 g. Other standard textbooks recommend 450 or 400-450 g. Nomogram (a graphical plot used for solving certain types of equations) can be used to relate the revolutions per minute (RPM) to RCF by measuring the radius (r) of the centrifuge head in centimeters from the center pin to the bottom of a horizontal cup or from the following formula:

\[
\text{RCF(g)} = (11.18 \times 10^{-6}) \times (r) \times \text{RPM}^2
\]

- **Concentration factor of the sediment.** This is based on the volume of well-mixed urine centrifuged and the final volume of sediment remaining after the supernatant urine is removed. A uniform amount of 0.5 to 1.0 ml should remain in the tube after decantation.

- **Sediment drop uniformity.** The drop transferred to the slide should be of uniform size, large enough to fill the coverslip without air bubbles, but small enough not to run over the slide. An excessive amount of fluid may cause casts to go out of the vision area.

- **Reporting Format.** Every person in an institution who performs a microscopic examination of the urinary sediment should use the same terminology, reporting format, and reference ranges.

- **Correlate** microscopic results with physical and chemical findings.
ROUTINE URINALYSIS CORRELATIONS

<table>
<thead>
<tr>
<th>Microscopic Elements</th>
<th>Physical</th>
<th>Chemical</th>
<th>Exceptions</th>
</tr>
</thead>
<tbody>
<tr>
<td>Red blood cells</td>
<td>Turbidity, Red</td>
<td>+ Blood</td>
<td>Number, Hemolysis</td>
</tr>
<tr>
<td></td>
<td>color</td>
<td></td>
<td></td>
</tr>
<tr>
<td>White blood cells</td>
<td>Turbidity</td>
<td>+ Protein, + Nitrite, +</td>
<td>Number, Lysis</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Leukocytes</td>
<td></td>
</tr>
<tr>
<td>Epithelial cells</td>
<td>Turbidity</td>
<td></td>
<td>Number</td>
</tr>
<tr>
<td>Casts</td>
<td>Turbidity</td>
<td>+ Protein</td>
<td>Number</td>
</tr>
<tr>
<td>Bacteria</td>
<td>Turbidity</td>
<td>pH, + Nitrite, + Leukocytes</td>
<td>Number and Type</td>
</tr>
<tr>
<td>Crystals</td>
<td>Turbidity, Color</td>
<td>pH</td>
<td>Number and Type</td>
</tr>
</tbody>
</table>


NORMAL URINE SEDIMENT

Normal urine contains urinary sediment. It is the amount and the type of constituent that determines abnormality. The following table is a reference guide for distinguishing normal from abnormal. Keep in mind all laboratories should establish their own normal values.

NORMAL REFERENCE VALUES FOR URINARY SEDIMENT

<table>
<thead>
<tr>
<th>SEDIMENT CONSTITUENT</th>
<th>12:1 CONCENTRATION</th>
</tr>
</thead>
<tbody>
<tr>
<td>Red blood cells</td>
<td>0 – 2 / hpf*</td>
</tr>
<tr>
<td>White blood cells</td>
<td>0 – 5 / hpf</td>
</tr>
<tr>
<td>Hyaline casts</td>
<td>0 – 2 / lpf</td>
</tr>
<tr>
<td>Renal epithelial cells</td>
<td>few / hpf</td>
</tr>
<tr>
<td>Transitional epithelial cells</td>
<td>few / hpf</td>
</tr>
<tr>
<td>Squamous epithelial cells</td>
<td>few / lpf</td>
</tr>
<tr>
<td>Bacteria</td>
<td>neg / hpf</td>
</tr>
<tr>
<td>Abnormal crystals</td>
<td>none / lpf</td>
</tr>
</tbody>
</table>

*hpf = high power (40x objective) field; *lpf = low power (10x objective) field.
CELLS FOUND IN URINE SEDIMENTS

1. RED BLOOD CELLS (RBC)

**Normal:** Colorless discs which may appear biconcave, crenated (concentrated urine) or swollen (dilute urine).

**Description:** Colorless discs which may appear biconcave, crenated (concentrated urine) or swollen (dilute urine).

**Clinical Significance:** Although the presence of 0-2 RBC’s/hpf is considered normal, the presence of RBC’s in the urine (hematuria) is an early indicator of renal disease. When blood is detected on the reagent strip, the microscopic is an important piece of information in distinguishing between hematuria, hemoglobinuria, and myoglobinuria, all conditions of different origin. Hematuria is the presence of intact RBC’s. Hemoglobinuria is the presence of free hemoglobin in the urine. Myoglobinuria is the presence of the muscle hemoglobin called myoglobin in the urine. When myoglobin is found in the urine, it is indicative of muscle wasting. In general, with hematuria, red blood cells are present in the urine sediment but absent with hemoglobinuria and myoglobinuria.

Bleeding can occur at any point in the urinary system. The presence of RBC’s only indicates a problem of the lower urinary system. When RBC’s are accompanied by casts in the urine sediment and protein on the reagent strip urinalysis, the bleeding may be of glomerular origin within the kidney. The amount and shape of the RBC’s also correlate with the severity of the problem. Dysmorphic or irregularly shaped RBC’s could indicate glomerular origin.

**Structures which can be misidentified as red blood cells:** yeast, calcium oxalate, bubbles, oil droplets, lymphocytic white blood cells.

2. WHITE BLOOD CELLS (WBC)

**MONOCYTE**

**LYMPHOCYTE**

**NEUTROPHIL**

**EOSINOPHIL**

**MACROPHAGE**

**Description:** Rounded, globular cells, most having lobed nuclei; larger than RBC’s. Supravital Stain may be helpful in identifying nuclear material.
Clinical Significance: Normal concentrated sediment may contain up to 5 leukocytes per high power field. Though the presence of WBC’s generally refers to neutrophils, the presence of lymphocytes and eosinophils are of diagnostic significance. An increase in WBC’s in the urine is called pyuria and indicates inflammation at some point in the urinary system. Neutrophils plus bacteria are generally seen together. Clumps of WBC’s indicate acute infection.

Glitter cells are a type of neutrophil seen in dilute urine that contains granules which exhibit Brownian motion. This motion results in a shiny or glittry appearance of the cell.

Eosinophils in urine sediment are identifiable only with supravital stains. They indicate drug induced nephritis including hypersensitivity to antibiotics. Lymphocytes can be seen with renal transplant rejection. Lymphocytes will not react with leukocyte esterase on the reagent strip.

Structures which can be misidentified as white blood cells: red blood cells, renal epithelial cells

3. EPITHELIAL CELLS

Epithelial cells are cells that line all the urinary and genital tracts. They are of three different types: squamous, transitional and renal. Identification is clinically significant.

SQUAMOUS EPITHELIAL CELLS

Description: Squamous epithelial cells are the largest cells seen in normal urine specimens. They are thin, flat cells, usually with an angular or irregular cell membrane and granular cytoplasm. The nucleus is small but prominent nucleus. They may be present as single cells or as variably-sized clusters.

Clinical significance: Squamous epithelial cells line the lower urethra and vaginal areas. Large numbers often represent vaginal contamination in females or foreskin contamination in males. A few squamous epithelial cells are seen in most urine specimens. Their presence has little clinical significance.

Clue cells are a special type of squamous cell seen in bacterial vaginitis caused by *Gardnerella vaginalis*, a rod-shaped bacterium. The bacteria encrust themselves to the surface of the epithelial cell. To be reported as a clue cell, most of the surfaces should be covered with bacteria.
TRANSITIONAL EPITHELIAL CELLS

Description - Transitional epithelial cells are round or pear shaped with a central nucleus. Most often they are round or polygonal because they take in water and swell spherically. Some transitional cells have tails of cytoplasm and are referred to as caudate cells. These originate from the renal pelvis or the bladder and are not clinically significant.

Clinical Significance - Transitional epithelial cells form the lining of the urinary tract from the pelvis of the kidney to the base of the bladder in female and the proximal part of the male urethra. Those cells closer to the kidney are deeper, thicker, and rounder. Normal urine may contain a few transitional cells. Numbers are increased in infections. Clusters or sheets may be seen after catheterization.

Structures which can be misidentified as transitional epithelial cells - white blood cells, renal epithelial cells.

RENAL TUBULAR EPITHELIAL CELLS

Description: Renal tubular cells are originally cubic in shape; but once exfoliated, they adopt a rounded or elongated oval shape with a granular cytoplasm. They have a single round eccentric nucleus. Renal tubular cells vary in size from slightly larger to twice as large as a WBC. Renal cells do not absorb water and swell as do transitional cells; therefore they tend to retain an oval rather than round shape.

Clinical significance - Renal epithelial cells originate from the proximal to the distal convoluted tubules, as well as the collecting ducts. Small numbers can be found in normal urine. Increased numbers, e.g., 15 renal tubular epithelial cells per hpf, provide evidence of active renal disease or tubular injury. Their clinical significance also includes tubular necrosis, viral kidney infections, renal transplant rejection and chemical toxins.

Oval fat bodies are degenerated or necrotic renal epithelial cells filled with fat droplets. They can be recognized with brightfield microscopy but may need special fat stains for identification (Sudan stains).

If cholesterol is present in the fat globules, they may be visualized with polarized light. The characteristic Maltese cross (white cross on black background) is seen when the specimen is viewed with crossed polarizing filters.

Structures which can be misidentified as renal tubular epithelial cells: transitional epithelial cells and white blood cells.

4. BACTERIA

Description - Bacteria are the smallest cellular elements of clinical importance found in urine sediment. Most bacteria present in urine sediment are rod-shaped (bacillus). Rounded forms (cocci) can be seen mostly due to contamination. Bacteria in a fresh specimen may be motile.

Clinical Significance - Bacteria are not normal constituents of urine. However, unless specimens are collected under sterile conditions, bacterial contamination will occur. Specimens that are stored at room temperature may have a significant amount of bacteria that represents nothing
diagnostically. Bacteria in a urine sediment that has been properly collected and stored indicates a urinary tract infection. White blood cells are present and the reagent strip is positive for nitrite, leukocyte esterase, and protein if a significant urinary tract infection is present. Most urinary tract infections are due to bacteria of fecal origin such as *Escherichia coli* or *Proteus* species, which are both gram-negative rods.

**Structures which can be misidentified as bacteria** - amorphous phosphates, amorphous urates.

### 6. YEAST

**Description**: Yeast can vary in appearance from ovoid shape to ovoid with buds and elongated branches called pseudohyphae. They have a smooth refractile appearance with no granules.

**Clinical Significance** - Yeast in the urine are most often Candida species. They result from urine contamination from a vaginal or skin yeast infection. They can be associated with the presence of glucose in urine, often with diabetic patients. The presence of pseudohyphae (elongated cells resembling mycelia) may indicate a true kidney infection caused by Candida species. For this reason, pseudohyphae when present in the urine sediment should be noted on the report along with yeast.

**Structures which can be misidentified as yeast** - red blood cells

### 6. PARASITES

*Trichomonas vaginalis* – the most commonly seen parasite.

**Description** - *Trichomonas vaginalis* is a flagellated protozoan that is pear shaped and about the size of a large white blood cell. The organism has a single nucleus, four anterior flagella, an anterior undulating membrane, and a sharp protruding axostyle. Their most distinguishing feature is a rapid jerky motility.

**Diagnostic Significance** - *Trichomonas vaginalis* is a contaminant of urine that is responsible for vaginal, urethral, bladder, periurethral, and prostate infections. If present, it resides in the vagina in women and the prostate in men.

**Structures which can be misidentified as Trichomonas** - white blood cells, transitorial epithelial cells.

Urine may rarely contain a variety of parasites other than *Trichomonas*. They are of fecal or vaginal origin with exception of *Schistosomiasis* which can reside in the urinary tract. If a parasite is suspected, consult with your microbiology personnel. In most cases, what looks like a parasite may be nothing more than a pollen grain or starch granule.
CASTS

Casts are the result of precipitation or clumping together of protein material in the tubules of the kidney. This produces solidified protein that is molded to the lumen of the distal convoluted tubule, ascending loop of Henle, and/or collecting ducts. Their shape generally consists of parallel sides and round or blunt ends. The major protein in casts is Tamm-Horsfall protein which is excreted by the renal tubular cells. Casts provide a microscopic view of the nephron. When the cast is formed any material present in the tubule (e.g. cells, bacteria, fat) is trapped within the cast. This helps to identify the type of cast and the condition within the kidney that is represented. Cast formation is enhanced by decreased urine flow, an acid pH and high concentration of solutes (increased specific gravity). Casts are the only element found in the microscopic examinations that are unique to the kidney.

**STEPS IN THE FORMATION OF A CAST:**

- Aggregation of Tamm-Horsfall protein into individual protein fibrils
- Attachment of the protein fibrils to the surface of the tubular epithelial cells to prevent them from being washed away
- Interweaving of protein fibrils to form a loose fibrillar network (urinary constituents may become enmeshed in the network at this time)
- Further protein fibril interweaving to form a solid structure
- Possible attachment of urinary constituents to the solid matrix
- Detachment of protein fibrils from the epithelial cells
- Excretion of the cast

**HYALINE CAST**

**Description:** Hyaline casts are colorless with parallel sides, uniform diameter, definite borders and rounded ends. They are difficult to see with brightfield microscopy and need careful light adjustment. The light must give contrast by lowering the condenser slightly. The use of supravital stains is helpful. The narrow diameter form of this cast with one end tapering off into a tail is referred to as a **cylindroid**. Cylindroids are formed at the loop of Henle and distal convoluted tubules, which causes the characteristic shape. They are reported as number of hyaline casts per low power field.

