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Modern Urine Chemistry
Application of Urine Chemistry and Microscopic Examination in Health and Disease

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Preface

Urine testing provides clinicians with valuable information used in the diagnosis and monitoring of disease. Such testing is useful not only in detecting individuals who have some clinical abnormality, but also in confirming normal urinalysis in the majority of individuals who do not have detectable abnormal values.

This fourth edition attempts to provide the reader with the latest information available at the time of printing. The intent is to provide the reader with a comprehensive understanding of the basic laboratory procedures used in routine urinalysis. The text describes the methodology and the purpose of selected tests. It also discusses expected values and the clinical significance of abnormal results with the goal of making clear the extent to which routine urinalysis and supplemental procedures can supply clinically valuable information about the health status of the patient.
Chapter 1:
Urinalysis History and Milestones

Introduction and Background
It has been known for centuries that abnormalities in urine may indicate diseases. Perhaps one of the earliest known records of a urine test was the technique of pouring urine on the ground and observing whether or not it attracted insects. The attraction of insects indicated “honey urine,” which was known to be excreted by people with boils. Today, checking for sugar in the urine is a test to detect diabetes.

In ancient times, before the existence of any alphabet, it was common to use symbols to denote important known elements of nature. The symbol shown in Figure 1 is an ancient sign used to denote urine.

![Figure 1. Ancient symbol denoting “urine” as one of the elements of nature.](image)

Around 1000 A.D., a Persian physician named Ismail of Jurjani described seven different observations made when analyzing urine:

- Quantity
- Consistency
- Color
- Odor
- Transparency
- Sediment
- Froth

During the Middle Ages, the great painters typically depicted physicians peering into a round-bottomed flask of urine. This practice became a common tool of quacks and charlatans, who came to be known as “Pisse Prophets.” They not only pretended to diagnose disease by visual examination of urine, but they also claimed to see into the future. Table I on the following page lists some of the milestones by various investigators who studied urine with physical and chemical tests.
Table I. Milestones in the Development of Urinalysis

<table>
<thead>
<tr>
<th>Era</th>
<th>Observation</th>
<th>Association</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ancient Times</td>
<td>Attraction of Insects</td>
<td>“Honey” Urine</td>
</tr>
<tr>
<td>400 B.C. - Hippocrates</td>
<td>Color/Odor Changes</td>
<td>Fever</td>
</tr>
<tr>
<td>1000 A.D. - Ismail of Jurjani</td>
<td>Seven Observations in Urine Study</td>
<td></td>
</tr>
<tr>
<td>Middle Ages Uroscopists, “Pisse Prophets”</td>
<td>Visual Observations</td>
<td>Health, Disease, Future</td>
</tr>
<tr>
<td>1609 - Scribonius</td>
<td>Black Urine</td>
<td>Alcaptonuria</td>
</tr>
<tr>
<td>1673 - Dekkers</td>
<td>Boiling/Acetic Acid</td>
<td>Protein</td>
</tr>
<tr>
<td>1674 - Willis</td>
<td>Sweet Taste</td>
<td>Diabetes</td>
</tr>
<tr>
<td>1674 - Boerhaave</td>
<td>Specific Gravity</td>
<td>Dehydration</td>
</tr>
<tr>
<td>1776 - Dobson</td>
<td>Sweet Taste/Diabetes</td>
<td>Sugar</td>
</tr>
<tr>
<td>1787 - Marabellini</td>
<td>Nitric Acid</td>
<td>Bilirubin</td>
</tr>
<tr>
<td>1790 - Horne</td>
<td>Yeast Fermentation</td>
<td>Sugar</td>
</tr>
<tr>
<td>1790 - Cruikshank</td>
<td>Nitric Acid</td>
<td>Protein</td>
</tr>
<tr>
<td>1810 - Wollaston</td>
<td>Chemical Tests</td>
<td>Kidney Stone Composition</td>
</tr>
<tr>
<td>1827 - Bright</td>
<td>Heat and Acid</td>
<td>Protein/Kidney Disease</td>
</tr>
<tr>
<td>1841 - Trometer</td>
<td>Alkaline Copper Reduction</td>
<td>Sugar</td>
</tr>
<tr>
<td>1911 - Benedict</td>
<td>Alkaline Copper Reduction</td>
<td>Sugar</td>
</tr>
</tbody>
</table>

It was not until the twentieth century that urinalysis became a practical laboratory procedure. Stanley Benedict provided the first stable, practical liquid test for urine sugar – the Benedict’s Solution of alkaline copper sulfate. His paper was published in the Journal of the American Medical Association (JAMA) in 1911 while he was a 17-year-old medical student. At about the same time, Victor Myers, Ph.D. was appointed head of the first clinical laboratory in the United States at the New York Postgraduate Hospital. Thus, Myers became the first practitioner of clinical chemistry, including urinalysis.

Walter Ames Compton, M.D. ushered in the modern era of urinalysis in the early 1940’s with the invention of Clinistix® tablets. These tablet tests are for the determination of reducing sugars in urine and remain in the Siemens portfolio today.

Other convenient tests were added during the next decade under the direction of Alfred H. Free, Ph.D., who was also responsible for one of the industry’s most significant inventions – the 1956 development of Clinistix® urinalysis test strips, the first dip-and-read urine test. Clinistix urinalysis test strips were unique in that they were based on the enzyme glucose oxidase, and thus were specific for glucose. Free’s research led the way for many other urinalysis test strips, culminating 30 years later with the ten-way urinalysis test strip, Multistix® 10 SG Reagent Strips for Urinalysis. Multistix® urinalysis test strips remain the cornerstone of the Siemens urinalysis product portfolio today.

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Chapter 2:
Kidney Structure and Formation of Urine

Overview
A basic knowledge of kidney structure and urine formation is an important aid in understanding urinalysis and test interpretation.

The primary functions of the kidneys are:
- Removal of waste products, primarily nitrogenous wastes from protein metabolism and acids
- Retention of essential nutrients such as water, glucose, protein, and electrolytes
- Acid-base balance
- Water and electrolyte balance
- Hormone production such as erythropoietin and renin
- Production of Vitamin D

Diseased kidneys cannot adequately regulate the volume and composition of body fluids, which makes the body unable to maintain homeostasis, the stabilization of bodily functions. Consequently, substances normally retained by a kidney, or excreted in small amounts, may appear in the urine in large quantities; or substances normally excreted may be retained. Disease processes that directly affect the kidney (such as infection, inflammation, or stones) may cause structural elements, such as red blood cells, leukocytes, urinary tract cells, and casts from the diseased kidneys, to appear in the urine.

Kidney Anatomy
The kidneys are situated on either side of the spinal column, in an area called the retroperitoneal space. They are bean-shaped organs that, in the adult, measure approximately 4-5 inches (10-12 cm) by 2-2.5 inches (5-6 cm) and weigh about 12 ounces (300 grams) (Figure 2). Each kidney consists of five segments, each with its own blood supply. The functional unit of the kidney is the nephron. There are approximately one million nephrons in each kidney.

Each nephron consists of a glomerulus, which is essentially a filtering system, and a tubule, through which the filtered liquid passes. As liquid moves through the tubule, various changes occur. Certain constituents are reabsorbed by the cells lining the tubule, while others are secreted into the lumen for eventual excretion. On average, nearly all the water that passes through the glomeruli is reabsorbed by the tubules (Figure 3).
Each glomerulus consists of a network of capillaries surrounded by a membrane called Bowman’s capsule. This membrane continues on to form Bowman’s space and the beginning of the renal tubule.

The afferent arteriole carries blood from the renal artery into the glomerulus. The afferent arteriole then divides to form a capillary network. These capillaries reunite to form the efferent arteriole, through which blood leaves the glomerulus. The blood vessels thus follow the course of the tubule, forming a surrounding capillary network.

The tubular portion of each nephron has several distinct structural and functional segments. The uppermost portion, continuous with the glomerulus, is the proximal convoluted tubule, followed by the thin-walled segment and the distal convoluted tubule, respectively. The descending limb of the proximal tubule (the thin-walled segment) and the distal tubule form a loop known as the loop of Henle.

The distal convoluted tubules from several nephrons drain into a collecting tubule. A number of these coalesce to form the collecting duct. The collecting ducts then join together to form the papillary ducts. The latter empty at the tip of the papillae into the calyces, which in turn drain into the renal pelvis. Urine passes from the pelvis of the kidney down the ureter and into the bladder, where it remains until voided.

**Formation of Urine**

The kidney is a highly discriminating organ that selectively excretes or retains various substances according to specific body needs. Approximately 1,200 mL of blood flow through the kidneys each minute. This represents about one-fourth of the body’s total blood volume. The blood enters the glomerulus of each nephron by passing through the afferent arteriole into the glomerular capillaries. The capillary walls in the glomerulus are highly permeable to water and to the low-molecular-weight components of the plasma. These constituents filter through the capillary walls and the closely adhering membrane of Bowman’s capsules into Bowman’s space, forming a plasma ultrafiltrate. From here, the ultrafiltrate passes into the tubule where reabsorption of some substances, secretion of others, and the concentration of urine occur.

Many components of the plasma ultrafiltrate, such as glucose, water, and amino acids, are partially or completely reabsorbed by the capillaries surrounding the proximal tubules. In the distal tubules, more water is absorbed and potassium and hydrogen ions are secreted. The loop of Henle and the system of collecting tubules are the principal sites where the urine is concentrated. As the urine is being formed, a mucoprotein, called Tamm-Horsfall protein, is produced by the tubules. As the urine is formed and concentrated, this protein coats and lubricates the tubules. Without this protection, the tubular epithelial cells would have great difficulty functioning, due to the harsh environment. If there is a reduction in the urine flow, this mucoprotein may fill the tubule and “gel,” forming a cast.
Chapter 3: Urine Collection

Overview
In order for urinalysis to be meaningful, the urine must be properly collected. Improper collection may invalidate the results of the laboratory procedures, regardless of how carefully and skillfully the tests are performed. Accurate urinalysis results require:

- Patient preparation prior to specimen collection
- Appropriate specimen collection
- Specific containers for the specimen
- Preservation and transportation of the specimen
- Proper labeling with all appropriate information
- Examination and testing of the specimen as soon as possible following accepted protocols

While urinalysis may be requested on specimens obtained by voiding, catheterization, needle puncture, post-operative urostomy, or via one of several types of collection vessels, such as bags or special receptacles for bed-bound patients, the most commonly obtained specimen is the random midstream urine. Diapers or nappies are sometimes suggested as collecting tools for clinical urine specimens from babies. However, while this method occasionally produces promising results for chemical constituents measured by qualitative strip examinations, morphological, and in particular, microbiological, investigations are impossible with this procedure. Appropriate containers need to be used, depending on the test required.

The patient should be told why a urine specimen is being requested, and given instructions on how it should be collected. To ensure uniformity of the collection procedure, it is recommended that the instructions be given both orally and in written form accompanied by illustrations where possible.

The requesting clinical staff must document the method of collection to allow correct interpretation of results. Proper preservation of the specimen (usually refrigeration) is required if the specimen cannot be immediately tested. Urine specimens should be brought to room temperature before testing. A lab should have a written protocol for the entire process of collection, transportation, and testing to ensure reliability.

Types of Specimens
The concentration of urine varies throughout a 24-hour period, depending partly on the patient’s water intake and activities. Various solutes may appear in greater or lesser amounts at various times of the day; glycosuria appears more often after meals; proteinuria may occur following activity or assumption of the orthostatic (upright) position; and hemoglobinuria may follow severe exertion. The number of bacteria in the urine of a person with a urinary tract infection also varies greatly throughout the day.

Table II lists some of the most common types of specimens used for urinalysis. In general, a more concentrated urine specimen is preferred for testing rather than a dilute specimen. Therefore, the first-voided morning urine, which is the most concentrated, is the best for routine analysis. However, it is often not practical to obtain the first morning specimen; in such cases, a randomly voided specimen of lesser concentration is usually obtained. Because concentration may affect results, the urine’s specific gravity (amount of dissolved substances present) should be considered during result interpretation.

<table>
<thead>
<tr>
<th>Type of Specimen</th>
<th>Characteristics and Uses</th>
</tr>
</thead>
<tbody>
<tr>
<td>Random</td>
<td>A portion of a single voided urine without defining the volume, time-of-day, or detail of patient preparation. While the most convenient and common type of specimen, it is also associated with many false negative and some false positive results. The specimen is good for chemical screens and microscopic examinations.*</td>
</tr>
<tr>
<td>First Morning</td>
<td>The specimen voided immediately after an overnight bed rest before breakfast and other activities. This has been traditionally recommended as the standard specimen for urinalysis, because it is more concentrated than the day urine and allows for possible bacterial growth in the urinary bladder. This specimen is best for nitrite, protein, and microscopic examinations.*</td>
</tr>
<tr>
<td>Second Morning</td>
<td>A single specimen voided 2-4 hours after the first morning urine. In contrast to the first morning urine, its composition may be affected by prior ingestion of food and fluids and by movement. This specimen may reflect blood glucose and typically contains intact formed elements.</td>
</tr>
<tr>
<td>Postprandial</td>
<td>A single specimen collected 1-2 hours after a meal. The specimen is good for glucose testing because the blood glucose is at its highest level 1-2 hours after eating and thus it is the specimen most likely to contain glucose.</td>
</tr>
</tbody>
</table>
| Timed Collection | A specimen collected at a specified time in relation to another activity, e.g., therapy, meals, and daytime or bed rest.
  - 24-hour – All urine produced during the next 24 hours is collected and preserved in a manner appropriate for the analyte(s) being tested. The collection can begin at any time of the day by emptying the bladder and noting the time.
  - Timed overnight – All urine produced during the bed-rest period. Sampling begins by emptying the bladder just before going to bed, noting the exact time, and then collecting all urine voided during the bed-rest period. At the end of the period, the last portion is collected, the exact time recorded, and the total volume of overnight urine noted. The specimen or a representative aliquot is then sent to the laboratory. |

*Formed elements may lyse or disintegrate if pH is high and/or specific gravity is low.
Routine tests and any other tests performed on a random specimen of urine are generally qualitative or semi-quantitative. At best, only the concentration of a substance in the specimen tested can be measured, but never the total amount being excreted unless the urine is collected over a precisely measured period of time and the volume is measured.

For example, two random urine specimens are tested for proteinuria. Both may show the same concentration of protein. If one is a large-volume sample and the other is a small-volume sample, the total amounts of protein are different between the two samples.

In the past, a 24-hour specimen was needed to provide a more precise measurement. Collection of a timed urine sample is inconvenient and may be associated with errors, largely due to improper timing and missed samples. This may lead to over-collections or under-collections.

Today, there are several dipsticks on the market that offer a protein-to-creatinine ratio (P:C ratio) and albumin-to-creatinine ratio (A:C ratio), both of which correct for varying urine concentrations. The ratio results are more reliable than albumin or protein measurements alone, and reduce both false positive and false negative results compared with reference assays. These new tests enable physicians to quickly assess protein excretion using a single random urine specimen.

Both the American Diabetes Association (ADA) and the National Kidney Foundation (NKF) recognize the convenience of ratio testing as compared to timed urine collection.

“The analysis of a spot sample for the A:C ratio is strongly recommended.
The other two alternatives (24h collection and a timed specimen) are rarely necessary. At least two of three tests measured within a 6-month period should show elevated levels before a patient is designated as having microalbuminuria.”

“The ratio of protein-to-creatinine or albumin-to-creatinine in an untimed, “spot” urine specimen corrects for variations in urinary concentration due to hydration and provides a more convenient method of assessing protein and albumin excretion than is involved with timed urine collection.”

Collection Containers
For urine collection, disposable containers of plastic or coated paper are available in many sizes; they are provided with lids to reduce bacterial and other types of contamination. Special pliable polyethylene bags are also available for collection of urine from infants and children who are not toilet-trained.

For some chemical constituents, quantitative excretion measurements are important. For this purpose, large, wide-mouthed plastic or glass containers with screw caps are used for cumulative collection of urine over a long period of time. Containers designed for a 24-hour or overnight urine collection typically hold 2-3 liters and are manufactured with materials that prevent:

- Adherence of urine constituents
- Exposure of urine to direct light, which might alter clinically significant metabolites
- Contamination from the exterior when closed

Preservatives and/or refrigeration are often used to prevent metabolic and other changes of urine constituents.

When urine is to be cultured for bacterial content, the specimen must be obtained under aseptic conditions (as discussed in the next section) and collected in a sterile container equipped with a tight-fitting, sterile cap. This cap is left in position until the actual time of urine collection and replaced immediately afterward.

Methods of Obtaining Specimens

Random Specimen
A freshly voided urine specimen is adequate for most urinalysis – except the microbiological culture. The procedure for obtaining a random specimen is described below.

Women
1) Wash hands well
2) Use an antiseptic towelette to wipe from front to back
3) Spread labia apart
4) Start urinating into toilet
5) Collect urine specimen (do not touch the inside of the container)
6) Finish urinating into toilet
7) Place cap on container

Men
1) Wash hands well
2) Retract foreskin and wipe with an antiseptic towelette
3) Start urinating into toilet
4) Collect urine specimen (do not touch the inside of the container)
5) Finish urinating into toilet
6) Place cap on container
Infants and Children
Specimens from infants and young children are typically collected in a disposable plastic bag that is equipped with an adhesive around the opening, which holds the bag in place so that the child voids directly into the bag.

Clean-Voided Midstream Catch
This is the most commonly used procedure for obtaining a urine specimen suitable for microbiological examination. It is also the procedure of choice for urine specimens likely to contain vaginal discharge or menstrual blood. In very rare and unusual circumstances, bladder catheterization or percutaneous suprapubic aspiration of the bladder may be used.