**Clinical Significance** - Hyaline casts are the least important cast. Less than 2 hyaline casts per lpf may be seen in normal urine. They result from Tamm-Horsfall protein secreted into the renal tubular epithelium that aggregates into fibrils. The reagent strip result for protein may be positive or negative. Strenuous exercise will produce an increased number of this type of cast but the urine sediment returns to normal after 48 hours. Hyaline casts may be seen in large numbers in moderate to severe renal disease.

**Structures which can be misidentified as hyaline casts:** mucus, epithelial cells rolled into a cigar shape, yeast with pseudohyphae.
**WHITE BLOOD CELL (WBC) CASTS**

**Description:** WBC casts have parallel sides, rounded ends, and WBC’s embedded within the protein material. WBC casts are relatively easy to see with brightfield microscopy or supravital stain. The number of WBC’s within the cast varies from packed to few. Clumps of WBC’s adhering to the exterior of a mucus thread are called a **pseudocyst** which may be difficult to distinguish from a WBC cast.

**Clinical Significance** - WBC casts are not a normal finding. They are most often made up of neutrophils. Pyelonephritis or acute interstitial nephritis will produce WBC casts. When WBC casts are present, the origin of the infection is in the kidney rather than the bladder.

**Structures which can be misidentified as WBC casts** - pseudocyst, epithelial cell casts

---

**EPITHELIAL CELL CAST**  
(Renal tubular)

**Description** - Epithelial cell casts have parallel sides, rounded ends and renal epithelial cells embedded within the cast. They vary in size and shape. The epithelial cell cast undergoes changes from cellular, to granular, and finally waxy.

**Clinical Significance** - Epithelial cell casts result from destruction of the cells that line the renal tubules. Damage may be irreversible depending on the disease severity. Epithelial cell casts may be seen after exposure to nephrotoxic substances such as mercury or antifreeze or associated with viral infections.

**Structures which can be misidentified as epithelial cell casts** - epithelial cells rolled into a cigar shape.

---

**RED BLOOD CELL CAST (RBC AND HEMOGLOBIN)**

**Description** - RBC casts have parallel sides, round ends and red blood cells entrapped within the protein matrix. White blood cells and epithelial cells also can be seen within the cast. They are refractile and have a characteristic color ranging from yellow to brown from released hemoglobin under brightfield illumination. They are often observed as fragments or portions because red blood cell casts are probably the most fragile cast. As the cast ages, cells lyse and the casts appear waxy but retain the characteristic yellow to brown color. The aged red blood cell cast is then called a hemoglobin cast. Hemoglobin casts should be reported as RBC casts 1pf. Differentiation between the hemoglobin cast and red blood cell cast is not necessary because diagnostically they represent the same disease states.

**Clinical Significance** - The presence of red blood cell casts show bleeding within the nephron. Primarily they are associated with glomerulonephritis or lupus nephritis; however anything that damages the glomerulus, tubules or renal capillaries may cause production of RBC casts. Very strenuous exercise can also cause RBC casts to be produced but it is only a temporary condition.

**Structures which can be misidentified as red blood cell casts** - granular casts with bilirubin or pyridium in the urine sediment.
GRANULAR CASTS

Description - Granular casts have parallel sides and rounded ends with coarse to fine granules. The size of the granules within the cast varies and becomes progressively smaller with degeneration. The number of granules also varies from only a few within a hyaline matrix to a cast that seems to be completely filled. Granular casts, whether the granules are coarse or fine, need not be further classified. Although many laboratories distinguish between coarse and fine granular casts, it is not necessary. Reporting the term granular is sufficient. If a cast has a definite hyaline matrix with only a very few granules, it should be reported as hyaline rather than granular.

Clinical Significance - A few granular casts may be present in normal urines, especially during vigorous exercise. The granules may result from disintegration of tubular cells, cellular casts or protein aggregates such as Tamm-Horsfall protein. Urinary stasis which allows the casts to remain in the tubules longer must occur to result in a granular cast. Granular casts may be seen in glomerular (glomerulonephritis) or tubular disease (interstitial nephritis). Renal graft rejection may also produce this cast.

Structures which can be misidentified as granular casts: cellular casts, rolled epithelial cells, clothing fibers, scratches on lens.

WAXY CASTS

Description - Waxy casts have a brittle texture that causes them to be fragmented, or cracked. They are homogeneous, like hyaline casts, but are easier to see because they are refractile with a broader width and blunt ends.

Clinical Significance - Waxy casts are the final stages of the degeneration process from cellular to granular to waxy. They indicate a state of extreme renal stasis and are associated with chronic renal disease. They may appear in telescoped sediment which is urine sediment that contains a range of casts in various stages of degeneration. This is seen during a period when the kidney resumes function after a period of shutdown.

Structures which can be misidentified as waxy casts - diaper fibers (NOTE: differentiate from casts fibers based on negative reagent strip results for protein and other sediment findings. Fibers will polarize with polarizing filters and casts will not polarize.)

BROAD CASTS (RENAL FAILURE CASTS)

Description - Broad casts are of all cast types but appear wider. Many times they are waxy casts of greater diameter.

Clinical Significance - Broad casts are formed in the collecting ducts when the flow from the kidney tubules becomes severely compromised. The finding of this type of cast represents renal failure.

CASTS: KEY POINTS & REVIEW

- Unique to the kidney, formed in the tubules and collecting ducts
- Acid pH, high specific gravity, increased protein, decreased urine flow
- Parallel sides and rounded or blunt ends
- Usually pathogenic
- Do not polarize as fibers do
# SUMMARY OF URINE CASTS

<table>
<thead>
<tr>
<th>Type</th>
<th>Origin &amp; Key Points</th>
<th>Clinical Significance</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hyaline</td>
<td>Tubular secretion of Tamm-Horsfall protein that aggregates into fibrils. Contain no granules or exclusions.</td>
<td>Glomerulonephritis, Pyelonephritis, Chronic renal disease, Congestive heart failure, Stress and exercise, 0-1/1pf normal</td>
</tr>
<tr>
<td>Red blood cell</td>
<td>Red blood cell enmeshed within the Tamm-Horsfall protein matrix. Cells are not just stuck to the outside.</td>
<td>Glomerulonephritis, Strenuous exercise</td>
</tr>
<tr>
<td>White blood cell</td>
<td>White blood cells enmeshed within the Tamm-Horsfall protein matrix. Cells are not just stuck to the outside.</td>
<td>Pyelonephritis, Acute interstitial nephritis</td>
</tr>
<tr>
<td>Bacterial</td>
<td>Bacteria attached to Tamm-Horsfall protein matrix.</td>
<td>Pyelonephritis</td>
</tr>
<tr>
<td>Epithelial cell</td>
<td>Renal Tubular cells enmeshed within Tamm-Horsfall protein fibrils.</td>
<td>Renal tubular damage</td>
</tr>
<tr>
<td>Granular</td>
<td>Disintegration of cellular casts resulting in course or fine granules.</td>
<td>Glomerulonephritis, Pyelonephritis, Stress and exercise</td>
</tr>
<tr>
<td>Waxy</td>
<td>Refractile fractures and very brittle or no granules exclusion.</td>
<td>Stasis of urine flow</td>
</tr>
</tbody>
</table>
| Fatty         | Urinary lipids
Oval fat bodies                                                                                                                                                                                                 | Nephrotic syndrome                                                                    |
| Broad casts   | Formation of all types of casts in the collecting ducts or distended distal tubules. They are 2-6 times wider.                                                                                                     | Extreme stasis of flow, Serious Prognosis                                              |

CRYSTALS

Crystals are commonly present in urine sediment. They are seldom of clinical significance. In some cases, they obscure more important pathological findings such as cells or casts. Some can be beautiful, especially under polarized light with their birefringent properties. Identification of crystals is necessary to ensure that they do not represent an abnormal condition.

Abnormal crystals are of metabolic origin. Crystals are formed by the precipitation of urine salts subjected to changes in pH, temperature, or concentration. The presence of crystals in the urine is called crystalluria. As urine cools to room temperature or is refrigerated, specimens may become cloudy due to crystal precipitation. Generally crystals are important only when present in freshly voided urine.

Crystal forms found in the urine sediment are classified as normal or abnormal. These are further classified as acid crystals or alkaline crystals. Other crystals are of drug origin.

Crystals are identified by morphology and urine pH. Normal crystals are generally reported as few, moderate or many per high power field. Abnormal crystals are reported as average number per low power field. Confirmation of some crystals may require a chemical test such as a diazo reaction for the sulfonamides or the cyanide nitroprusside reaction for cystine. Knowledge of medication or procedures that involve radiographic contrast media is also helpful.

NORMAL CRYSTALS

ACID URINE (pH of 6.5 or less)

1. URIC ACID

Description - Uric acid crystals are seen in a variety of shapes, including four-sided and rhombic plates, rosettes, wedges, and needles. They may be relatively thick or thin. Uric acid crystals are birefringent and give a beautiful display of colors when viewed with polarized light. Uric acid becomes soluble at 60°C and in 10% sodium hydroxide.

Clinical significance - Although of little clinical significance, the presence of uric acid may be associated with gout, stone formation, gouty nephritis (in large numbers), leukemia patients (particularly in chemotherapy), and Lesch-Nyland syndrome. Together with amorphous urates, uric acid crystals are the most common crystals seen in acid urine. The pH of the urine needs to be less than 6 for the crystals to be present. They are dependent on dietary intake of purines and breakdown of nucleic acids.
2. **AMORPHOUS URATES**

**Description** - Amorphous means without shape. Amorphous urates are yellow to red to brown colored microcrystals that polarize light. They precipitate in refrigerated or room temperature urine. The physical appearance of urine containing amorphous urates resembles bloody urine. Amorphous urates are soluble at 60°C and in 10% sodium hydroxide. They will change to uric acid when acidified with acetic acid.

**Clinical significance** - Amorphous urates can often be seen in concentrated urine associated with dehydration and fever. Generally, they are of no diagnostic significance and are one of the most common crystals.

**Structures which can be misidentified as amorphous materials:** granular casts (when amorphous appears in clumps or adhere to mucus).

3. **CALCIUM OXALATE**

**Description** - Calcium oxalate is seen as a colorless octahedron - an eight-sided form that may be thought of as two four-sided pyramids joined at far ends. They are often described as envelopes or squares with a cross in the center. Large and small forms may be seen. Occasionally, calcium oxalate is present in a small ovoid form. The ovoid shape is troublesome to identify since the size and shape resembles a red blood cell. It may be distinguished from RBC’s by polarized light. Calcium oxalate is birefringent when viewed with polarized light.

**Clinical Significance** - The presence of calcium oxalate without symptoms is of little clinical significance. However, with symptoms, the crystals may give evidence of urinary stones, which can contain calcium. Large calcium oxalate crystals in clusters are associated with stones and ethylene glycol poisons. Excess oxalate from food containing oxalic acid (spinach, rhubarb and Vitamin C (ascorbic acid) may cause formation of calcium oxalate crystals. Calcium oxalate may rarely appear in neutral and alkaline urine.

**Structures which can be misidentified as calcium oxalate:** red blood cells.

4. **CALCIUM CARBONATE**

**Description** - Calcium carbonate crystals are small and colorless with a characteristic dumbbell shape. They may appear in clumps or singly. When acetic acid is added to urine sediment containing this crystal, calcium dioxide gas is released which produces bubbles. Calcium carbonate is strongly birefringent.

**Clinical significance** - Calcium carbonate is of little clinical significance. It is an uncommon crystal in normal urine.

**Structures which can be misidentified as calcium carbonate:** amorphous phosphates.
ALKALINE URINE

1. **AMORPHOUS PHOSPHATES**

   **Description** - Amorphous phosphates are shapeless microcrystals that lack color and tend to be finer than amorphous urates. Unlike urates, phosphates do not dissolve when heated to 60°C and dissolve when treated with acetic acid and dilute hydrochloric acid. Macroscopically, amorphous phosphates appear as a fluffy white precipitate that causes cloudiness in alkaline urine.

   **Diagnostic significance** - Amorphous phosphates have little clinical significance. They are a nuisance because they obscure the presence of bacteria.

   **Structures which can be misidentified as amorphous phosphates**: bacteria.

2. **CALCIUM PHOSPHATE**

   **Description** - Calcium phosphates are colorless prisms with a wedge-like end which can appear as plates or needles. They are weakly birefringent when viewed with polarized light. Calcium phosphate is not soluble when heated to 60°C, but is soluble in dilute hydrochloric acid.

   **Clinical Significance** - Calcium phosphate is of little clinical significance.

   **Structures which can be misidentified as calcium phosphate**: sulfonamides.

3. **TRIPLE PHOSPHATE - (AMMONIUM MAGNESIUM PHOSPHATE)**

   **Description** - Triple phosphate is a colorless crystal, characteristically a three-to-six-sided prism. They are often referred to as a “coffin lid” shape. They can be small or large in size. Triple phosphate is birefringent when viewed with polarized light. Occasionally, triple phosphates will appear as a fern leaf when they start to dissolve into solution. Triple phosphates are soluble in acetic acid.

   **Clinical Significance** - Triple phosphate is of little clinical significance. However, some urinary stones contain triple phosphate.

   **Structures which can be misidentified as triple phosphate**: calcium oxalate.

4. **AMMONIUM BIURATE**

   **Description** - Ammonium biurate crystals are yellow-brown colored spheres often described as “thorny apples”. They are soluble at 60°C and convert to uric acid when treated with acetic acid or hydrochloric acid.

   **Clinical Significance** - Ammonium biurate is not clinically significant. It precipitates as urine stands at room temperature. Ammonium biurate is the alkaline counterpart to uric acid.

   **Structures which can be misidentified as ammonium biurate**: sulfonamides.
ABNORMAL URINE CRYSTALS

In most cases, abnormal crystals are present in urine specimens with an acid pH - 6.5 or less.