A Clean-Void Midstream Catch helps avoid or minimize:

• Contamination of specimen by commensal bacteria
• Growth of bacteria following specimen collection
• Damage or death of diagnostically relevant bacteria
• Disintegration of diagnostically valuable formed elements

To avoid contamination of the specimen by organisms in the areas adjacent to the urethral meatus, these areas must be cleansed thoroughly before the patient voids. To avoid contamination by organisms normally found in the distal urethra, the initial stream of voided urine, which clears these organisms from the urethra, is discarded and the subsequent midstream urine is collected.

Women
A satisfactory technique for the female consists of spreading the labia and cleansing the area with a packaged, premoistened towelette. The washing is accomplished by making a single front-to-back motion with the towelette. The towelette is used to cleanse the areas on both sides and directly across from the meatus. While the labia is still held apart, a small amount of urine will be passed into the toilet or bedpan and should be discarded. Then a midstream specimen should be collected in the sterilized container, which should be immediately capped with its sterilized cover.

Men
A comparable technique is used for males. First, the foreskin should be retracted. Then the tip of the penis, particularly the area directly across the point of urine void, should be cleansed with a packaged, premoistened towelette. With the foreskin still retracted, the small amount of urine passed into the toilet or bedpan should be discarded. From the subsequent midstream urine, the specimen should be collected in a sterilized container, which should be immediately capped with its sterilized cover.

Infants and Children
For infants and children who have not been toilet-trained, a sterilized, plastic, disposable collection bag can be applied to the perineal region after the area has been suitably cleansed. Once in place, the collection bag should be checked frequently for urine flow. The collection bag should be in situ for a maximum of one hour, after which the probability of contamination increases greatly. Negative culture results reliably exclude urinary tract infection. Borderline results need to be re-investigated from a suprapubic aspiration or single catheterized urine specimen.

All Methods
Regardless of the method used to obtain a specimen, once obtained it should be immediately covered and taken to the laboratory.

Preservation of Specimens
The time elapsing between voiding and examination of urine is a major obstacle to diagnostic accuracy in most laboratories. Therefore, precise collection times must be documented and delays exceeding the specified limits should be stated on reports. For many chemical constituents examined with urinalysis test strips, no preservatives are needed – provided the analysis is performed within 24 hours and the specimen has been refrigerated. When rapid or refrigerated transportation is not possible, strip examination should be performed on-site. If the specimen has become contaminated with external bacteria and has not been refrigerated, compromised nitrite or protein results may be obtained with urinalysis test strips. In addition, the bacteria may possibly cause false negative glucose results. This may cause erroneous results, as casts decompose in alkaline and/or hypotonic urine and red blood cells may lyse. Marked changes in pH may also affect other cellular components.

Preservation Options
There is no substitute for the use of fresh urine in routine urinalysis. Whenever possible, urine should be macroscopically examined and the urinalysis test strip analyzed within two hours after voiding. If this cannot be done, then refrigeration at 5°C is the preferred storage condition. The urine specimen should be brought to room temperature before testing. Preservatives should be used cautiously, as preservatives suitable for some test procedures may interfere with others, and preservatives added to small amounts of urine will increase specific gravity, may have minor effects on pH, and may also slightly inhibit the leukocyte esterase reaction.

For Microscopic Examinations
For particle examination, the specimen should be refrigerated if not examined within 1 hour, although urate and phosphate precipitation will occur in some specimens. If precipitation disturbs interpretation, a new specimen should be examined at +20°C. The longer the testing delay, the more likely it is for elements to lyse, especially when the urinary
pH is alkaline and the relative density is low. The WBC count may be questionable after 2-4 hours, even with refrigeration. Traditionally, ethanol (50% volume fraction) is used to preserve the cells but this only partially prevents lysis of red and white blood cells. Commercial preservatives, such as formaldehyde-based solutions, buffered boric acid and formate-based solutions, and mercuric chloride-based tablets, have gained renewed interest following the development of automated systems.

For Microbiological Examinations
Specimens requiring microbiological investigation must be collected in a clean, sterile container and examined in the laboratory within 2 hours. They should be refrigerated at 4°C without preservative if a delay >2 hours is expected. Then, they should be examined within 24 hours. If delay is unavoidable and refrigeration not possible, containers pre-filled with boric acid preservative, alone or in combination with formate or other stabilizing media, ideally in a lyophilized form, may be used. Boric acid will stabilize white blood cell numbers and bacterial concentration in urine held at +20°C for 24 hours. Boric acid concentration may be critical for successful preservation without bacterial inhibition. It is suggested that containers containing boric acid should be filled to the indicated line to achieve a correct borate concentration. The specimen should be examined within 24-48 hours of production. It should be noted that borate may inhibit growth of Pseudomonas sp.

During Transport
Chemical preservatives may also be necessary when a patient transports a specimen from home to office or laboratory.

Chapter 4: Purpose and Components of Routine Urinalysis

Overview
The analysis of urine has two purposes:

1. Detect body disturbances, such as endocrine or metabolic abnormalities, in which the kidneys function normally but excrete abnormal amounts of metabolic end-products specific to a particular disease

2. Detect intrinsic conditions that may adversely affect the kidneys or urinary tract

Urinalysis is routine for many patients seen in the physician’s office or medical clinic and for every physical examination. The testing is usually repeated annually or as frequently as the physician deems necessary. It is one of the most useful procedures available to the physician as an indicator of health or disease, especially in the areas of metabolic and kidney disorders.

Urinalysis is frequently performed at the time of hospital admission and is often repeated to evaluate health status while the patient is hospitalized. The major observations and determinations in routine urinalysis are listed in Table III.

Table III. Observations and Determinations in Routine Urinalysis

| Visual Examination | • Color  
|                   |  • Appearance 
|                   |  • Volume |
| Chemical Examination |  • pH  
|                   |  • Glucose  
|                   |  • Bilirubin  
|                   |  • Nitrate  
|                   |  • Specific Gravity  
|                   |  • Blood (Hemoglobin)  
|                   |  • Urobilinogen  
|                   |  • Albumin  
| Microscopic Examination |  • Protein  
|                   |  • Ketones  
|                   |  • Leukocyte Esterase  
|                   |  • Creatinine  
|                   |  • Cells  
|                   |  • Crystals  
|                   |  • Other Organisms  
|                   |  • RBCs  
|                   |  • Yeast |

Components of Routine Urinalysis
In the laboratory, the first procedure is to note the physical characteristics of the urine; the second is to run a series of chemical tests; the third, which is not always necessary, is to examine the sediment. After the initial tests have been performed, the remainder of the urine specimen should be saved until all procedures are completed so the tests can be repeated or special tests performed, if necessary. All the procedures outlined in this section are described in detail in the following chapters.
Physical Examination
The first observation usually made in a urine specimen is its appearance, which can provide useful clues to the presence of many substances, as seen below. For example, dark color may indicate concentrated urine; pale color, dilute urine; reddish-brown color, blood. A turbid specimen may suggest alkaline urine. Table IV gives examples of the common urine colors that can be observed.

Table IV. Urine Color and Indications

<table>
<thead>
<tr>
<th>Color</th>
<th>May Indicate</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dark</td>
<td>Concentrated Urine</td>
</tr>
<tr>
<td>Pale</td>
<td>Diluted Urine</td>
</tr>
<tr>
<td>Reddish-Brown</td>
<td>Blood in Urine</td>
</tr>
</tbody>
</table>

Chemical Tests
Chemically impregnated reagent strips or urinalysis test strips are available for rapid determination of urine specific gravity, pH, protein, albumin, creatinine, glucose, ketones, bilirubin, blood, nitrite, leukocyte esterase, and urobilinogen. These strips are used in basic urinalysis and have virtually replaced older, more cumbersome methods. In addition, other urinalysis test strips, chemical tablets, selectively treated slides, and simplified culture tests are available for special determinations. Standardized results can be achieved by processing the reacted urinalysis test strips with special instrumentation, such as one of the CLINITEK® instruments.

Microscopic Examination
The examination of centrifuged sediment under the microscope is performed to detect those elements, particularly cells that do not give chemical reactions. In some circumstances, microscopic examination can also serve as a confirmatory test.

The microscopic examination might not be standardized. When it is not a standardized determination, the actual number of cells counted should be interpreted with caution.

Confirmatory Tests
The purpose of confirmatory testing is to increase the confidence that the result is correct. Confirmatory tests are used when the results are positive, unexpected, or inconsistent with the clinical diagnosis or other results. General approaches to confirmatory testing include:

- Repeat the test on the same specimen, and run a positive and negative control
- Test a new specimen

- Use a different method on the same specimen
- Gather additional information (i.e., testing the urine for another component, conducting a relevant serum test, collecting further information from the patient)

Quality Assurance and Controls
Quality assurance is an important part of clinical laboratory procedures. Its use has expanded since the early 1940s when surveys first revealed that not all laboratories obtained the same results on blood specimens subjected to hematology or chemistry assays.

The extreme precision and accuracy required for some clinical chemistry procedures is not required for routine urinalysis. The important aspects of quality assurance in regard to urinalysis include:

- Specimen identification
- Specimen transport and preservation
- Quality assurance
- Written procedures
- Instrument maintenance and reagent handling
- Record-keeping

Specimen Identification
One of the most likely laboratory errors is testing the wrong specimen or recording results into the wrong record. Accordingly, each testing facility must establish a specimen identification system that starts when the specimen is voided and continues through the time it is delivered to the laboratory, centrifuged, treated (when necessary), tested, and the results are recorded and released.

Specimen Transport and Preservation
Transportation and preservation procedures for urine specimens are test specific. For instance, if tests for urine bilirubin and urobilinogen are important for a person with compromised liver function, the specimen should be hand-carried to the laboratory immediately after voiding, since these urine constituents are easily destroyed, even at room temperature, and can be affected by laboratory light. In contrast, glucose and ketones are relatively stable in urine unless there are large numbers of organisms in the specimen that metabolize these urine constituents. Refrigeration is the best way to temporarily store specimens to be tested for these constituents. Refrigerated urine specimens should be brought to room temperature before testing.
Quality Assurance
Control solutions are used to help improve laboratory performance, and to help assure the clinician and the patient that the results reported are of the highest quality. In addition, they increase the self-confidence of personnel performing the testing. Guidelines for urinalysis quality assurance and control have been established for Europe by the European Confederation of Laboratory Medicine (ECLM), and for the United States by the National Clinical Chemistry Ligand Society (NCCLS) and the Clinical Laboratory Improvement Amendments (CLIA).

Urinalysis controls can be run using:

a) Commercially available controls with known positive or negative values

b) External quality control materials with unknown values as part of an external proficiency program

The use of control materials with known values helps assure the personnel that they are properly performing the test and that the reagents have been protected from heat and moisture and, therefore, have not deteriorated to give false negative or false positive results.

Chek-Stix® Positive and Negative Urine Controls are available for use in routine urinalysis, which can easily be used to assure proper handling, as well as proper testing. Chek-Stix Controls are useful as composite positive and negative solutions to ensure that expected results will be obtained for several reagent areas of urinalysis test strips.

External quality control materials may be tested as part of a proficiency program. The objective of a proficiency program is to provide laboratories with information regarding:

a) How well the laboratory performs urinalysis testing

b) How well the laboratory compares to peer laboratories participating in the program

There are professional organizations that administer proficiency programs. These organizations prepare external quality control materials for testing by participating laboratories, on a quarterly basis, for example. The quality control materials have test values established by the administering organizations, but the test values are blind or unknown to participating laboratories.

The participating laboratories test the quality control materials during their routine urinalysis testing, and record the results obtained. The laboratories send the results to the organization administering the proficiency program, where the results are analyzed statistically. Reports are prepared and shared with all participating laboratories to confirm that their processes are in control.

Siemens offers a Urinalysis Proficiency Survey as part of a value-added service to users of CLINITEK urinalysis instruments and Siemens urinalysis test strips (Multistix family), check with your local Siemens representative.

While each laboratory should establish its own quality control ranges, urinalysis controls are typically considered acceptable if a positive result is obtained on the positive control specimen 95% of the time and a negative result is obtained on the negative control specimen 95% of the time. Positive specimens used for control should not be borderline positive or contain trace levels of the analytes in question. Instead, positive controls should be definitely positive, recognizable as such by a variety of testing personnel. Control values should be posted to easily detect any abnormal trends and to proudly acknowledge good, stable quality control practices.

The testing facility must also have written procedures that detail the steps to be taken when a urine test is “out of control.” The first step may simply be repeating the control test and then deciding whether or not to conduct further testing. In order to pass laboratory inspection, all corrective procedures must appear in writing, be followed exactly, and documented properly.

Written Procedures
In the United States every urinalysis procedure must appear in writing and be kept up-to-date in the laboratory's procedure manual. This manual should include procedures for dipstick methods, microscopic examination of urine sediment, confirmatory procedures, and special tests.

Siemens offers a CLIA compliance manual for selected urinalysis instruments. Contact your Siemens representative for more information.

Instrument Maintenance and Reagent Handling
The system for instrument maintenance, as well as handling and storage of reagents, is also an important part of quality control. Even ready-to-use, dip-and-read urinalysis test strips must be protected from moisture and heat by carefully choosing the area in which they will be stored and by proper handling during use. This includes removing only the necessary number of strips at a time and making certain the cap is tightly replaced after each use.

If instruments are used in the urinalysis area, it is helpful to maintain a log of items, which must be checked daily, weekly, monthly, or at other intervals. One person in the laboratory should be responsible for keeping this log and should see that the instructions are maintained as specified.

Record-Keeping
Records should be kept for each shift. They should include patient and control test results, and identification of the person(s) who performed the tests. They should also include written procedures for detection and correction of errors as well as for out-of-control results.
Chapter 5: Physical Analysis

Overview
This portion of urine testing involves visual examination of the urine for color and clarity, as well as a notation of any obvious odor and, in some cases, a measurement of volume. Some of the new automated urinalysis instruments are also capable of determining color and clarity.

Specific Gravity
Specific gravity is a physical measurement, which can be performed with a refractometer or urinometer. The procedure is now also performed using a chemical dip-and-read colorimetric technique and is therefore discussed later in this chapter in the section titled Chemical Analysis.

Color
The color of the urine is affected by many elements, including concentration, food pigments, dyes, drugs, and blood. The yellow or amber color of normal urine is caused by the yellow pigment urochrome.

Pigments produced by particular diseases may cause the urine to change color. Bile pigments may produce a yellow-brown or greenish color; porphyrins produce a dark brown-red color upon standing; and hemoglobin gives a reddish-brown color. Melanins cause urine to turn a brown-black color upon standing. Alcaptonuria is identified by urine that turns dark brown or black upon standing.

The CLINITEK Atlas® and the CLINITEK Advantus™ determine and report urine color results such as yellow (or straw), dark yellow (or amber), red, orange, green, or other.

Odor
The smell of normal, freshly voided urine is believed to be due to the presence of volatile acids. Urine that has been standing for a long time develops an ammonia-like odor, which is due to the decomposition of urea by bacteria. The urine of people with diabetes mellitus may have a fruity odor due to the presence of ketones. The urine of people with urinary tract infections may be foul-smelling, especially when the infecting organism is a coliform bacillus. Certain foods such as asparagus may produce a characteristic odor.

And while urine may have many characteristic odors, as a rule these odors are not considered diagnostically significant. However, there are some well-known exceptions. The classical exception is the mother who was instrumental in identifying phenylketonuria by noticing a peculiar odor to her child’s diaper. Other innate metabolic disorders that may also cause distinctive odors in urine include isovaleric acidemia, which produces a “sweaty feet” odor, and maple syrup urine disease, which was named for its odor.

Clarity
Normal, freshly voided urine is usually clear or transparent. If the specimen is alkaline, it may have a cloudy or turbid appearance due to the presence of phosphates and carbonates. This cloudiness will usually disappear when the urine is acidified. A pinkish turbidity frequently indicates the presence of urates. Abnormal turbidity of urine may occur with urinary tract infections, but this is usually due to the alkalinity rather than the actual number of bacteria or leukocytes present. In about 10% of urine specimens, turbidity formed during refrigeration will not dissolve when the urine is brought to room temperature. The CLINITEK Atlas Analyzer optically determines the clarity of each urine sample and reports it as Clear, Slightly Cloudy, Cloudy, or Turbid.

Volume
As mentioned in Table II, it is sometimes advantageous to measure specific analytes in a patient’s urine in relation to some activity such as therapy, meals, and daytime or bed rest. In such cases, the patient is requested to collect all urine voided for a specific time period, usually 24 hours or overnight. The urine voided at the beginning of the time period is discarded and the exact time is noted. All urine voided after this time is collected; urine voided at the end of the time period is added to the collection and the exact time is recorded. The volume is determined by pouring the entire urine collection into a large graduated cylinder and recording the volume in milliliters. The total volume noted is reported as urine volume per unit of time.

The normal volume of urine voided by an adult in a 24-hour period ranges from 750 to 2,000 mL, but averages about 1,500 mL. The amount voided over any period is directly related to the individual’s fluid intake, the temperature and climate, and the amount of perspiration that occurs. Children void somewhat smaller quantities than adults, but the total volume voided is greater in proportion to their body size.
Polyuria
Polyuria refers to an increase in the excretion of urine. It is a physiologic response to increased fluid intake; the ingestion of diuretic medications; certain diuretic drinks, such as coffee, tea, and alcohol; chilling of the body; nervousness and anxiety; and the intravenous infusion of fluids. Polyuria occurs in several disease states, particularly in diabetes mellitus and diabetes insipidus. It is a symptom of chronic kidney disease and has been noted in patients with certain tumors of the brain and spinal cord, as well as acromegaly and myxedema. Polyuria may indicate the loss of concentrating ability by the kidneys.