ABNORMAL CRYSTALS OF METABOLIC ORIGIN

1. CYSTINE

Description - Cystine crystals are colorless hexagonal plates which are refractile. Cystine crystals do not polarize.

Clinical Significance - Cystine crystals are found in persons who inherit a metabolic defect that prevents the reabsorption of cystine by the proximal convoluted tubules. Patients with cystinuria form cystine stones which may cause kidney damage. Cystine stones may fill the renal collecting system, resulting in calculi. Cystine crystals are soluble in dilute hydrochloric acid and ammonia. They are insoluble in acetic acid, alcohol and boiling water. Confirmation of cystine may be done with the cyanide-nitroprusside test.

Structures which can be misidentified as cystine: uric acid.

2. CHOLESTEROL

Description - Cholesterol crystals are colorless rectangular plates with a characteristic notched end in one or more corners. Their presence is sometimes associated with proteinuria, free fat, oval fat bodies and fatty casts.

Clinical significance - Cholesterol crystals are rare in freshly voided urine sediment. They are seen more often in refrigerated urine. Droplets of cholesterol are present in nephrotic syndrome and lipoid nephrosis.

Structures which can be misidentified as cholesterol crystals: radiographic media used in I.V. pyelogram.

3. LEUCINE

Description - Leucine crystals are yellow-brown, oily looking spheres with radial and concentric striations and sheets of fine needles. Confirmation of leucine is by liquid chromatography. They are birefringent and exhibit a pseudo-Maltese cross when polarized.

Clinical Significance - Leucine is a rare crystal associated with liver disease and disorders of amino acid metabolism.
4. **TYROSINE**

**Description** - Tyrosine crystals are colorless needles that appear black as the microscope is focused. They are soluble in ammonia, dilute acids, and heat. Chemical confirmation may be done with the nitrosonaphthal test.

**Clinical significance** - Tyrosine crystals are rare in the urine, but may be seen in patients with liver disease and hereditary tyrosinosis.

**Structures which can be misidentified as tyrosine**: bilirubin crystals, sulfonamides, ampicillin.

5. **BILIRUBIN CRYSTALS**

**Description** - Bilirubin crystals appear as reddish brown needles that cluster in clumps or as spheres.

**Clinical significance** - Rarely do bilirubin crystals appear in urine sediment. It is present in patients with liver disease or hepatitis. The detection of bilirubin on the dipstick helps in confirming identification.

**Structures which can be misidentified as bilirubin**: tyrosine crystals.

**ABNORMAL CRYSTALS OF DRUG ORIGIN**

*NOTE*: Patient drug history is important in identifying all crystals of drug origin.

1. **SULFONAMIDES**

**Description** - Sulfonamides are yellow to brown (sometimes colorless) needles arranged in sheaves or rosettes. They sometimes appear as brownish stocks of wheat with central binding. Greenish brown fan shapes also are seen.

**Clinical significance** - Sulfadiazine drugs administered to patients may not be soluble. Precipitated sulfonamides can damage the kidney. These crystals can be accompanied by blood and oliguria. It is important to identify them so patients can be given sufficient fluid for adequate hydration and the urine made alkaline to dissolve them.

**Structures which can be misidentified as sulfonamide**: ampicillin, tyrosine.
2. **AMPICILLIN**

**Description** - These colorless needles that appear in acidic urine are crystal clusters in refrigerated urine.

**Clinical significance** - Ampicillin crystals may be found in the urine after administration of high doses of ampicillin.

**Structures which can be misidentified as ampicillin**: tyrosine, sulfonamide.

3. **RADIOGRAPHIC CONTRAST MEDIA (MEGLUMINE DIATRIZOATE)**

**Description** - Crystals of radiographic contrast media are chemically meglumine diatrizoate. They are flat rectangular plates often notched at the corner. Long, thin prism forms also occur. They are strongly birefringent when viewed with polarized light. Macroscopically, urine containing radiographic contrast media appears cloudy with a very high specific gravity by refractometer (greater than 1.035). If the specific gravity is measured by the reagent strip method, it is *not* high because radiographic contrast media does not ionize. Information provided by the patient as to recent radiographic imaging procedures is an important confirmation of this crystal.

**Clinical significance** - Radiographic contrast media is seen in the urine of patients for a brief period after injection of these compounds for diagnostic radiographic procedures. Their appearance can be of clinical significance in dehydrated patients with renal blockage from crystals.

**Structures which can be misidentified as radiographic contrast media**: cholesterol crystals.

**ARTIFACTS**

Contaminants of all types can be found in the urine, particularly when specimens are collected improperly or in dirty containers. Most confusing are starch granules, oil droplets, air bubbles, and fibers.

1. **STARCH (TALC)**

**Description** - Starch is generally a large round granule that sometimes has an uninflated beach ball appearance. Starch is birefringent and exhibits a Maltase cross pattern under polarized light.

**Sources of contamination are**: (1) latex gloves with powder used in the collection and specimen processing and, (2) talc that may have been applied to the patient’s hands or urogenital area.

**Structures which can be misidentified as starch**: cholesterol crystals and droplets, urate crystals, parasites.
2. **FIBERS (INCLUDING DISPOSABLE DIAPER FIBERS)**

**Description** - Fibers are large, long cylindrical shapes that may have a twisted appearance. Fibers are birefringent and will polarize light.

**Source of contamination** - cotton threads, synthetic fibers, and hair may enter the urine at the time of collection and at any time during the treatment process. In children and incontinent patients, the diaper is a major source.

**Structures which can be misidentified as fibers**: casts - differentiate casts from fibers with the polarizing properties of fibers.

3. **AIR BUBBLES**

**Description** - Air bubbles are round, colorless, and highly refractile.

**Source** - Air bubbles are introduced as the coverslip is applied to the sediment slide. Care must be taken to apply the coverslip from one edge and gradually covering the sediment drop so air is pushed out rather than gathered under the coverslip.

**Structures which can be misidentified as air bubbles**: red blood cells, parasites, fat globules.

4. **OIL DROPLETS**

**Description** - Oil droplets are highly refractile and colorless and usually round or oval.

**Sources of contamination** - contamination from vaginal areas, catheter lubricant, microscopic oil immersion, vaginal creams.

**Structures which can be misidentified as misidentified as oil droplets**: red blood cells or fat globules.

5. **GLASS FRAGMENTS**

**Description** - Glass fragments are colorless, highly refractile and take on different shapes and sizes.

**Source of contamination** - slivers of glass from the coverslip or microscope slide.

**Structures which can be misidentified as misidentified as glass**: crystals.

6. **POLLEN GRAINS**

**Description** - Pollen grains have a very large raised shape with thick cell walls.

**Source of contamination** - Pollen grains are mostly a seasonal contaminant from external sources.

**Structures which can be misidentified as misidentified as pollen**: parasite eggs.
<table>
<thead>
<tr>
<th>CRYSTAL</th>
<th>pH</th>
<th>COLOR</th>
<th>SOLUBILITY</th>
<th>GENERAL APPEARANCE</th>
</tr>
</thead>
<tbody>
<tr>
<td>uric acid</td>
<td>acidic</td>
<td>yellow-brown</td>
<td>alkali soluble</td>
<td></td>
</tr>
<tr>
<td>amorphous urates</td>
<td>acidic</td>
<td>brick-dust or</td>
<td>alkali &amp; heat</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>yellow-brown</td>
<td></td>
<td></td>
</tr>
<tr>
<td>calcium oxalate</td>
<td>acidic/neutral (alkaline)</td>
<td>colorless (envelopes)</td>
<td>dilute HCl acid</td>
<td></td>
</tr>
<tr>
<td>amorphous phosphates</td>
<td>alkaline neutral</td>
<td>white-colorless</td>
<td>dilute acetic acid</td>
<td></td>
</tr>
<tr>
<td>calcium phosphate</td>
<td>alkaline neutral</td>
<td>colorless</td>
<td>dilute acetic acid</td>
<td></td>
</tr>
<tr>
<td>triple phosphate</td>
<td>alkaline</td>
<td>colorless (coffin lids)</td>
<td>dilute acetic acid</td>
<td></td>
</tr>
<tr>
<td>ammonium biurate</td>
<td>alkaline</td>
<td>yellow-brown (thorny apples)</td>
<td>acetic acid with heat</td>
<td></td>
</tr>
<tr>
<td>calcium carbonate</td>
<td>alkaline</td>
<td>colorless (dumbbells)</td>
<td>gas from acetic acid</td>
<td></td>
</tr>
</tbody>
</table>

## MAJOR CHARACTERISTICS OF ABNORMAL URINARY CRYSTALS

<table>
<thead>
<tr>
<th>CRYSTAL</th>
<th>pH</th>
<th>COLOR</th>
<th>SOLUBILITY</th>
<th>GENERAL APPEARANCE</th>
</tr>
</thead>
<tbody>
<tr>
<td>cystine</td>
<td>acidic</td>
<td>colorless</td>
<td>ammonia, dilute HCl acid</td>
<td></td>
</tr>
<tr>
<td>cholesterol</td>
<td>acidic</td>
<td>colorless</td>
<td>chloroform</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>(notched plates)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>leucine</td>
<td>acidic/neutral</td>
<td>yellow</td>
<td>hot alkali or alcohol</td>
<td></td>
</tr>
<tr>
<td>tyrosine</td>
<td>acidic/neutral</td>
<td>colorless-yellow</td>
<td>alkali or heat</td>
<td></td>
</tr>
<tr>
<td>bilirubin</td>
<td>acidic</td>
<td>yellow</td>
<td>acetic acid, HCl, NaOH, ether, chloroform</td>
<td></td>
</tr>
<tr>
<td>sulfonamides</td>
<td>acidic/neutral</td>
<td>green</td>
<td>acetone</td>
<td></td>
</tr>
<tr>
<td>radiographic dye</td>
<td>acidic</td>
<td>colorless</td>
<td>10% NaOH</td>
<td></td>
</tr>
<tr>
<td>ampicillin</td>
<td>acidic/neutral</td>
<td>colorless</td>
<td>refrigeration forms bundles of needles</td>
<td></td>
</tr>
</tbody>
</table>

QUALITY ASSURANCE
CONFIRMATORY URINE SCREENING TESTS

CHAPTER 3

CHAPTER OBJECTIVES

At the conclusion of Chapter 3, participants should be able to:

- Define quality assurance
- Describe the components of a quality assurance program
- Discuss causes of errors: pre-analytical, analytical, post-analytical
- Know when to perform confirmatory urine screening tests

Like other laboratory tests, urine results must be accurate if the physician is to treat the patient properly. A quality assurance program is necessary to assure that errors are prevented and urinalysis test results are accurate. This chapter will discuss the components of a quality assurance program.

In addition, this chapter will discuss the importance of the three most commonly performed confirmatory urine screening tests, Clinitest, Ictotest and Sulfosalicylic acid.

QUALITY ASSURANCE IN URINALYSIS

Quality Assurance (QA) is a comprehensive set of policies, procedures, and practices necessary to make sure that the laboratory’s results are reliable. It is essential that all persons working in the laboratory be committed to the quality assurance concepts with patient service being the top priority. For a QA program to be successful, interaction and communication between the laboratory staff and health care providers is essential.

According to the federal laboratory regulations (CLIA-88), “Each laboratory performing moderate or high complexity testing must establish and follow written policies and procedures for a comprehensive QA program. This program must evaluate the effectiveness of its policies and procedures identify and correct problems, assure accurate, reliable, and prompt reporting of test results, and competency of the staff.”
QUALITY ASSURANCE COMPONENTS

- Complaints and communication
- Patient test management
- Personnel competency
- Procedures
- Proficiency testing
- Quality control
- Record keeping
- Test comparisons

I. QUALITY CONTROL

Quality Control (QC) is the most familiar component of a quality assurance program. Often the terms quality control and quality assurance are incorrectly interchanged. Quality control is only one portion of a quality assurance program and it is important to make the distinction.

Quality control validates and monitors the testing process which involves checking the integrity of the microscope, urine reagent strip reading instrument, reagent strips, and the technique of testing personnel. This is done primarily through the use of control specimens which are similar in composition to patient specimens. The controls must be carried through the entire test procedure and treated in the same manner as patient specimens. CLIA-88 federal laboratory regulations states that “at least two levels of control material must be run each day of testing and are tested in the same manner as patient specimens.” For laboratories performing urine microscopics and/or using instruments for reading reagent strips, a quality control program is required. For laboratories performing a manual reagent strip urinalysis only, quality control is recommended but not required. Waived laboratories are to follow manufacturer’s quality control recommendations (at a minimum).

The Ames (Bayer) Multistix package inserts state:

“For best results, performance of reagent strips should be confirmed by testing known negative and positive specimens or controls whenever a new bottle is first opened, randomly, or if questionable results are obtained. Each laboratory should establish performance standards and should question handling and testing procedures if these standards are not met.”

Urinalysis controls are purchased commercially in ready-to-use or in dried form. Care must be taken in reconstituting dried control material. The correct amount of distilled or deionized water must be added to the control bottle and dissolved completely. Laboratories that do not have pipettes or distilled water available should purchase the ready-to-use control material for optimal control performance.
Control material must have the following properties:

- Similar to patient samples
- Acceptable range - instrument specific
- Focused at medical decision points
- At least 2 concentrations
- Available in aliquots convenient for use
- Validates the “run”
- Troubleshooting tool

All QC results must be documented immediately on a log or chart or in a computer. This documentation provides a link between control and patient results, and serves as a permanent record for the director to review. The chart, whether it is manually recorded or computerized, should include the following information:

- Name and location of laboratory site
- Test title (e.g. Urinalysis Control Chart)
- Reagent Strip information:
  - Name
  - Manufacturer
  - Lot Number
  - Expiration Date

- Control Material Information:
  - Product
  - Control level - Normal and Abnormal
  - Manufacturer
  - Lot number
  - Expiration Date
  - Control Ranges

- Date tested
- Time collected or (Time In)
- Time Out
- Initials of who tested control
- Control Results
- Corrective Action (may be separate form)
Other elements of quality control can include preventive maintenance and calibration of microscope and/or reagent strip instruments, and reagent strip storage requirements. All must be documented.

Quality control does not:

- Ensure the proper test is performed
- Ensure the correct patient is tested
- Ensure the urine specimen is properly collected
- Ensure the results are timely
- Ensure the results are properly evaluated

However, with proper documentation you can clearly track when:

- Lots of quality control reagent strips and controls are introduced
- A different laboratorian begins to perform the test
- Instrument maintenance is performed
- Responses are made to “out of control” results
- Proficiency testing is performed

II. PROFICIENCY TESTING

Proficiency testing (PT) is an external form of quality control that compares test results from each participating site to all participating laboratories that use the same methodology. The practice of testing unknown specimens from an outside agency provides additional means to assure quality laboratory test results. Every four months the PT provider sends unknown specimens to their subscribers. These specimens are tested in the same manner as patient specimens. The test results are returned to the PT provider. The results are reviewed to determine whether each participant passes or fails performance levels as established by CLIA-88 and other accrediting agencies.

Under CLIA-88, laboratories performing moderate or high complexity testing must be enrolled in an approved PT program. Laboratories that perform urine sediment examination, or use a reagent strip instrument, fall into the moderate complexity category of testing. A few of the CLIA approved PT programs available for urinalysis is listed in the following chart:
## URINALYSIS PROFICIENCY TESTING PROVIDERS

<table>
<thead>
<tr>
<th>Proficiency Testing Provider</th>
<th>Program Names</th>
<th>Description</th>
<th>Ordering Code (If applicable)</th>
</tr>
</thead>
<tbody>
<tr>
<td>College of American Pathologists (CAP) 800-323-4040</td>
<td>Clinical Microscopy, Excell-Urinalysis</td>
<td>Specimens for reagent strip and analytes transparencies for microscopics</td>
<td>CM, XL-A</td>
</tr>
<tr>
<td>American Association of Bioanalysts (AAB) 800-234-5315</td>
<td>Urinalysis</td>
<td>Specimens for reagent strip analytes and transparencies for microscopics</td>
<td>N/A</td>
</tr>
<tr>
<td>Wisconsin State Laboratory of Hygiene 800-462-5261</td>
<td>Urinalysis, Urine Sediment Identification</td>
<td>Specimens for reagent strip only Transparencies</td>
<td>UR, SU</td>
</tr>
<tr>
<td>American Proficiency Institute (API) 800-333-0958</td>
<td>Urinalysis, Urine sediment</td>
<td>Specimens for reagent strip only Transparencies</td>
<td>#232, #234</td>
</tr>
<tr>
<td>American Society of Internal Medicine (MLE) 800-338-2746</td>
<td>Urinalysis</td>
<td>Transparencies</td>
<td></td>
</tr>
<tr>
<td>American Academy of Family Physicians (AAFP) 800-274-2237</td>
<td>Urinalysis</td>
<td>Transparencies</td>
<td></td>
</tr>
</tbody>
</table>

Proficiency testing is not complete without a system of monitoring the results received from the PT provider.

Proficiency testing assessment involves:

- Regular monitoring of PT results
- Corrective action taken and documented for each unacceptable or unsatisfactory PT result
- Monitoring the effectiveness of corrective action
- Verifying that PT is treated in the same manner as patient samples
- Checking that reagent strips used for PT match those used for patient testing, and those described the procedure manual.
### EXAMPLE OF A PROFICIENCY TESTING REPORT (CAP)

<table>
<thead>
<tr>
<th>CONSTITUENT METHOD</th>
<th>SPECIMEN</th>
<th>YOUR RESULT</th>
<th>EVAL CODE</th>
<th>GOOD PERFORMANCE</th>
<th>ACCEPTABLE PERFORMANCE</th>
</tr>
</thead>
<tbody>
<tr>
<td>pH IN URINE</td>
<td>CM-19</td>
<td>8.0</td>
<td>61</td>
<td>8.0</td>
<td>7.0</td>
</tr>
<tr>
<td>AMES CLINITEK 10/100</td>
<td>CM-20</td>
<td>8.5</td>
<td>61</td>
<td>8.5 – 9.0</td>
<td>8.0</td>
</tr>
<tr>
<td>PROTEIN QUAL URINE</td>
<td>CM-19</td>
<td>30 MG/DL (1+)</td>
<td>61</td>
<td>30 MG/DL (1+)</td>
<td>100 MG/DL (2+)</td>
</tr>
<tr>
<td>AMES CLINITEK 10/100</td>
<td>CM-20</td>
<td>300-500 MG/DL (3+)</td>
<td>61</td>
<td>300-500 MG/DL (3+)</td>
<td>100 MG/DL (2+)</td>
</tr>
<tr>
<td>GLUCOSE REDUC SUB-UR</td>
<td>CM-19</td>
<td>NEGATIVE</td>
<td>61</td>
<td>NEGATIVE</td>
<td></td>
</tr>
<tr>
<td>AMES CLINITEK 10/100</td>
<td>CM-20</td>
<td>1000 MG/DL</td>
<td>61</td>
<td>500 MG/DL</td>
<td>250 MG/DL 2000 MG/DL OR MORE</td>
</tr>
<tr>
<td>KETONES , URINE</td>
<td>CM-19</td>
<td>NEGATIVE</td>
<td>61</td>
<td>NEGATIVE</td>
<td></td>
</tr>
<tr>
<td>AMES CLINITEK 10/100</td>
<td>CM-20</td>
<td>MODERATE (2+, 40 MG/DL)</td>
<td>61</td>
<td>MODERATE (2+, 40 MG/DL)</td>
<td>SMALL (1+, 15 MG/DL) LARGE (3+, &gt;80 MG/DL)</td>
</tr>
<tr>
<td>BILIRUBININ, URINE</td>
<td>CM-19</td>
<td>POSITIVE (MOD OR 2+)</td>
<td>61</td>
<td>LARGE AMOUNT (3+)</td>
<td>POSITIVE (MOD OR 2+) TRACE (SMALL OR 1+)</td>
</tr>
<tr>
<td>AMES CLINITEK 10/100</td>
<td>CM-20</td>
<td>POSITIVE (MOD OR 2+)</td>
<td>61</td>
<td>POSITIVE (MOD OR 2+) LARGE AMOUNT (3+)</td>
<td>TRACE (SMALL OR 1+)</td>
</tr>
<tr>
<td>BLOOD/HEMOGLOBIN, URINE</td>
<td>CM-19</td>
<td>POSITIVE (50 ERY/UL)</td>
<td>61</td>
<td>POSITIVE (50 ERY/UL)</td>
<td>TRACE (5-10 ERY/UL) MARKED POS (250 ERY/UL)</td>
</tr>
<tr>
<td>AMES CLINITEK 10/100</td>
<td>CM-20</td>
<td>NEGATIVE</td>
<td>61</td>
<td>NEGATIVE</td>
<td></td>
</tr>
<tr>
<td>LEUKOCYTE ESTERASE</td>
<td>CM-19</td>
<td>TRACE</td>
<td>61</td>
<td>NEGATIVE</td>
<td></td>
</tr>
<tr>
<td>AMES CLINITEK 10/100</td>
<td>CM-20</td>
<td>MODERATE (2+)</td>
<td>61</td>
<td>MODERATE (2+)</td>
<td>SMALL (1+)</td>
</tr>
<tr>
<td>NITRITE , URINE</td>
<td>CM-19</td>
<td>POSITIVE</td>
<td>61</td>
<td>POSITIVE</td>
<td></td>
</tr>
<tr>
<td>AMES CLINITEK 10/100</td>
<td>CM-20</td>
<td>NEGATIVE</td>
<td>61</td>
<td>NEGATIVE</td>
<td></td>
</tr>
<tr>
<td>URINE SEDIMENT IDENT</td>
<td>CM-21</td>
<td>GRANULAR CAST</td>
<td>71</td>
<td>GRANULAR CAST</td>
<td></td>
</tr>
<tr>
<td>CM-22</td>
<td>WAXY CAST</td>
<td>71</td>
<td>WAXY CAST</td>
<td></td>
<td></td>
</tr>
<tr>
<td>CM-23</td>
<td>HYALINE CAST</td>
<td>71</td>
<td>HYALINE CAST</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
III. PROCEDURE MANUAL

Another essential component of a quality assurance program is the procedure manual. The procedure manual contains the operational protocols that standardize laboratory testing. It must be accessible to all of the laboratory staff. A good procedure manual should follow NCCLS format. This section will review the NCCLS format with examples of urinalysis sediment examination information included under each heading. This is a guide for each facility to develop its own site-specific procedure manual.

Example:

I. Urinalysis - Sediment Examination Procedure Manual

II. Principle

The kidneys are paired organs which are located in the small of the back on each side of the spine. They function in maintaining the fluid balance of the body and forming urine which is excreted. Urine can be an indicator of health or disease especially in metabolic or renal disorders. A microscopic examination of urinary sediment is helpful in confirming the presence of red blood cells (RBC’s), white blood cells (WBC’s), bacteria, yeast, crystals, or casts. A qualitative or semi-quantitative evaluation of urine sediment provides adequate information for the majority of diagnostic and clinical needs.

III. Specimen Collection and Handling

- Specify the condition for patient preparation, e.g., clean catch instructions.
- Indicate the preferred type of specimen and any other acceptable specimen, e.g., first morning, random urine.
- List the acceptable collection containers, e.g., sterile urine container.
- Specifications for home collected specimens - clean leak proof container.
- Specify urine volume requirements, optimal (50 ml or more) and minimal (12 ml).
- State the storage and handling requirements for stability of the specimen, e.g., Urine specimens must be delivered to the laboratory within 1 hour of collection and processed within 2 hours held at room temperature.
- Specify requirements for specimen labeling, e.g., Urine specimen container must be labeled immediately after collection by person responsible for specimen collection. The specimen must be labeled with name, date, and time of collection.
- Include criteria for unacceptable specimens and the action taken by the laboratory to correct the problem, e.g., unlabeled specimens and specimens over 1 hour old are rejected. Document on specimen rejection log.
- Include information for notifying the person responsible for collecting a rejected specimen.
IV. Reagents, Supplies and Equipment

- Clean, dry containers for collecting the urine sample
- Antiseptic wipes for patient preparation before collecting urine
- Microscope slides 1" x 3"
- Cover slips 22 x 22 mm - #1 or #2
- Centrifuge tube - 15 ml capacity with conical or constricted bottom
- General purpose centrifuge
- Microscope with mechanical stage and low power (10X) and high power (40X) objectives.
- Include a brief description of preventive maintenance of centrifuge, microscope, etc.

V. Calibration

- Step-by-step instructions on calibration of microscopes and centrifuges.
- Specify acceptable performance and corrective action to be taken when limits are exceeded.
- Indicate where to record calibration results.

VI. Quality Control:

- List the manufacturer and product name of each level of control.
- List control storage requirements.
- Describe step-by-step instructions for reconstituting lyophilized control samples and testing controls.
- Indicate how control results are recorded (charts, computer, logs).
- State limits of acceptability and corrective action when control results exceed these limits.

VII. Test Procedure:

- Give detailed instructions in a step-by-step manner for preparation of urine sediment, slides and microscopic scanning technique.
- Keep instructions free of explanation
- Include clean-up and disposal of specimens and any other contaminated material.

VIII. Results:

- Include criteria for identification of specific elements in the urine sediment, e.g., red and white blood cells, epithelial cells, yeast, crystals, and casts.
- State the normal versus abnormal elements found in the urine sediment.
- Describe critical values which require immediate physician notification.
- Describe site-specific reporting protocol.
GENERAL REPORTING SYSTEM FOR URINE SEDIMENT

Average number per low power field (X100)

<table>
<thead>
<tr>
<th></th>
<th>Average number per low power field (X100)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0-2</td>
</tr>
<tr>
<td>Casts</td>
<td>Negative</td>
</tr>
<tr>
<td>Abnormal crystals</td>
<td>Negative</td>
</tr>
<tr>
<td>Squamous epithelial cells</td>
<td>Rare</td>
</tr>
</tbody>
</table>

Average number per low power field (X400)

<table>
<thead>
<tr>
<th></th>
<th>Average number per low power field (X400)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0-2</td>
</tr>
<tr>
<td>Red blood cells</td>
<td>0-2</td>
</tr>
<tr>
<td>White blood cells</td>
<td>0-2</td>
</tr>
<tr>
<td>Normal crystals</td>
<td>Rare</td>
</tr>
<tr>
<td>Epithelial cells (renal tubular, oval fat bodies, transitional)</td>
<td>Rare</td>
</tr>
<tr>
<td>Miscellaneous (bacteria, yeast, Trichomonas, fat globules)</td>
<td>Rare</td>
</tr>
<tr>
<td>Sperm (males only)</td>
<td>Present</td>
</tr>
</tbody>
</table>

NOTE: Few means some are present; moderate means easily seen; many means prominent.

Adapted from Linhe JJ, Ringsrud KM: Basic Techniques in Clinical Laboratory Science, 3rd edition, St. Louis, 1992, Mosby

IX. Sources of Error

List interferences and sources of error. Some examples are described later in this chapter.

X. Notes/Remarks

Provide helpful hints and miscellaneous information.