Oliguria and Anuria
Oliguria refers to decreased urinary output (i.e., less than 200 mL/24 hours). The extreme form, anuria, refers to a total lack of urine. Oliguria occurs when there is excessive loss of body fluids as in vomiting and diarrhea and when there is kidney shutdown through inflammation (nephritis), poisoning, or in cardiac insufficiency. Occasionally, oliguria is due to a mechanical obstruction of the urinary flow. Physiologic forms of oliguria occur with decreased fluid intake, increased ingestion of salt, and excessive perspiration.

Chapter 6:
Urinalysis Tests

Overview
Urinalysis testing allows a physician to perform a single test and obtain results for a number of different test parameters. These test results provide insightful clues to the physician regarding a person’s health. Urinalysis is a fast, simple, inexpensive, and reliable tool for ruling in or ruling out many medical conditions and diseases related to carbohydrate metabolism, urinary tract health, kidney and liver function, cardiovascular health, and many other medical conditions.

Test Procedure
The procedure listed below represents the general procedure used. However, to obtain reliable results the procedure listed on the package insert or in the manufacturer’s manual must be followed exactly.

1. Collect a fresh urine specimen in a clean, dry container. Mix well immediately before testing.
2. Remove one strip from the bottle and replace the cap.
3. Completely immerse the reagent areas of the strip into the urine and remove immediately to avoid dissolving out the reagents.

NOTE: If there is insufficient urine available to completely immerse all of the reagent pads, tip the container so that the urine reaches a sufficiently high level to be able to wet all reagent areas.

4. Start timing.
5. While removing the strip from the urine container, run the edge of the strip against the rim of the urine container to remove excess urine.
6. Hold the strip in a horizontal position to prevent possible mixing of chemicals from adjacent reagent areas and/or contaminating the hands with urine.

7. A - If reading the urinalysis test strips visually, compare the reagent areas to the corresponding color chart on the bottle label at the times specified. Hold the strip close to the color blocks and match carefully. Avoid laying the strip directly on the color chart, as this will result in the urine soiling the color chart.

B - If reading the urinalysis test strips instrumentally, carefully follow the directions provided in the manufacturer’s manual.

NOTE: When using the CLINITEK Atlas system, the urinalysis test strips are in a continuous roll. The on-board precision pipette automatically dispenses the urine samples.
**Leukocyte Esterase**

The presence of a significant number of white blood cells (leukocytes) in the urine (≥10 cells/μL) indicates bacteriuria or a urinary tract infection. Granules of neutrophilic leukocytes release esterase into the urine, which can be detected by chemical means.

The detection of leukocyte esterase as an indication of bacteriuria is an indirect test for infection. Pyuria (the presence of white blood cells in urine in significant numbers) has long been an indication of the possibility of urinary tract infections. When WBCs are found in the sediment, experienced technologists will look harder to find bacteria and correlate other indications of urinary tract infections, such as high pH or positive nitrite tests.

It is usually agreed that the combination of positive results for both nitrite and leukocyte esterase is a good indicator of the need to perform a microscopic examination of the urine sediment for bacteria. Often, however, the combination of two positive chemical tests leads directly to confirmation of bacteriuria by microbiological culture testing.

**Clinical Significance**

Like the nitrite test, the leukocyte esterase test is useful in detecting urinary tract infections. This is because normal urine does not contain large numbers of white blood cells, but infected urine does. Leukocyte esterase is often used in conjunction with the nitrite test when the clinician suspects infection.

Leukocyte esterase testing on the initial portion of a first-morning urine sample has also been proven a reliable testing method for detecting organisms responsible for sexually transmitted diseases (STDs).

**Method of Determination**

The leukocyte esterase test on the Siemens Multistix urinalysis test strips and other Siemens urinalysis test strips detects the esterase released from white blood cells, for example polymorphonuclear leukocytes (mostly neutrophils). The principle of the reaction is that the enzyme splits an ester to form a pyrrole compound that reacts with a diazo reagent to form a highly colored azo dye. The intensity of color is proportional to the amount of enzyme in the urine and, in turn, proportional to the number of white blood cells in the urine.

Siemens leukocyte esterase test results range from negative, trace, small (+), moderate (++), and large (+++). The test may detect levels as low as 5-15 cells/μL in clinical urines.

Nitrite

The nitrite test is fast and inexpensive, and it provides an indirect method for early detection of significant bacteriuria. Common infecting organisms include species of Enterobacter, Citrobacter, Escherichia, Proteus, Klebsiella, and Pseudomonas. These infecting organisms contain reductase enzymes that reduce nitrate in the urine to nitrite.

It is usually agreed that the combination of positive reactions for both nitrite and leukocyte esterase is a good indication for the need to perform a microscopic examination of the urine sediment for bacteria. Often, however, the combination of two positive chemical tests leads directly to confirmation of bacteriuria by microbiological culture testing. And while all positives should be confirmed by microbiological testing, a positive test for nitrite on any random urine specimen always indicates bacteriuria and often prompts the physician to immediately initiate therapy.

However, while a positive result from a nitrite test is an indication of significant bacteriuria, a negative test result should never be interpreted as an absence of bacteriuria. There are several reasons for this:

- First-morning urine, or urine that has remained in the bladder for four hours or more, is more likely to yield a positive nitrite test result in the presence of significant bacteriuria than a random urine sample that may have been in the bladder only a short time
- In the latter type of specimen, there may have been insufficient time for the conversion of nitrate to nitrite by the infecting bacteria
- Some strains of urinary pathogens do not produce the enzyme necessary to reduce nitrate to nitrite, but these organisms are the least common pathogens (see Table V)
- A negative test result may occur if dietary nitrates are absent

**Table V. Organisms That Cause Bacteriuria**

<table>
<thead>
<tr>
<th>Organism</th>
<th>Approximate % of Infections</th>
</tr>
</thead>
<tbody>
<tr>
<td>E.coli</td>
<td>72</td>
</tr>
<tr>
<td>Klebsiella/Enterobacter</td>
<td>16</td>
</tr>
<tr>
<td>Proteus</td>
<td>5</td>
</tr>
<tr>
<td>Staphylococcus</td>
<td>5</td>
</tr>
<tr>
<td>Pseudomonas</td>
<td>1</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Organisms that do not reduce Nitrate to Nitrite</th>
</tr>
</thead>
<tbody>
<tr>
<td>Streptococcus Faecalis</td>
</tr>
</tbody>
</table>

**Clinical Significance**

Bacteriuria is considered significant when microbiological laboratory findings show the presence of 100,000 (10^5) or more bacteria per mL in three separate urine specimens. If bacteria from an external source contaminates an otherwise “sterile specimen,” the count may be as low as 10,000 (10^4) or even 1,000 (10^3) or less per mL.
When the count is between 10^3 and 10^4, the possibility of an incipient urinary tract infection is suggested and the physician may request that another clean-voided midstream urine specimen be obtained for repeat testing.

Significant urinary tract infections may be present in people experiencing no symptoms. Despite an absence of symptoms, these infections are serious because they have the potential for causing severe kidney damage before the person is aware of them. This condition is known as significant asymptomatic bacteriuria. The availability of simple, inexpensive, semi-quantitative methods for detecting bacteriuria makes it easy for physicians to test high-risk patients with and without symptoms for bacteriuria. High-risk patients include pregnant women, school children (especially girls), people who are pregnant or elderly, or people who have diabetes or a previous history of urinary tract infections.

Gram-negative bacteria of the types normally present in the large intestine are the organisms most commonly identified in urinary tract infections.

Method of Determination
The nitrite area of the Multistix 10 SG Reagent Strips for Urinalysis, and all other Siemens urinalysis test strips, has a chemical compound, para-arsanilic acid, impregnated into the strip. This reagent forms a diazonium compound with nitrite which, in turn, couples with a tetrahydro quinoline derivative to produce a pink-colored compound.

Blood
Although protein in urine is the most important indication of kidney dysfunction, the presence of blood in urine is also an indication of damage to the kidney or urinary tract. Blood may appear as intact cells (non-hemolyzed) or as free hemoglobin (hemolyzed or lysed). Usually the presence of free hemoglobin indicates that the cells have ruptured because of the traumatic passage through the kidney and urinary tract to the bladder, or because the cells have been exposed to dilute urine in the bladder, which has caused them to hemolyze. Free hemoglobin is excreted from the blood into the urine in rare cases, such as transfusion reactions.

Hematuria is defined as the presence of red blood cells in urine. In contrast, hemoglobinuria is defined as the presence of free hemoglobin. Most urine containing red blood cells will also contain some hemolyzed occult blood. Thus, the differentiation of only trace amounts of blood as cells versus free hemoglobin is of little interest, although the detection of intact cells can prevent false negative blood results.

Expected Values
Normally, there is no detectable amount of occult blood present in urine, even with very sensitive chemical methods.