XI. References

- Include sources such as:
  - package inserts
  - textbooks
  - NCCLS publications
  - scientific journals

XII. Review and Update

Each procedure should be signed by the director, with date of approval.
XIII. **Supplemental Materials:**

- color atlases, textbooks, flowcharts
- reporting results
- procedure for notification of erroneous results
- panic values and reporting protocols
- review and update
- references

Keep in mind that each site must include site-specific information for that site. According to CLIA-88 regulations, "manufacturer's instructions, and reference material can be used for a procedure but the following information should be supplied by each laboratory:"

---

**IV. PERSONNEL COMPETENCY**

The competence of personnel is an important determinant of the quality of any laboratory including urinalysis. Only well trained personnel who are skilled in the proper use of the microscope should perform urine sediment examination. Policies on how employees are trained and assessed for competency must be developed and recorded.

**Activities to Assure Personnel Competency** *(Document all activities.)*

- Develop standardized orientation training policies for new employees. Competence verification checklists provide documentation that a new employee is competent to perform specific tasks or procedures independently.

- Provide periodic opportunities for upgrading the technical skills for all personnel. This can be accomplished through in-service classes, continuing education courses, and by encouraging study of textbooks and audiovisuals.

- Make color atlases of urine sediment and textbooks readily available for review.

- Instruct laboratory personnel to share with each other all unusual or abnormal urine sediment findings.

- Evaluate personnel periodically; for test result reporting, QC records, and preventive maintenance records.

- Make direct observation of personnel performing patient preparation, specimen handling, processing, and testing.

- Assess technical competency through proficiency testing. Each employee, sometime within the year, must complete a PT survey including any monitoring of unsatisfactory results. This provides the employee with valuable problem solving competence.
V. PATIENT TEST MANAGEMENT

Patient test management is the process to assure that patient specimens and test results are not confused with other patient results. This component of quality assurance tracks patient information from the time the specimen is collected to the time the test result is reported. The laboratory must employ and maintain a system that provides for proper specimen submission and handling by assessing the following elements:

- patient preparation
- specimen collection
- labeling
- specimen preservation
- specimen transportation
- specimen processing
- reporting of correct results

Patient test management must be documented using the records described below:

1. **Test requisition**

   The test requisition may have one of several different formats depending upon the testing site. Traditionally, it has been a preprinted form printed with multiple carbon copies. In computerized laboratories, it may be computer generated form with accompanying specimen labels. At small volume testing sites, the patient chart may serve as the test requisition. Regardless of format, the test requisition must include the following information:

   - patient name or unique identifier
   - patient sex
   - authorized person requesting test
   - test ordered
   - date and time of specimen collection
   - relevant medical information

2. **Labeling**

   Patients must be identified before the specimen is collected. Once the patient is identified, the specimen can be collected and properly labeled. All containers must be labeled by the person doing the collection to make certain that the specimen has been collected from the patient whose identification is noted on the label. The label must be placed on the container and must adhere during refrigeration. Never label the lid because once the lid is removed, identity is lost. The label should include the patient's full name, unique identification number, date and time of collection. The complete request form must accompany the specimen to the laboratory. The request form must be clean and legible. The information on the specimen container must match exactly the patient identification on the request slip. All specimens received by the laboratory without proper identification must be rejected. It is better to recollect the specimen than report false results.
3. **Test Records**

Devise a specimen log-in book to record:

- patient name
- identification number
- accession number
- date and time of receipt into laboratory
- test results
- initials of person performing the test
- time the analysis is finished (time out)
- explanation for rejected specimens

When the specimen is received by the laboratory, the information listed above should be completed. These records can be handwritten or computer generated.

4. **Test Report**

The test report may be done on the same form as the test requisition or patient chart. Duplicate copies of the test report should be kept, one in the laboratory and one on the patient chart.

The test report must contain the following information:

- identity of the location of laboratory performing testing
- test results
- units of measurements
- information regarding specimen collection
- reference or normal ranges

All test reports should be accurately reported in a timely manner to the health care provider who ordered the test. Once the test report reaches the patient chart, the health care provider review should be documented either by initials or comments to confirm that the results were noted on the correct chart. Periodic meetings with laboratory staff and health care providers are necessary to discuss test reporting problems.

5. **Specimen Referral Log**

Any urine specimen that is referred to another laboratory for further study must be documented on a reference laboratory log. The reference log should have the following information:

- date collected
- patient name or unique identifier
- test requested
- date mailed
- date results were received back in the laboratory
- name and address of reference laboratory
VI. COMMUNICATION AND COMPLAINT INVESTIGATION

In order for laboratories to survive managed care competition, consumers (patients, physicians) must be pleased with the service. Communication of problems is essential. The laboratory must have a system in place that documents and resolves complaints that occur as the result of communication breakdowns, e.g., incorrect test, patient name, test results and unacceptable specimens.

After communication problems are detected and documented, the laboratory must investigate the complaints to determine a course of action. All complaints should be taken seriously. No matter how insignificant the complaint may seem at the time, it may be a symptom of a bigger problem. The communication or complaint log documents the communication process. This log should answer the following questions:

- Was the problem documented?
- Was the problem corrected?
- Has a policy or procedure changed?
- If a policy change occurred, has the change been evaluated to determine that the action taken was effective?
- Were the appropriate people notified?
- Was each step of the correction processed initialed (if more than one person was involved)?

VII. COMPARISON OF TEST RESULTS

An important quality assurance component is comparison of test results. The information generated for each patient should be correlated with other results obtained by the laboratory. In urinalysis, it is important to correlate the physical appearance, chemical (reagent strip) screen and microscopic results.

Inspect the test results for inconsistencies and investigate the possible cause before releasing the results to the physician. Inconsistencies may or may not be erroneous and may lead to clues for determining specific clinical problems. Although there are exceptions, the physical, chemical and microscopic portions of a urinalysis should correlate. The chart below summarizes some common urinalysis test comparisons.

### COMMON URINALYSES TEST COMPARISONS

<table>
<thead>
<tr>
<th>Appearance</th>
<th>Reagent Strip Screen</th>
<th>Sediment examination</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hazy to cloudy</td>
<td>Protein - trace to large amount</td>
<td>Casts</td>
</tr>
<tr>
<td>Pink to red color</td>
<td>Blood - trace to large amount</td>
<td>Red blood cells</td>
</tr>
<tr>
<td>Hazy to cloudy</td>
<td>Leukocyte esterase - trace to large amount</td>
<td>White blood cells</td>
</tr>
<tr>
<td>Hazy to cloudy</td>
<td>Nitrite - Positive</td>
<td>Bacteria</td>
</tr>
</tbody>
</table>
RELATIONSHIP OF PATIENT INFORMATION TO PATIENT TEST RESULTS

Laboratories should develop a system to identify patient test results that appear inconsistent with patient status or medical information.

EXAMPLES OF RELATING PATIENT CHART INFORMATION TO PATIENT URINALYSIS TEST RESULTS

<table>
<thead>
<tr>
<th>EXAMPLE</th>
<th>URINALYSIS RESULTS</th>
<th>PATIENT CHART INFORMATION</th>
<th>INVESTIGATION</th>
</tr>
</thead>
</table>
| #1      | Color - Yellow     | Patient had a radiographic procedure performed one day prior to the urinalysis | Clues:  
| #2      | Appearance - cloudy|                            | - Cloudy appearance  
|         | Reagent strip reactions - negative |                            | - Specific gravity inconsistency  
|         | Specific gravity:  |                            | - Crystals  
|         | By refractometer - 1.035 |                            | Without knowing the radiographic procedure information, the crystals may have been reported as cholesterol.  
|         | By reagent Strip - 1.020 |                            |  
|         | Urine Sediment Exam: |                            |  
|         | Crystals resembling cholesterol |                            |  
|         |                      |                            |  |
|         |                      |                            |  |
| #2      | Color - yellow     | Routine examination with no symptoms of a urinary tract infection | Clues:  
|         | Appearance - hazy |                            | - Hazy appearance  
|         | Reagent strip -    |                            | - Nitrite - positive  
|         | Nitrite - positive |                            | - Leukocyte esterase - negative  
|         | Protein - trace    |                            | - Large amount of bacteria  
|         | All other reactions - negative or normal |                            | Patient asymptomatic - question urinalysis review procedure  
|         | Specific gravity:  |                            | - Suspect: Contamination  
|         | By refractometer - 1.020 |                            |  
|         | By reagent strip - 1.020 |                            |  
|         | Urine sediment examination: |                            |  
|         | Large amount of bacteria present |                            |  
|         |                      |                            |  |
In each of the above examples, investigation is needed to determine whether or not the urinalysis results are clinically correct or are erroneous. All action must be documented. In small testing sites, the patient charts are available for investigation. In larger institutions, patient medical information is limited. In this case, open communication with nurses and physicians is essential.

VII. RECORD KEEPING

Thorough record keeping is essential to quality assurance. The phrase “If it isn’t documented, it isn’t done,” is reiterated by inspectors and supervisors in every laboratory. This last section lists some of the most common urinalysis records. Retain these records for a minimum of two years.

Types of Laboratory Records

- Control logs - two levels of control along with strip information
- Temperature charts:
  - refrigerators
  - room temperature freezers
- Instrument preventive maintenance
- Centrifuge calibration records and preventive maintenance
- Patient logs
- Test requisitions
- Refractometer:
  - preventive maintenance
  - calibration
  - controls
- Proficiency testing – to include:
  - results submitted
  - summary reports
  - corrective action on deficiencies.
- Corrective active logs for controls and instruments
- Review of test results
- Personnel training
- Unsatisfactory specimens/Collection of additional specimens
- Instrument service & repair
- Microscope preventive maintenance
- Complaint logs
- Communication reviews with staff and clients (memos, meeting agenda)
- Quality Assurance Monitors and Review
- Retain package insert information on controls and reagent strips (one per lot or revision)
SCOPE OF QUALITY ASSURANCE

A good quality assurance program incorporates all of the components discussed in this chapter into a program to prevent errors and assure accuracy. Errors can occur in any phase of the testing process – pre-analytic, analytic, post-analytic. According to a survey conducted by Q probe, the least amount of errors occurs in the analytic phase: 7.3%. The pre-analytic phase has an error distribution of 45.5%, and the post-analytic phase accounted for 47.2% of laboratory errors. This data indicates that laboratories do very well with their accuracy and precision during the actual testing phase but may be faulty in specimen collection or reporting of results. The lists below summarize the common errors associated with urinalysis in each phase of testing.

PRE-ANALYTIC ERRORS

- Patient physiology factors:
  - Foods which produce abnormal urine color
  - Medications
  - Strenuous exercise which results in excretion of cells and protein

- Failure to use a clean collection container
- Failure to deliver specimen to the laboratory promptly (within 1 hour) and failure to perform test promptly
- Contamination of urine with skin cleansers
- Failure to provide patients with instructions on how to properly collect specimen (e.g. clean-catch method)
- Failure to wash hands before collecting specimen
- Patient misidentified or not identified
- Specimen cup not labeled or incorrectly labeled
- The wrong type of urine specimen collected, for example, random urine specimen collected for a urine culture.
- Random or short volume urine specimens (may not truly represent the patient’s condition)

ANALYTICAL ERRORS

- Improper dipping of a reagent strip.
- Proper timing of the reactions ignored.
- Touching the reagent strip test pads.
- Failure to protect the reagent strips from light and humidity (leaving the cap off the bottle)
- Failure to test reagent strips with controls
- Failure to thoroughly mix the urine specimen immediately before testing or before pouring urine specimen into a centrifuge tube for sedimentation
- Control specimens improperly reconstituted or poorly mixed
- Failure to maintain and perform function checks on refractometer
- Refrigerated specimen tested before allowing it to reach room temperature
- Using expired reagent strips and controls
- Failure to test specimen promptly
- Technical errors in using the microscope
- Failure to QC and properly maintain microscope
- Urine centrifuge RPM’s and time not checked.
- Lack of a written and updated procedure manual
- Clerical errors, e.g., results recorded for incorrect patient
- Confirmatory test not preformed for glucose, bilirubin, and protein
- Technical problems with urine strip instrument
- The human eye’s perception of color
- Failure to reject an unsatisfactory specimen
- Poor quality water supply
- Unstable power supply and temperature
- Failure to correlate the visual, chemical, and microscopic results

POST ANALYTICAL ERRORS

- Failure to report results in a timely manner
- Not identifying critical results (panic values)
- Transcription errors
- Failure to report result to health care provider
- Results placed on incorrect patient chart
- Mistiling or disorganized patient records
- Failure to provide laboratory interpretive information to health care provider
- Failure to refer specimens
- Illegible results
- Lack of communication and interaction between the laboratory and those directly involved with patient care (physicians, nurses).

CONFIRMATORY URINE TESTS

Errors can occur if confirmatory urine tests are not performed as part of a chemical screen. In general, problems arise with the human eye’s perception of color and the color of the urine specimen masking the color interpretation of the reagent strip. Confirmatory urinalysis tests detect the same substance with greater sensitivity or specificity. They may use a different chemical reaction to detect the analyte. Repeating a reagent strip reaction is not a confirmatory test. Beyond reagent strip urinalysis, microscopic sediment examinations represent confirmatory tests for blood, leukocyte esterase, and nitrite detected by reagent strips. The three most common confirmatory tests involve reducing sugars, protein and bilirubin. These tests are described in this section.

REDUCING SUGARS (CLINITEST® COPPER REDUCTION TEST)

Principle: The copper reduction test is a nonspecific test for reducing sugars in the urine which include: glucose, galactose, fructose, lactose, and pentose.

The method is based on the ability of sugar to reduce copper sulfate to cuprous oxide in the presence of alkali and heat (Benedict’s Principle). The color change which correlates with the amount of sugar present in the urine progresses from blue (negative) to yellow and then to red. It is important to observe this reaction closely because high levels of glucose may cause a “pass through phenomenon” to occur. When the “pass through phenomenon” occurs, the color produced by the Clinitest reaction passes through the red-color and returns to a blue color. In this situation, a high glucose level may be reported as negative. Using the 2-drop Clinitest method instead of the traditional 5-drop method can prevent the “pass through phenomenon” from occurring. However, the ability to detect low levels of reducing sugars may be compromised by the 2-drop Clinitest method.
Sensitivity:

Be aware that the reagent strip screen for glucose is more sensitive than the Clinitest method. The reagent strip will detect glucose levels as low as 40 mg/dl while the Clinitest glucose sensitivity is 250 mg/dl.

Clinical Reasons for Performing Clinitest

Galactosuria - The most important reason to perform the Clinitest method is to detect galactosuria in pediatric patients. All children under 2 years of age (especially newborns) should be screened using the reagent strip and Clinitest method. The presence of galactose in the urine results from a metabolic error in which the enzyme galactose-1-phosphate uridyl transferase is lacking. This results in a failure to metabolize galactose, with increased levels in the blood (galactosemia) and urine. Galactosemia will result in permanent physical and mental deterioration. This deterioration can be prevented by early detection and dietary restriction of galactose.

Lactose Intolerance - Lactose intolerance can be detected by the Clinitest method. Lactose intolerance will cause gastrointestinal distress in adults. In infancy, a failure to gain weight may be because of intestinal lactose deficiency. Lactose may also be seen in the urine in late pregnancy or early lactation.

BILIRUBIN (ICTOTEST®)

Principle: The Ictotest is the confirmatory test for urine bilirubin which is based on a diazo reaction. The test is performed by placing 10 drops of urine on an absorbent mat. The bilirubin (if present) will remain on the mat surface as the urine filters into the mat. A reagent tablet is then placed on the mat and one drop of water is added to the tablet. After 5 seconds, a second drop is added and allowed to run down the side of the tablet. After 60 seconds, the reaction between the tablet and bilirubin produces a blue or purple color appearing on the mat. Colors other than blue or purple appearing on the mat are interpreted as negative.

Clinical Reasons for Performing Ictotest

Sensitivity - The Ictotest is more sensitive for bilirubin than the reagent strip method. Urine suspected of containing bilirubin due to the urine appearance or clinical history should be confirmed by the Ictotest method.

Color interpretation - The color changes in the reagent strip bilirubin test pad can be very subtle. The purple color reaction of Ictotest is easier to interpret than the brown colored reaction of the bilirubin reagent strip test pad. When in doubt about bilirubin color interpretation, perform an Ictotest.

PROTEIN (SULFOSALICYLIC ACID TEST)

The sulfosalicylic acid test (SSA) is a cold precipitation test of protein. Various concentrations of sulfosalicylic acid have been described. In general, equal amounts of urine and SSA are placed in a test tube and protein (if present) precipitates.
PROCEDURE: Sulfosalicylic Acid Test for Protein

1. Centrifuge a 10 - 12 ml aliquot of well mixed urine.
2. Decant 4 - 5 ml of the urine supernatant into a 16 x 125 mm test tube. Note clarity of the supernatant.
3. Add equal parts 3% sulfosalicylic acid reagent (SSA).
4. Stopper the tube and mix by inverting twice.
5. Let stand exactly 10 minutes.
6. Invert tube once again.
7. Observe the degree of precipitation and grade.

GRADE PRECIPITATED PROTEIN AS FOLLOWS:

<table>
<thead>
<tr>
<th>SSA result</th>
<th>Description</th>
<th>Approximate protein Concentration mg/dL by reagent strip*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Negative</td>
<td>No turbidity, or no increase in turbidity</td>
<td>&lt;5 mg/dl</td>
</tr>
<tr>
<td>Trace</td>
<td>Barely perceptible turbidity in ordinary room light Printed material distorted but readable through the tube Cannot see a ring at bottom of tube when viewed from above.</td>
<td>5-20 mg/dL</td>
</tr>
<tr>
<td>1+</td>
<td>Distinct turbidity but no distinct granulation</td>
<td>30 mg/dL</td>
</tr>
<tr>
<td>2+</td>
<td>Turbidity with granulation but no flocculation</td>
<td>100 mg/dL</td>
</tr>
<tr>
<td>3+</td>
<td>Turbidity with granulation and flocculation</td>
<td>300-500 mg/dL</td>
</tr>
<tr>
<td>4+</td>
<td>Clumps of precipitated protein or solid precipitate</td>
<td>&gt;500 mg/dL</td>
</tr>
</tbody>
</table>

*Based on a comparison of SSA values by reagent strip (Multistix) and Cobas protein results UMHC Jan. 1993. Unpublished.

Clinical Reasons for Performing SSA

Difficulty in color interpretation - The protein area of the reagent strip is one of the most difficult to interpret, particularly in relation to the “trace” reading. Questionable results should be confirmed using the SSA method.

Specificity of the reagent strips - Reagent strips measure primarily albumin and may not detect tubular proteins and Bence Jones protein which are pathological. The SSA method will detect albumin, glycoprotein globulins, hemoglobin and light chain immunoglobulin.

Reaction Interference

Any substance precipitated by acid will produce false turbidity in the SSA method. The most highly encountered substances are radiographic dyes, cephalosporins, penicillin and sulfonamide. The patient history will provide the necessary information to rule out these interferences. This is an example of why communication among laboratories, nurses and physicians is crucial to quality patient care. Important pieces of information prevent misinterpretation.
<table>
<thead>
<tr>
<th>Glossary of Quality Assurance Terms</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>acceptable control range</strong></td>
<td>The range of results that indicate adequate performance when analyzing a control sample. The range is shown in the control’s product insert.</td>
</tr>
<tr>
<td><strong>accuracy</strong></td>
<td>Correctness; freedom from error. The extent to which measurements agree with the true value of the quality being measured.</td>
</tr>
<tr>
<td><strong>analysis</strong></td>
<td>The laboratory procedure that enables you to measure the amount of an analyte in a specimen.</td>
</tr>
<tr>
<td><strong>analyte</strong></td>
<td>The substance or constituent being measured, e.g., glucose.</td>
</tr>
<tr>
<td><strong>assayed values</strong></td>
<td>The measurement of the amount of a constituent in a specimen; a test.</td>
</tr>
<tr>
<td><strong>bias (inaccuracy)</strong></td>
<td>A measure of the departure from accuracy. A numerical difference between the mean of a set of replicate measurements and the true value of the sample.</td>
</tr>
<tr>
<td><strong>calibration</strong></td>
<td>The process by which readings obtained from an instrument or other measuring device are related to known concentrations of an analyte.</td>
</tr>
<tr>
<td><strong>calibrator</strong></td>
<td>A material, solution, or freeze-dried preparation used in calibration. The concentration of the analyte in a calibrator is known to be within a particular range.</td>
</tr>
<tr>
<td><strong>centrifuge</strong></td>
<td>A machine using centrifugal force (proceeding in a direction away from the center) for separating substances of different densities.</td>
</tr>
<tr>
<td><strong>clean-catch urine</strong></td>
<td>A urine sample collected after the urethral opening and surrounding tissues have been cleansed.</td>
</tr>
<tr>
<td><strong>CLIA 88</strong></td>
<td>Clinical Laboratory Improvement Amendments. Laws passed by federal government in 1988 to improve the quality of laboratory testing.</td>
</tr>
<tr>
<td><strong>coarse adjustment</strong></td>
<td>Adjusts position of microscope objectives; used to bring objects into focus.</td>
</tr>
<tr>
<td><strong>coefficient of variation</strong></td>
<td>An index used to describe the precision of a laboratory method. It is calculated with this formula: CV = standard deviation/mean. Expressed as a percentage.</td>
</tr>
<tr>
<td><strong>control</strong></td>
<td>A material, solution, or lyophilized preparation used in quality control. The concentration of the analyte in the control is known to be within certain limits, i.e., some value.</td>
</tr>
<tr>
<td><strong>corrective action (laboratory)</strong></td>
<td>Action taken when quality control tests or instrument calibration are out of control; must be documented.</td>
</tr>
<tr>
<td><strong>critical limits</strong></td>
<td>Test results of a patient’s specimen that is dangerously low or high; used for emergency notification of clinicians.</td>
</tr>
<tr>
<td><strong>critical results (critical values, panic values)</strong></td>
<td>Test results that fall outside of the low or high critical limit for the particular test in question. URGENT notification of the appropriate medical personnel must be made if critical results are obtained.</td>
</tr>
<tr>
<td><strong>deficiency</strong></td>
<td>Noncompliance to a standard.</td>
</tr>
<tr>
<td><strong>distilled water (dist. H2O)</strong></td>
<td>The condensate collected when water has been boiled (distilled) to remove impurities.</td>
</tr>
<tr>
<td><strong>equivocal</strong></td>
<td>A laboratory result of a doubtful or uncertain nature which requires retesting.</td>
</tr>
<tr>
<td><strong>expiration date</strong></td>
<td>The last day a reagent, kit, control, etc. can be used; the unopened expiration date is printed on product by manufacturer. If the expiration date changes when the product is opened, the laboratorian must record the new expiration date.</td>
</tr>
<tr>
<td>Term</td>
<td>Definition</td>
</tr>
<tr>
<td>-----------------------------------------</td>
<td>--------------------------------------------------------------------------------------------------------------</td>
</tr>
<tr>
<td>false negative (result)</td>
<td>A negative test result for a patient who is positive for the condition or constituent being tested for.</td>
</tr>
<tr>
<td>false positive (result)</td>
<td>A positive test result for a patient who is negative for the condition or constituent being tested for.</td>
</tr>
<tr>
<td>in control</td>
<td>Term used to describe the testing procedure when the results from a control sample or series of control samples are within the acceptable control range.</td>
</tr>
<tr>
<td>Levy-Jennings chart</td>
<td>Quality control chart; a graph or table that shows results of control results over a period of time; used in a quality control program.</td>
</tr>
<tr>
<td>linearity</td>
<td>The measure of the range (the linear range) of concentration of an analyte over which a method or test produces consistent (i.e., linear, straight line) and accurate results.</td>
</tr>
<tr>
<td>lot number (control number)</td>
<td>The number given to a batch of a product by the manufacturer. Lot number must be recorded when product is used in testing. Lot number is important if product fails to perform adequately.</td>
</tr>
<tr>
<td>lyophilized</td>
<td>Freeze-dried; a lyophilized calibrator, control, or reagent has been specially dried to make its analytes more stable. It must be refrigerated to maintain its stability and is reconstituted by adding an appropriate diluent.</td>
</tr>
<tr>
<td>maintenance (preventive maintenance)</td>
<td>Steps followed to keep instruments in good state of repair; must be documented.</td>
</tr>
<tr>
<td>mean</td>
<td>The average of the numerical results obtained from a series of analyses.</td>
</tr>
<tr>
<td>method</td>
<td>Analytical method; the instructions including procedures, material, equipment, and everything else needed for an analyst to perform an analysis.</td>
</tr>
<tr>
<td>NBS thermometer</td>
<td>National Bureau of Standards thermometer; readings of thermometers used in laboratory instruments must be compared to reading with NBS thermometer. Results must be within 2 degrees.</td>
</tr>
<tr>
<td>NCCLS</td>
<td>National Committee for Clinical Laboratory Standards - committee of experts that develops standards for laboratory procedures. Standards are published to be followed by laboratories.</td>
</tr>
<tr>
<td>nocturia</td>
<td>Excessive urination at night</td>
</tr>
<tr>
<td>normal values (expected values, reference values)</td>
<td>A range of values established for each analyte which includes results expected when performing a test on a healthy person.</td>
</tr>
<tr>
<td>out of control</td>
<td>Term used to describe the testing procedure when the results from a control sample are outside the acceptable control range.</td>
</tr>
<tr>
<td>panic values</td>
<td>Test results that fall outside of the low and high critical limit. URGENT notification of the appropriate personnel must be made if a panic value is obtained.</td>
</tr>
<tr>
<td>parallel testing</td>
<td>Comparison testing of a new product with product currently being used.</td>
</tr>
<tr>
<td>percentage</td>
<td>Parts per 100.</td>
</tr>
<tr>
<td>precision (reproducibility)</td>
<td>The measure of the closeness of the results obtained when analyzing the same sample more than once; the measure of agreement between replicate measurements. The smaller the variation between results, the better the precision.</td>
</tr>
<tr>
<td>procedure manual</td>
<td>A laboratory manual that contains the methods, materials, and other information needed to perform a test.</td>
</tr>
<tr>
<td>product insert</td>
<td>Informational material that comes with instrument, reagents, and other laboratory products giving instructions for the use of product and other information required of the manufacturer by the U.S. Food and Drug Administration</td>
</tr>
<tr>
<td>Term</td>
<td>Definition</td>
</tr>
<tr>
<td>------------------------------------------</td>
<td>------------------------------------------------------------------------------------------------------------------------------------------</td>
</tr>
<tr>
<td>proficiency testing</td>
<td>A program in which samples are sent to a group of laboratories for analysis. The results are tabulated by the program sponsor, and a participating laboratory can compare its results with those of other laboratories that use the same method.</td>
</tr>
<tr>
<td>quality assurance</td>
<td>A comprehensive set of policies, procedures, practices necessary to make sure that the laboratory’s results are reliable. QA includes record-keeping, calibration and maintenance of equipment, quality control, proficiency testing, and training.</td>
</tr>
<tr>
<td>quality control</td>
<td>The set of laboratory procedures designed to ensure that the test method is working properly and that the results meet the diagnostic needs of the physician. QC includes testing control samples, charting results, and analyzing the results statistically.</td>
</tr>
<tr>
<td>qualitative</td>
<td>This term is applied to tests that detect whether a particular analyte, constituent, or condition is present.</td>
</tr>
<tr>
<td>quantitative</td>
<td>This term is applied to tests that give results expressing the numerical amount of an analyte in a specimen.</td>
</tr>
<tr>
<td>reactivity</td>
<td>The ability of a reagent to produce its proper chemical reaction. Reagents can lose their reactivity if they are misused, mishandled, or too old.</td>
</tr>
<tr>
<td>reagent</td>
<td>A substance that produces a chemical reaction in a sample that allows an analyte to be detected and measured.</td>
</tr>
<tr>
<td>reconstitute</td>
<td>To add a diluent to a freeze-dried calibrator, control, reagent.</td>
</tr>
<tr>
<td>reliability</td>
<td>The method’s capacity to maintain both accuracy and precision.</td>
</tr>
<tr>
<td>replicate</td>
<td>To repeat an experiment or an analysis in order to check for accuracy of the results. Each repeat is a replicate test or measurement.</td>
</tr>
<tr>
<td>reproducibility</td>
<td>See precision.</td>
</tr>
<tr>
<td>result</td>
<td>The value obtained by analysis for a particular analyte in a particular sample.</td>
</tr>
<tr>
<td>run (analytical run)</td>
<td>A group of measurements by a particular method over a given period of time during which the accuracy and precision of the method are expected to be stable.</td>
</tr>
<tr>
<td>saline</td>
<td>An isotonic solution of sodium chloride and distilled water; normal saline; physiological saline; usually made in 0.85 or 0.9% concentration for use in medical laboratory procedures.</td>
</tr>
<tr>
<td>sample</td>
<td>The part of a specimen that is used for an analysis.</td>
</tr>
<tr>
<td>sediment</td>
<td>Solid substances which settle to the bottom of a liquid.</td>
</tr>
<tr>
<td>sensitivity</td>
<td>The ability of a test to give a positive result for patients that have the disease or condition being tested for; measured as the ratio of positive tests to the total number of tests in those that have the disease; expressed as percentage.</td>
</tr>
<tr>
<td>specific gravity</td>
<td>Ratio of weight of a given volume of a solution to the weight of the same volume of water; a measurement of density.</td>
</tr>
<tr>
<td>specificity</td>
<td>The ability of a test to give a negative result for patients that do not have the disease or condition they are tested for; measured as the ratio of negative tests to the total number of tests in those that do not have the disease or condition; expressed as a percentage.</td>
</tr>
<tr>
<td>split-sample testing</td>
<td>Dividing a sample in half, and testing half in your laboratory and having the other half tested in another laboratory, and then comparing the results. This is a technique for testing accuracy.</td>
</tr>
</tbody>
</table>
MULTIPLE CHOICE: CIRCLE THE BEST ANSWER