Clinical Significance
The presence of blood in urine, as indicated by a positive test for occult blood, most likely indicates bleeding in the urinary tract. This may occur in a variety of kidney disorders, infectious disease, neoplasms, or trauma affecting any part of the urinary tract. Free hemoglobin is likely to be found in any of the above disorders. Free hemoglobin may also indicate transfusion reaction, hemolytic anemia, or paroxysmal hemoglobinuria. It may also appear in various poisonings, or following severe burns. A positive chemical test without the presence of red blood cells may indicate myoglobinuria as a result of traumatic muscle injury.

Method of Determination
The urinalysis test strip method is the simplest and most direct test for the presence of blood in urine. The blood reagent area on Siemens urinalysis test strips is impregnated with tetramethylbenzidine and buffered organic peroxide. The composition forms a green to dark blue compound when hemoglobin catalyzes the oxidation reaction of tetramethylbenzidine with a peroxide. Development of green spots indicates non-hemolyzed (intact) erythrocytes.

If read visually, the Siemens urinalysis test strips are compared with a color chart 60 seconds after the strip is dipped into the urine. The color ranges from orange through green, indicating negative, non-hemolyzed trace, non-hemolyzed moderate, hemolyzed trace, small (1+), moderate (2+), and large (3+) amounts of blood. Most urinalysis test strips with four or more reagent areas include a test for occult blood (see Appendix I). The test is usually capable of detecting 0.015 to 0.062 mg/dL of free hemoglobin, which is equivalent to 5 to 20 intact red blood cells per microliter of urine.

Further discussion on determination of red blood cells in urine with microscopic examination of urine sediment is found in Chapter 8.

Proteins
Approximately one-third of normal urinary protein is albumin, which appears to be identical to serum albumin. However, the majority of normal proteins in the urine are globulins, primarily alpha-1 and alpha-2 globulins, with smaller amounts of beta and gamma globulins.

Urine globulins have lower molecular weight than the corresponding serum globulins, but are closely related antigenically. Trace quantities of other proteins may also be found in most urine. A high-molecular-weight mucoprotein, the Tamm-Horsfall protein (described in Chapter 2), occurs in normal urine in quantities up to 2.5 mg/dL. In nephrosis, this protein may occur in higher concentrations. It is not found in plasma and is thought to originate in the kidneys.
Bence Jones protein is a specific, low-molecular-weight protein excreted in the urine of >50% of people with multiple myeloma. It is also found in the urine of many people with macroglobulinemia. This protein represents a portion of the high-molecular-weight plasma myeloma globulin. It is different from all other urinary proteins in that it coagulates on heating to temperatures between 45° and 60°C and then dissolves when further heated to the boiling point.

The determination of proteins in urine is made difficult by the variability of urine excretion. The volume of urine excreted can be highly variable depending mainly on the individual’s fluid intake and physical activity. In dilute urine, the protein excretion may be underestimated. If the urine is concentrated, as frequently occurs after strenuous physical activity, an increased protein concentration could be misinterpreted.

To avoid this problem, accurately timed 24-hour urine specimens have been used to express protein excretion in units of Kg/min. However, 24-hour urine specimens are difficult to accurately collect, necessitating a means to correct for urine concentration and/or volume when the collection accuracy is in doubt. Urinary creatinine excretion is now being used for this purpose, as the ratio of albumin-to-creatinine or protein-to-creatinine permits the estimation of the 24-hour protein excretion. (See Creatinine and P:C Ratio later in this Chapter.)

Expected Values

Normally, between 40 and 80 mg of protein are excreted daily, but as much as 100 to 150 mg per day may be considered normal. Since the average daily urine volume may range from 1,000 to 1,500 mL, the average normal concentration of protein in the urine varies from 2 to 8 mg/dL. This wide range of normal values is the result of biological variations and differences in the methods used for the determination of protein.

Albuminuria is indicated at 20 mg/dL albumin and may be detected in people with kidney damage either early or late in the course of the disease.

Clinical Significance

Proteinuria refers to an increased amount of protein in the urine and is one of the most important indicators of kidney disease. Detection of protein in the urine, combined with the microscopic examination of the urinary sediment, forms the basis of the differential laboratory diagnosis of kidney disorders. Proteinuria may, at times, reflect urinary tract or physiological conditions rather than intrinsic kidney disorders.

The types of proteins excreted in disease states are typically related to serum proteins. In fact, in severe cases, they are the serum proteins. Smaller proteins, such as albumin and alpha-1 globulin, are excreted more readily than larger proteins. Albumin constitutes between 60% and 90% of protein excreted in most disease states. Certain diseases are characterized by the excretion of specific globulins rather than by a diffuse proteinuria. The urine of people with multiple myeloma contains increased amounts of a low molecular weight globulin (Bence Jones protein). Table VI lists some important proteins, along with conditions in which they may be found.

Proteinuria depends on the precise nature of the clinical and pathological disorder and upon the severity of the specific disease. Proteinuria may be intermittent or continuous. Transient, intermittent proteinuria is usually caused by physiological or functional conditions rather than by kidney disorders.

### Table VI. Proteins in Urine

<table>
<thead>
<tr>
<th>Protein</th>
<th>Conditions(s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Albumin</td>
<td>Glomerulonephritis</td>
</tr>
<tr>
<td></td>
<td>Strenuous Physical Exercise</td>
</tr>
<tr>
<td></td>
<td>Emotional Stress</td>
</tr>
<tr>
<td></td>
<td>Pregnancy</td>
</tr>
<tr>
<td></td>
<td>Infections</td>
</tr>
<tr>
<td></td>
<td>Newborns (first week)</td>
</tr>
<tr>
<td>Globulins</td>
<td>Glomerulonephritis</td>
</tr>
<tr>
<td></td>
<td>Tubular Dysfunction</td>
</tr>
<tr>
<td>Hemoglobin</td>
<td>Hematuria</td>
</tr>
<tr>
<td></td>
<td>Hemoglobinuria</td>
</tr>
<tr>
<td>Fibrinogen</td>
<td>Severe Kidney Disease</td>
</tr>
<tr>
<td>Nucleoproteins</td>
<td>WBCs in Urine</td>
</tr>
<tr>
<td></td>
<td>Epithelial Cells in Urine</td>
</tr>
<tr>
<td>Bence Jones</td>
<td>Multiple Myeloma</td>
</tr>
<tr>
<td></td>
<td>Leukemia</td>
</tr>
</tbody>
</table>

Marked proteinuria is characterized by the excretion of more than 4 grams of protein per day. This is typical with nephrotic syndrome, and severe cases of glomerulonephritis, nephrosclerosis, amyloid disease, and systemic lupus erythematosus. It also can occur with severe venous congestion of the kidney produced by renal vein thrombosis, congestive heart failure, or constrictive epicarditis.

Moderate proteinuria refers to the daily excretion of between 0.5 and 4 grams of protein. It is found in the vast majority of kidney diseases, as well as all of the disorders listed above. It is also found in chronic glomerulonephritis; diabetic nephropathy; multiple myeloma; toxic nephropathy; and pre-eclampsia, as well as inflammatory, malignant, degenerative, and irritative conditions of the lower urinary tract, including the presence of calculi.

Minimal proteinuria is the excretion of less than 0.5 gram of protein per day. It is associated with chronic glomerulonephritis, polycystic disease of the kidneys, renal tubular disorders, the healing phase of acute glomerulonephritis, latent or inactive stages of glomerulonephritis, and various disorders of the lower urinary tract.

Postural proteinuria is excretion of protein by people who are in an upright position. The proteinuria is intermittent and disappears when the individual lies down. The daily protein excretion is usually less than 1 gram. Postural proteinuria, which occurs in 3% to 5% of healthy adults, may be differentiated from other forms of proteinuria by testing for protein in urine specimens collected before and after the individual has been in an upright position. The patient voids and discards his urine at bedtime.
He collects a urine specimen immediately after awakening and before he is upright for more than one minute. He collects another specimen after he has remained upright or walking for at least 2 hours. The first specimen should contain no protein. The second will be positive if the patient has postural proteinuria.

Functional proteinuria is protein excretion associated with fever, exposure to heat or cold, excessive exercise, or emotional stress. The underlying physiological mechanism that induces proteinuria in all of these situations is renal vasoconstriction.

**Method of Determination**
A number of simple, semi-quantitative tests and more complex quantitative tests are available for the determination of all proteins in urine. Specific methods are used for the detection and quantification of albumin, globulins, Bence Jones protein, and others. The majority of these methods, with the notable exception of the simple colorimetric reagent strip test, depend on the precipitation of protein as the basis for quantitative determinations.

**Colorimetric Reagent Strip Test**
The colorimetric reagent strip test is based on the ability of proteins to alter the color of some acid-base indicators without altering the pH known as the "protein error of indicators" principle. When an indicator, such as tetrabromphenol blue, is buffered at pH 3, it is yellow in solution without protein but, in the presence of protein, the color will change to green and then to blue with increasing protein concentrations.