1. The normal kidney performs all of the following functions except:
   a. removes metabolic waste products from the blood
   b. regulates acid-base balance in the body
   c. removes excess protein from the blood
   d. regulates the water and electrolyte content in the body

2. Each kidney is composed of approximately:
   a. 100 nephrons
   b. 1000 nephrons
   c. 15,000 nephrons
   d. 1,500,000 nephrons

3. The essential function(s) of the proximal convoluted tubules include:
   a. secretion
   b. reabsorption
   c. renal blood flow
   d. secretion and reabsorption

4. Maintaining a blood pH of approximately 7.4 is dependent on secretion of:
   a. hydrogen ions
   b. potassium ions
   c. ammonium ions
   d. water
5. Name the hormone which is produced by the pituitary that helps to control the reabsorption process.
   a. renin
   b. vasopressin
   c. angiotensin II
   d. angiotensin I

6. The mechanism by which water moves down the descending loop and into the blood stream is called:
   a. active transport
   b. counter - current
   c. passive transport
   d. renal threshold

7. The amount of glomerular filtrate produced daily is approximately:
   a. 1 - 2 liters
   b. 180 liters
   c. 600 ml/min
   d. 70 liters

8. The glomerular filtrate is described as:
   a. a protein filtrate of plasma
   b. a glucose and protein-containing filtrate of plasma
   c. a plasma filtrate without glucose and protein
   d. an ultra filtrate of plasma that does not contain protein and fats

9. The renal threshold for glucose is:
   a. 50 - 100 mg/dl
   b. 160 - 180 mg/dl
   c. 220 - 240 mg/dl
   d. over 240 mg/dl
10. Active transport is responsible for the reabsorption of all of the following except:

- a. glucose
- b. albumin
- c. bicarbonate
- d. amino acids

11. A woman is suffering from recurrent urinary tract infections. The following results were obtained from her urinalysis:

<table>
<thead>
<tr>
<th>REAGENT STRIP</th>
<th>MICROSCOPIC</th>
</tr>
</thead>
<tbody>
<tr>
<td>color</td>
<td>yellow</td>
</tr>
<tr>
<td>appearance</td>
<td>cloudy</td>
</tr>
<tr>
<td>pH</td>
<td>8.0</td>
</tr>
<tr>
<td>protein</td>
<td>2+</td>
</tr>
<tr>
<td>occult blood</td>
<td>trace</td>
</tr>
<tr>
<td>glucose</td>
<td>negative</td>
</tr>
<tr>
<td>ketones</td>
<td>negative</td>
</tr>
<tr>
<td>specific gravity</td>
<td>1.030</td>
</tr>
<tr>
<td>nitrite</td>
<td>positive</td>
</tr>
<tr>
<td>leukocyte esterase</td>
<td>positive</td>
</tr>
</tbody>
</table>

WBC’s | 30 - 40/hpf
WBC clumps | occasional
bacteria | large
WBC casts | 5-10/hpf

Based on the above results, what renal disease is most likely?
- a. acute glomerulonephritis
- b. nephrotic syndrome
- c. pyelonephritis
- d. minimal change disease

12. The inflammatory kidney disease in which circulatory antibodies from group A streptococci are deposited on the glomerular membrane is called:

- a. glomerulonephritis
- b. acute interstitial nephritis
- c. pyelonephritis
- d. nephrotic syndrome
13. An image that is in focus under the low power becomes out of focus under the high power objective. What could be the cause of the problem?

   a. condenser is not lowered  
   b. objective not screwed into nose piece fully  
   c. bulb filament is about to burn out  
   d. dirty specimen

**SHORT ANSWERS: FILL IN THE BLANK**

14. List the four main parts of the urinary system.

   __________________________________________________________
   __________________________________________________________
   __________________________________________________________
   __________________________________________________________

15. List the two functions of tubular secretion.

   __________________________________________________________
   __________________________________________________________
   __________________________________________________________
   __________________________________________________________
16. Label the various parts of the microscope that are indicated.
TRUE OR FALSE: CIRCLE THE LETTER CORRESPONDING TO THE CORRECT ANSWER

17. To adjust the amount of light in the brightfield microscope to perform a urine sediment examination, the condenser should be lowered to its lowest point to achieve proper contrast.
   a. True                      b. False

18. The proper technique in scanning a urine sediment slide involves a systematic back-and-forth pattern to the edges of the cover slip with the 40X (high dry) objective.
   a. True                      b. False

19. Polarizing microscopy technique is used in identifying crystals, fat, starch and fibers in urine sediment.
   a. True                      b. False

20. The 10X objective is used primarily to view casts and crystals, scan the slide, and locate elements for more detailed examination.
   a. True                      c. False
MICROSCOPIC EXAMINATION OF URINE SEDIMENT

CHAPTER 2

MULTIPLE CHOICE: CIRCLE THE LETTER CORRESPONDING TO THE BEST ANSWER

1. RBC’s are often confused microscopically with all of the following, except:
   a. renal epithelial cells
   b. yeast cells
   c. calcium oxalate
   d. oil droplets

2. The most valuable aid in the identification of crystals is the knowledge of urinary:
   a. protein
   b. specific gravity
   c. pH
   d. color

3. The most frequently encountered parasite in the urine is:
   a. *Trichomonas vaginalis*
   b. *Schistosoma haematobium*
   c. *Giardia lamblia*
   d. *Entamoeba coli*

4. What protein is the major constituent of casts?
   a. Tamm-Horsfall
   b. albumin
   c. globulin
   d. myoglobin
5. Which type of cast is primarily associated with pyelonephritis?
   a. white blood cell
   b. epithelial cell
   c. granular
   d. red blood cell

6. If the same urine has specific gravity of greater than 1.035 by refractometer method and a specific gravity of 1.015 using reagent strip method, which of the following would be the most likely cause for the discrepancy?
   a. the strips are faulty
   b. radiographic contrast media
   c. casts
   d. white blood cells

7. A useful technique to differentiate ovoid forms of calcium oxalate from red blood cells is:
   a. brightfield microscopy
   b. interference contrast microscopy
   c. polarizing microscopy
   d. phase contrast microscopy

8. Which of the following casts is most difficult to see under brightfield microscopy due to its refractive index being similar to urine?
   a. fine granular casts
   b. hyaline casts
   c. hemoglobin casts
   d. RBC casts

9. Broad casts are:
   a. formed by the disintegration of waxy and fatty casts
   b. formed in the distal convoluted tubules instead of the proximal convoluted tubules
   c. formed at the juncture of the ascending loop of Henle and distal convoluted tubule
   d. formed in the collecting ducts
MATCHING: WRITE THE APPROPRIATE LETTER(S) IN THE BLANKS PROVIDED

10. Match the following crystals with their best descriptions:

<table>
<thead>
<tr>
<th>CRYSTALS</th>
<th>DESCRIPTIONS</th>
</tr>
</thead>
<tbody>
<tr>
<td>ammonium biurate</td>
<td>a. sheaths of fine needles</td>
</tr>
<tr>
<td>calcium carbonate</td>
<td>b. dumbbell</td>
</tr>
<tr>
<td>cholesterol</td>
<td>c. spherical, concentric</td>
</tr>
<tr>
<td>tyrosine</td>
<td>d. thorny apple</td>
</tr>
<tr>
<td>leucine</td>
<td>e. notched corners</td>
</tr>
<tr>
<td>uric acid</td>
<td>f. yellow-brown microcrystals</td>
</tr>
<tr>
<td>amorphous phosphates</td>
<td>g. yellow-brown rhombic, 4-sided plates</td>
</tr>
<tr>
<td>amorphous urates</td>
<td>h. white microcrystals</td>
</tr>
<tr>
<td>calcium oxalate</td>
<td>i. octahedral envelopes</td>
</tr>
<tr>
<td>triple phosphate</td>
<td>j. coffin lids</td>
</tr>
</tbody>
</table>

SHORT ANSWER: FILL IN THE BLANK

11. For each crystal listed below, indicate whether it is found primarily in acid or alkaline urine by writing acid or alkaline in the spaces provided.

a. calcium oxalate
b. triple phosphate
c. calcium carbonate
d. uric acid
e. ammonium biurate
f. cholesterol

12. List the three types of epithelial cells in order of least significant to most pathologic.

________________________________________________________________________
________________________________________________________________________
________________________________________________________________________

13. A large leukocyte that appears to sparkle because of the Brownian movement of its cytoplasmic granules is called:

________________________________________________________________________
14. Indicate which crystals are normal (N) or abnormal (A) by writing an (N) or (A) in the blank provided.
   a. cholesterol ___________
   b. leucine ___________
   c. uric acid ___________
   d. calcium oxalate ___________
   e. triple phosphate ___________
   f. tyrosine ___________

15. Draw a simple sketch of the following crystals:

   a. calcium oxalate

   b. cystine

   c. uric acid

   d. triple phosphate

   e. cholesterol
16. What are clue cells and what do they indicate?

________________________________________________________________________
________________________________________________________________________
________________________________________________________________________

TRUE OR FALSE: CIRCLE THE CORRECT RESPONSE

17. The presence of a large number of squamous epithelial cells in a urine sediment is an indication of a poorly collected specimen.
   a. True    b. False

18. Casts containing RBCs and those containing hemoglobin represent different diseases, therefore differentiation between them should be reported.
   a. True    b. False

19. Urinary sediment examination may not be necessary if the urine specimen is yellow, clear, and the reagent strip tests are normal.
   a. True    b. False
QUALITY ASSURANCE / CONFIRMATORY URINE TESTS

CHAPTER 3

MULTIPLE CHOICE: CIRCLE THE LETTER CORRESPONDING TO THE BEST ANSWER

1. All children under the age of two (2) should be screened for reducing sugars with:
   a. Ictotest®
   b. Clinitest®
   c. glucose oxidase
   d. sulfosalicylic acid

2. This component of quality assurance tracks patient information from the time a specimen is drawn, to the time the test result is reported.
   a. patient test management
   b. quality control
   c. complaint investigation
   d. procedure manual

3. Which of the following is the best definition of a quality control material?
   a. material provided by the manufacturer which sets the instrument before you can perform a laboratory test
   b. material provided by the manufacturer or ordered separately which goes through every step just like a patient test sample and is used to determine whether a test procedure is working before patient tests are performed
   c. material ordered separately from the reagent strips which is tested three or four times a year and sent to a “referee group” for grading

4. Controls could help me: (check all that apply)
   a. _________ know if a testing instrument is working properly
   b. _________ know that reagent strips are working properly
   c. _________ know if I performed a testing procedure correctly
   d. _________ know if I collected the proper type of sample for testing
5. Correlation of the information obtained from the physical, chemical, and microscopic findings is which component of quality assurance?
   a. quality control
   b. proficiency testing
   c. patient test management
   d. comparison of test results

6. Ways to assess personnel competency is through: (check all that apply)
   a. __________ proficiency testing
   b. __________ direct observation
   c. __________ personnel evaluations
   d. __________ procedure manuals

7. A comprehensive set of policies, procedures, and practices necessary to make sure that the laboratory’s results are reliable is called:
   a. quality control
   b. reliability
   c. quality assurance
   d. accuracy

8. How would the results of an SSA protein test result in turbidity with granulation but no flocculation be reported?
   a. trace
   b. 1+
   c. 2+
   d. 3+
   e. 4+

9. Proficiency testing is a quality control process which:
   1. establishes a link between laboratories using the same method of testing;
   2. is required for moderate and high complexity laboratories under CLIA-88;
   3. uses aliquotes of the same sample pool;
   4. is an internal quality control; or,
   5. is not available for urine microscopics.
    
    a. all are correct
    b. 1, 2, 3, and 4 are correct
    c. 1 and 2 are correct
    d. 1, 2, and 3 are correct

10. A urine specimen is collected at 1:15pm. It is tested at 2:00pm without refrigeration or preservation. Would the results be accurate?
    ( ) Yes       ( ) No
11. The operational protocol that standardizes laboratory testing for each testing site is called:
   a. quality control
   b. communication / complaint investigation
   c. the procedure manual
   d. test comparisons

MATCHING: WRITE THE APPROPRIATE LETTER(S) IN THE BLANKS PROVIDED

12. Match the following sources of error with when it is most likely to occur in the testing process.

   _____ ignoring time requirements           a. pre-analytical
   _____ strenuous exercise of patient        b. analytical
   _____ failure to perform confirmatory tests on questionable strip results  c. post-analytical
   _____ using expired reagent strips
   _____ ingestion of medications by patient
   _____ failure to provide interpretive information to physician
   _____ placing results on incorrect patient chart
   _____ using a contaminated urine container
   _____ incorrect information on test requisition form

13. Match the analyte with the confirmatory test method.

   _____ protein                               a. sulfosalicylic acid precipitation
   _____ glucose                                b. modified copper reduction
   _____ bilirubin                              c. sediment examination
   _____ white blood cells                      d. Diazo reaction

14. Indicate which tests are waived or moderate complexity under the CLIA-88 laboratory regulations. Place a (W) beside waived tests and an (M) beside moderate complexity tests.

   a. __________ urinalysis with sediment examination
   b. __________ manual reagent strip urinalysis
   c. __________ urinalysis using the reagent strip instrument
15. List the components of a quality assurance program.

16. Give two (2) reasons for performing a confirmatory bilirubin test.
   a. 
   b. 

17. List two (2) reasons for urine specimen rejection.
   a. 
   b. 

18. Most laboratory errors occur in the analytical phase of testing.
   a. True  
   b. False  

19. Laboratory records are to be kept for a minimum of two (2) years.
   a. True  
   b. False  

20. When urine has a hazy appearance and the nitrite is positive on the reagent strip, the urine sediment should contain bacteria.
   a. True  
   b. False
URINE MICROSCOPICS

FINAL EXAMINATION

MULTIPLE CHOICE: CIRCLE THE LETTER CORRESPONDING TO THE BEST ANSWER

1. Which of the following formed elements found in the urine sediment is unique to the kidney?
   a. crystals
   b. epithelial cells
   c. amorphous material
   d. casts

2. A comprehensive set of policies, procedures, and practices necessary to make sure that the laboratory’s results are reliable is called:
   a. quality assurance
   b. quality control
   c. accuracy
   d. CLIA-88 regulations

3. The portion of the microscope which focuses the lens in small increments is called the:
   a. condenser knob
   b. field diaphragm
   c. iris diaphragm
   d. fine adjustment knob

4. Which of the factors below must be standardized in a urine sedimentation procedure?
   1. a volume of 12 ml, generally recommended for centrifugation
   2. centrifugation time of 5 minutes
   3. centrifugal force (RCF) of 400-450 g
   4. uniform drop size
      a. all statements are correct
      b. 1, 2, 3 are correct
      c. 1, 2, 4 are correct

5. The process to assure that patient specimen test results are not confused with other patient test results is called:
   a. patient preparation
   b. comparison of test results
   c. patient test management
   d. personnel competency
6. Insufficient antidiuretic hormone (ADH) will result in a urine that has a:
   a. low specific gravity
   b. high solute concentration
   c. dark yellow color
   d. decreased daily volume

7. Oval fat bodies are a degenerated form of which of the following cells:
   a. renal epithelial cells
   b. white blood cells
   c. red blood cells
   d. transitional epithelial cells

8. In a concentrated urine specimen, red blood cells may appear:
   a. dysmorphic
   b. crenated
   c. ghost cells
   d. swollen

9. After performing an Ictotest, a blue or purple color appears on the surface of the Ictotest mat. How should this reaction be interpreted?
   a. negative reaction for bilirubin
   b. positive reaction for bilirubin
   c. equivocal, retest

10. Blood enters the glomerulus from the renal artery through the:
    a. efferent arteriole
    b. Bowman’s capsule
    c. afferent arteriole
    d. peritubular capillaries

11. The presence of white blood cells in the urine is called:
    a. hematuria
    b. pyuria
    c. oliguria
    d. proteinuria

12. The portion of the nephron where approximately 80% of the fluid and electrolytes filtered by the glomerulus are reabsorbed:
    a. proximal convoluted tubule
    b. distal convoluted tubule
    c. collection duct
    d. loop of Henle
13. Which of the following statements apply to the proper adjustment of the brightfield microscope for viewing urine sediment?

1. light is adjusted by opening and closing the aperture iris diaphragm
2. light must be reduced to given contrast
3. lower the condenser only slightly (1 - 2 mm)
4. light must be bright to enhance the features of the sediment constituents
5. lower the condenser to its lowest point

a. all statements are correct
b. 1, 2, 3 are correct
c. 1, 2, 5 are correct
d. 1, 2, 4 are correct

14. The elongated branches which are associated with urinary tract infections caused by Candida species are called:

a. budding yeast
b. cylindroid
c. pseudo hyphae
d. oval fat bodies

**SHORT ANSWER: COMPLETE THE BLANK**

15. List the normal reference values for the following urine constituents:

<table>
<thead>
<tr>
<th>Constituent</th>
<th>Normal Reference Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Red blood cells</td>
<td></td>
</tr>
<tr>
<td>Hyaline casts</td>
<td></td>
</tr>
<tr>
<td>White blood cells</td>
<td></td>
</tr>
</tbody>
</table>

16. The ability of a urine constituent to polarize light is called:

________________________________________________________

________________________________________________________

17. What is the smallest cellular element of clinical importance found in the urine sediment?

________________________________________________________

________________________________________________________
18. List five (5) preventive maintenance activities that should be performed on the microscope at the completion of the workday.

a. 

b. 

c. 

d. 

19. The medulla or central portion of the kidney consists of the:

a. 

b. 

c. 

20. Transitional epithelial cells which originate from the bladder and structurally have tails of cytoplasm are referred to as:

TRUE OR FALSE: CIRCLE THE CORRECT ANSWER

21. Tissues such as Kimwipes® should be used to clean the microscope lenses.

   a. True          b. False

22. Testing quality control materials daily will ensure that the correct patient was tested.

   a. True          b. False

23. The terms quality assurance and quality control can be used interchangeably.

   a. True          b. False
CASE STUDIES

Read the patient information and the urinalysis results.
Answer the corresponding questions using the information provided.

24. A 35 year old woman visits her health clinic with complaints of abdominal pain and frequency of urination. A urinalysis was performed using a clean catch urine specimen obtained from the woman. The following urinalysis results were reported:

<table>
<thead>
<tr>
<th>PHYSICAL AND CHEMICAL RESULTS</th>
</tr>
</thead>
<tbody>
<tr>
<td>Color - pink</td>
</tr>
<tr>
<td>Appearance - hazy</td>
</tr>
<tr>
<td>Specific gravity - 1.025</td>
</tr>
<tr>
<td>pH - 6.5</td>
</tr>
<tr>
<td>Protein - strip = 1+ ; SSA = 1+</td>
</tr>
<tr>
<td>Glucose - negative</td>
</tr>
</tbody>
</table>

Microscopic:
- Red blood cells - 5 – 10 / hpf
- White blood cells - 10 – 25 / hpf
- Casts - none seen
- Epithelial cells - few transitional epithelial cells
  few squamous epithelial cells
- Bacteria - many

Which renal disease would most likely generate the above urinalysis results?

a. pyelonephritis
b. nephritis syndrome
c. glomerulonephritis
d. cystitis
25. The following urinalysis information was obtained from an 18 month child.

<table>
<thead>
<tr>
<th>PHYSICAL AND CHEMICAL RESULTS</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Color</strong> - straw</td>
</tr>
<tr>
<td><strong>Appearance</strong> - clear</td>
</tr>
<tr>
<td><strong>Specific gravity</strong> - 1.010</td>
</tr>
<tr>
<td><strong>pH</strong> - 6.5</td>
</tr>
<tr>
<td><strong>Protein</strong> - trace</td>
</tr>
<tr>
<td><strong>Glucose</strong> - negative</td>
</tr>
<tr>
<td></td>
</tr>
</tbody>
</table>

25a. What inconsistency(ies) is(are) present in the case above?

_________________________________________________________________
_________________________________________________________________
_________________________________________________________________
_________________________________________________________________
_________________________________________________________________

25b. What most likely caused the inconsistency(ies)?

_________________________________________________________________
_________________________________________________________________
_________________________________________________________________
_________________________________________________________________
PARTICIPANT QUESTIONNAIRE

Please complete the following information and return to Training & Evaluation with the completed exams. Completion is voluntary and information gathered is used for training and research purposes only.

1. Name of participant: ____________________________________________________________

2. Home address: Street: ____________________________________________________________
    City: __________________________ State: _____ Zip: __________________________

3. Phone number: Home: __________________________ Business: ______________________

4. Professional title: ___ MT ___ MLT ___ CLA(CLT) ___ RN ___ LPN ___ PAC-C ___ MED. ASST. ___ RT ___ DO ___ MD

5. Formal education (check highest level completed):
   ___ High school ___ Associate degree
   ___ Hospital training program ___ Bachelor’s degree
   ___ Vocational training program ___ Master’s degree
   ___ Some college (# of yrs ___) ___ Other (specify): __________________________

6. Clinical laboratory practitioner’s license: Number: __________________________ Category: __________________________

7. Length of laboratory experience: ______ years

8. Facility (current employer): ______________________________________________________

9. Type of facility (check one): ___ Hospital ___ POL/Group practice ___ Health Dept
   ___ Primary care ___ Rural health clinic ___ Reference lab ___ Other (specify):

10. Does your facility have a CLIA-88 certificate to perform laboratory testing? ( ) YES ( ) NO
    In what complexity category?
    ___ Waived ___ High
    ___ Moderate ___ Physician performed microscopy
    ___ Other accreditation (specify): _____________________________________________

11. Does your facility have a procedure manual designed for your facility? ( ) YES ( ) NO

12. Do you currently have a written QA plan or policy? ( ) YES ( ) NO

13. Does your laboratory have any QA monitoring activities either in progress now or in the past? ( ) YES ( ) NO
    If “Yes”, list the QA monitoring activities: __________________________________________
How did you find out about this course? ____________________________________________
______________________________________________________________________________
______________________________________________________________________________

PLEASE RATE EACH OF THE ITEMS BELOW USING THE FOLLOWING SCALE:

<table>
<thead>
<tr>
<th>Scale</th>
<th>Excellent (5)</th>
<th>Good (4)</th>
<th>Acceptable (3)</th>
<th>Marginal (2)</th>
<th>Poor (1)</th>
</tr>
</thead>
</table>

HOW SUCCESSFUL WAS THIS COURSE IN:

1. Increasing you knowledge and/or skills in the subject matter presented?
   
<table>
<thead>
<tr>
<th>Scale</th>
<th>Excellent (5)</th>
<th>Good (4)</th>
<th>Acceptable (3)</th>
<th>Marginal (2)</th>
<th>Poor (1)</th>
</tr>
</thead>
</table>

2. Providing material that is directly applicable to your work?
   
<table>
<thead>
<tr>
<th>Scale</th>
<th>Excellent (5)</th>
<th>Good (4)</th>
<th>Acceptable (3)</th>
<th>Marginal (2)</th>
<th>Poor (1)</th>
</tr>
</thead>
</table>

3. Meeting the stated objectives?
   
<table>
<thead>
<tr>
<th>Scale</th>
<th>Excellent (5)</th>
<th>Good (4)</th>
<th>Acceptable (3)</th>
<th>Marginal (2)</th>
<th>Poor (1)</th>
</tr>
</thead>
</table>

4. How worthwhile was this program in terms of time and money invested?
   
<table>
<thead>
<tr>
<th>Scale</th>
<th>Excellent (5)</th>
<th>Good (4)</th>
<th>Acceptable (3)</th>
<th>Marginal (2)</th>
<th>Poor (1)</th>
</tr>
</thead>
</table>

5. Overall, I consider this course:
   
<table>
<thead>
<tr>
<th>Scale</th>
<th>Excellent (5)</th>
<th>Good (4)</th>
<th>Acceptable (3)</th>
<th>Marginal (2)</th>
<th>Poor (1)</th>
</tr>
</thead>
</table>

1. What would you add to this course to make it more beneficial to you? ________________________________

2. What would you delete from this course? _______________________________________________________

3. Based on what you learned in this course, do you intend to make any changes in your laboratory practices?  
   ( ) YES  ( ) NO

4. Which type of course do you prefer?  ( ) Correspondence  ( ) One day workshop/seminar held at various locations throughout the state

5. Suggestions for topics of future courses: ________________________________

<table>
<thead>
<tr>
<th>Scale</th>
<th>Excellent (5)</th>
<th>Good (4)</th>
<th>Acceptable (3)</th>
<th>Marginal (2)</th>
<th>Poor (1)</th>
</tr>
</thead>
</table>

OPTIONAL INFORMATION

Name: ________________________________

Clinical laboratory practitioner category (CLP) (please check one):  ( ) MT  ( ) MLT  ( ) POCT  ( ) CYTO

CLP license number: ________________________________ 
If not CLP licensed, other: ________________________________