The Siemens Albustix® urinalysis test strip contains a single protein test area. This area consists of a small square of absorbent paper impregnated with a buffered solution of tetrabromphenol blue. Most urinalysis test strips with multiple parameters, from Uristix® to Multistix 10 SG, and including the CLINITEK Atlas reagent, contain an area for protein determination, along with test areas for other urinary constituents. In visual reading, protein is determined simply by dipping the strip into well-mixed uncentrifuged urine and comparing the resulting color with the chart provided on the reagent strip bottle.

The protein results on Siemens urinalysis test strips are reported as negative (yellow color), trace, or 1+ to 4+ positive range. Trace readings may detect 15 to 30 mg/dL of protein. Plus (+) readings approximate protein concentrations of 30, 100, 300, and over 2,000 mg/dL, respectively. These readings are reliable indicators of increasingly severe proteinuria. Albumin reacts with the indicator more strongly than do the other proteins. Highly buffered, alkaline urine may give false positives when the buffer system in the reagent area is overcome.

A creatinine test pad on the Siemens Multistix PRO® and CLINITEK® Microalbumin urinalysis test strips is used to calculate P:C and A:C ratios, respectively, as a means of improving strip result correlation to actual analyte excretion rates. The ratio allows for the use of single-void specimens in the discrimination of normal and abnormal levels of protein. More information on the creatinine, P:C, and A:C ratios can be found later in this chapter.

*Bence Jones Protein Determination*
While any significant amount of Bence Jones protein will be detected by colorimetric or turbidimetric screening tests, the simplest method for screening is gradual heating of a urine specimen to the boiling point. When this protein is present, a precipitate will first appear, then dissolve as the urine is further heated. The presence of large amounts of other proteins or phosphates decreases the accuracy of this test. These interfering proteins can usually be removed by cooling the heated urine to room temperature, filtering, and repeating the heating process on the filtrate.

**Creatinine**
Creatinine is derived from the non-enzymatic dehydration of creatine in skeletal muscle. The amount of creatine per unit of muscle mass is constant, and thus the breakdown of creatine to creatinine is constant in health. It is this consistency that has enabled creatinine to be used to correct for urine concentration and/or volume when the collection accuracy of a 24-hour urine specimen for protein excretion is in question.

Recently, a creatinine test pad was developed for Siemens Multistix PRO and CLINITEK Microalbumin urinalysis test strips that improves strip result correlation to actual protein excretion rates. The creatinine test pad enables technicians to determine the P:C or A:C ratios, which allow for the use of single-void specimens in the discrimination of normal and abnormal levels of protein. Testing for the A:C ratio on random urine samples has been found to be as valid an indicator of microalbuminuria as a timed 24-hour sampling.

**Expected Values**
The normal creatinine concentration in adults is 0.6 to 2.0 g of creatinine per day (strip results of approximately 50 to 200 mg/dL), with men having higher values than women due to muscle mass. Random urines may have strip results that vary from 10 to 300 mg/dL. Concentrated urines from dehydrated individuals, or first-morning specimens, will typically have elevated concentrations (strip results of >200 mg/dL). Diuresis will typically result in lower concentrations (strip results of <50 mg/dL).

A P:C ratio of 150 mg/g is considered abnormal and should prompt the physician to evaluate the patient for early kidney disease. Values >300 mg/g are indicative of clinical proteinuria.

**Clinical Significance**
The value of the creatinine test is that it can be used to calculate a P:C ratio that corrects for varying urine concentrations. It provides meaningful results from random samples and minimizes the need for inconvenient timed or 24-hour urine samples. Creatinine is reported as a separate result for quality control purposes.
Method of Determination
The colorimetric urinalysis test strip is based on the peroxidase-like activity of copper-creatinine complexes. With 3,3',5,5'-tetramethylbenzidine (TMB) and disopropyl benzene dihydroperoxide (DBDH), the peroxidase-like activity of copper-creatinine complexes is measured.

Albumin-to-Creatinine (A:C) Ratio
Testing for microalbuminuria (low levels of albumin in the urine) can help physicians detect early kidney damage in people with diabetes, and is recommended by the American Diabetes Association.

Figure 4. A flow chart by the American Diabetes Association that can be used as a guide to microalbuminuria testing.

Expected Values
Albuminuria and microalbuminuria both refer to the presence of albumin in the urine. The difference between the two is in the quantity of albumin detected.

Microalbuminuria is indicated at 20-200 mg/L albumin per urine sample. It describes the very low levels of albumin found in the urine in the absence of other clinical signs or symptoms of kidney damage, and is a good indicator of early kidney damage, especially in people with diabetes.

An A:C ratio of 30-300 mg/g (SI units = 3.4-33.9 mg/mmol) is abnormal, and defined as microalbuminuria. An A:C ratio of >300 mg/g (SI units >33.9 mg/mmol) is defined as albuminuria.

Clinical Significance
Microalbuminuria is an accepted early marker for kidney damage in groups such as those with diabetes. Microalbuminuria constitutes the critical factor in the routine test for glomerular kidney damage, especially in the case of hypertension and diabetes.

If the microalbuminuria test is positive, it is recommended that the test be repeated within six to eight weeks. A positive test result should be defined as two out of the three tests being positive to assure persistent rather than intermittent positive results.

Method of Determination
Siemens urinalysis product, the CLINITEK Microalbumin Reagent Strips for Urinalysis, are available for microalbuminuria testing (using the Siemens CLINITEK urine chemistry analyzers) based on bis (3',3"-diido-4',4"-dihydroxy-5',5"-dinitrophenyl)-3,4,5,6-tetram bromosulfonphthalein (DIDNTB). This dye has the chemical sensitivity to detect microalbuminuria (20-200 mg/L).

The A:C ratio is calculated from the albumin and creatinine results. CLINITEK urine chemistry analyzers calculate the results automatically.

Protein-to-Creatinine (P:C) Ratio
Testing for proteinuria and obtaining a P:C ratio result is a useful tool for the early detection of kidney disease in people with diabetes and hypertension, and people at general risk for kidney disease.

Expected Values
Clinical proteinuria is indicated at a P:C ratio result of >300 mg/g or SI units of 33.0 mg/mmol. A "normal" ratio result indicates that the P:C ratio of the sample is below the cutoff.

A "normal dilute" ratio result indicates that the sample may be too dilute to reliably detect protein. If a "normal dilute" result is obtained, consider recollecting the sample, preferably a first-morning collection to obtain a more concentrated sample.
Clinical Significance
The P:C ratio result is adjusted for varying urine concentration, therefore, an abnormal P:C ratio is an indicator of kidney disease. The patient should be tested with a quantitative method and if the abnormal result is confirmed, a nephrologist or other specialist should be consulted.

Method of Determination
Siemens Multistix PRO Reagent Strips for Urinalysis,* is available for semi-quantitative measurement of the P:C ratio which is calculated from the protein and creatinine results. Siemens CLINITEST urine chemistry analyzers calculate the ratios automatically.

Siemens Multistix PRO Reagent Strips for Urinalysis report the protein and creatinine results and the P:C ratio and provide clinicians with a convenient and efficient manner to obtain all results simultaneously.

Glucose
Glucose is the sugar most commonly found in urine although other sugars, such as lactose, fructose, galactose, and pentose, may also be found under certain conditions.

Expected Values
Small amounts of glucose are normally excreted into the urine. This level is normally below the sensitivity of the test, but on occasion may produce a color that is between the negative color block and 100 mg/dL color block. Certain individuals have a reduced kidney threshold that results in glucose being excreted into the urine when the blood glucose level has not reached the "normal" kidney threshold level of approximately 180 mg/dL. In these cases, physiologically normal blood glucose levels can result in glucose being found in the urine.

Clinical Significance
The presence of detectable amounts of glucose in urine is known as glycosuria. Glycosuria occurs whenever the blood glucose level exceeds the reabsorption capacity of the kidney tubules (kidney threshold); that is, when the glomerular filtrate contains more glucose than the tubules are able to reabsorb. The condition may be either benign or pathological; the physician must distinguish between the two types.

Diabetes mellitus, a pathological state, is the chief cause of glycosuria. This condition is associated with a marked elevation of blood glucose and usually an increase in urine volume. The glucose content of diabetic urine may reach as high as 10% (10,000 mg/dL), though 2-5% (2,000-5,000 mg/dL) is more common.

Kidney glycosuria occurs with normal blood glucose levels when tubular reabsorption of glucose is below normal, thus permitting some glucose to spill into the urine. This is a benign condition, as is the occurrence of glycosuria after eating a heavy meal or in conjunction with emotional stress.

Method of Determination
While there are various glucose urine tests, two are most frequently used:

- Enzymatic tests based on the action of glucose oxidase on glucose
- Reduction tests based on the reduction of certain metal ions by glucose

Enzymatic Tests
Enzymatic glucose oxidase tests, as applied to urine, are specific for glucose. In these tests, glucose oxidase catalyzes the oxidation of glucose to gluconic acid and hydrogen peroxide. The peroxide, in the presence of peroxidase, oxidizes an indicator that produces a color change. Other sugars, such as lactose, fructose, galactose, and pentose, are not substrates for glucose oxidase and, therefore, do not react with this test.

The glucose reagent area on Siemens urinalysis test strips (i.e., Multistix family) contain a glucose oxidase test system that is specific for glucose. In practice, the strips are dipped into the urine sample; then the edge of the strip is run against the rim of the urine container to remove excess urine. The resulting glucose color reaction is compared to a six-block color chart ranging from blue, indicating less than 0.1% concentration of glucose, to brown, indicating 2.0% or more.

Reduction Tests
The reduction of metallic ions, such as Cu++, is non-specific for glucose because the reaction may be initiated by any reducing substance present in the urine, such as large quantities of creatinine, uric acid, ascorbic acid, or some other reducing sugar. While non-carbohydrate components seldom interfere with results, some interference occasionally occurs in concentrated urine. The non-specificity of this test has advantages and disadvantages. The advantage is that it detects important sugars, such as galactose and lactose; the disadvantage is that it detects reducing substances other than glucose.

The copper reduction test has been greatly simplified by Bayer Clinistest® Reagent Tablets, which consist of copper sulfate compounded into an effervescent tablet containing sodium carbonate, citric acid, and sodium hydroxide. When the tablet is added to a small test tube containing 10 drops of water and 5 drops of urine, it dissolves and produces carbon dioxide and heat. In the process, if a reducing substance such as glucose is present, the color changes from blue to orange, depending on the amount of sugar present. The amount of reducing substance in the urine can be estimated by comparing the color produced with the reference color chart. The 2-drop method is the same as above except that 2 drops of urine are used and the results are compared to a special 2-drop color chart that expands the color range from 2% to 5% (2,000-5,000 mg/dL).

* Available in the U.S. and Japan
Clinitest tablets are sensitive to approximately 0.25% (250 mg/dL) glucose in urine. Because it is somewhat less sensitive than Benedict’s reagent, it produces fewer false positive reactions.

**Non-Glucose Reducing Sugars**

**Galactose**
Galactose is found in the urine of infants afflicted with galactosemia. These children are deficient in the enzyme necessary for converting galactose into glucose. While this is a severe condition, it can be treated by eliminating lactose and other sources of galactose from the diet. If not treated properly, the infant will rapidly deteriorate physically and mentally, and early death will result.

Galactose can be detected with Clinistix tablets and identified by paper chromatography and by the galactose oxidase test. Some pediatricians screen infants for galactosemia using Clinistix tablets and Clinistix urinalysis test strips to detect the presence of non-glucose reducing substances (positive Clinistix tablet, negative Clinistix urinalysis test strip).

**Lactose**
Lactose in urine is usually considered physiological rather than pathological. The sugar may appear in the urine of lactating women. This is usually a temporary condition, which corrects itself when lactation ends. Children and adults who are deficient in intestinal lactase may also excrete lactose.

Lactose in urine can be detected by Clinistix tablets. Identifying the sugar as lactose, although not a routine procedure, can be done by paper chromatography.

**Fructose**
Fructose sometimes occurs in the urine of people with hepatic disorders. It can be detected with Clinistix tablets. It can be identified by Selivanoff’s test and by paper chromatography, neither of which is a routine procedure.

**Pentose**
Pentosuria is associated with certain types of drug therapy and with some hereditary conditions. In both cases, its presence in urine is considered benign. It can be detected with Clinistix tablets. Identification of a pentose can be accomplished by chromatography.

**Ketone**
Naturally, the body completely metabolizes fats to carbon dioxide and water. Whenever there is inadequate carbohydrate in the diet or a defect in carbohydrate metabolism or absorption, however, the body metabolizes increasing amounts of fatty acids. When this increase is large, fatty acid utilization is incomplete, causing intermediary products of fat metabolism to appear in the blood and be excreted in the urine. These intermediary products are the three ketone bodies: acetoacetic acid (which is also called diacetic acid), acetone, and beta-hydroxybutyric acid. Acetone and beta-hydroxybutyric acid are derived from acetoacetic acid. All three ketone bodies are present in the urine of people with ketonuria in the relative proportions of 20% acetoacetic acid, 2% acetone, and 78% beta-hydroxybutyric acid.

**Expected Values**
Detectable levels of ketones may occur in urine during physiological stress conditions such as fasting, pregnancy, and frequent strenuous exercise. In ketoacidosis, starvation, or with other abnormalities of carbohydrate or lipid metabolism, ketones may appear in urine in large amounts before serum levels are elevated. High protein/low carbohydrate diets are also known to produce ketones in the urine.

**Clinical Significance**
Diabetes mellitus is the most important disorder in which ketonuria occurs. Diabetes mellitus is a disorder of glucose metabolism, and in insulin-deficient diabetes (usually the juvenile-onset type) glucose metabolism is so impaired that fatty acids are utilized to meet the body’s energy requirements. When diabetes is untreated or inadequately treated, excessive amounts of fatty acids are metabolized, resulting in the accumulation of ketone bodies in the blood (ketosis), which are excreted in urine (ketonuria).

Ketone bodies are excreted in combination with normal basic ions, leading to a reduction in the carbon dioxide-combining power and causing systemic acidosis. Progressive diabetic ketosis is the cause of diabetic acidosis, which can eventually lead to coma and even death. (The term ketoacidosis is frequently used to designate the combined ketosis and acidosis of diabetes.)

Detection of ketonuria in people with diabetes mellitus may suggest the need to change the patient’s insulin dosage or implement other management procedures. Thus, during periods of acute infections, surgery, gastrointestinal disturbances, or other stress, and whenever the management routine does not adequately control the disease, the urine of people with diabetes should be checked for the presence of ketone bodies.

Ketonuria also accompanies the restricted carbohydrate intake that occurs in association with fevers, anorexia, gastrointestinal disturbances, fasting, starvation, cyclic vomiting, puerperal vomiting of pregnancy, and cachexia. It also occurs following anesthesia and as a result of certain neurological disorders.

**Method of Determination**
In ketonuria, acetoacetic acid, acetone and beta-hydroxybutyric acid are all excreted in the urine. Consequently, a general test procedure that indicates the presence of one of these components is usually satisfactory for the diagnosis of ketonuria. While specific tests do exist for the determination of each of these substances, they are not widely used because they tend to be more cumbersome, less reliable, and less sensitive.
Nitroprusside Reactions

Nitroprusside usually reacts with both acetone and acetoacetic acid in the presence of alkali to produce a purple-colored compound. This forms the basis of a number of different tests.

The urinalysis test strip method is the simplest technique for determination of ketonuria. The ketone reagent area on Siemens urinalysis test strips is impregnated with sodium nitroprusside and alkaline buffers. The strip is dipped into fresh urine, then the underside of the strip is dragged along the inside of the container to remove excess urine. The reagent pad color is compared to the color chart after exactly 15 seconds. The chart has six color blocks ranging in color from buff to lavender and maroon and indicating negative, trace (5 mg/dL), small (15 mg/dL), moderate (40 mg/dL), or large (80 mg/dL) and (160 mg/dL) concentrations of ketone. The test is sensitive only to acetoacetic acid. It does not react with betahydroxybutyric acid or acetone. Compounds that contain sulfhydryl groups, such as mesna (2-mercaptoethane sulfonic acid) may cause false positive results or an atypical reaction.

Bilirubin

Bilirubin in the urine indicates the presence of hepatocellular disease or intra- or extra-hepatic biliary obstruction. It is an early sign of these disorders and, therefore, a useful diagnostic tool.

Bilirubin is formed by the breakdown of hemoglobin in the reticuloendothelial cells of the spleen and bone marrow. It is linked to albumin in the bloodstream and transported to the liver. This albumin-bound form, which is also known as indirect bilirubin, is insoluble in water and does not appear in the urine. In the liver cells, it is separated from the albumin and conjugated with glucuronic acid to form water-soluble conjugated bilirubin, also known as direct bilirubin. The liver cells that form the conjugated bilirubin excrete it into the bile, where it is then excreted into the intestinal tract through the bile duct. This conjugated bilirubin in the intestinal tract is converted by bacterial action to urobilinogen. Being water-soluble, conjugated bilirubin can be excreted by the kidneys, although normally its level in the blood is not high enough to cause significant amounts to appear in the urine.

Expected Values

Bilirubin appears in the urine at a concentration of approximately 0.02 mg/dL, reflecting the normally low blood levels of conjugated bilirubin. This amount is not detected by routine qualitative or semi-quantitative techniques.

Clinical Significance

Bilirubin excretion in the urine will reach significant levels in any disease process that increases the amount of conjugated bilirubin in the bloodstream. In some liver diseases due to infectious or hepatotoxic agents, liver cells are unable to excrete all of the conjugated bilirubin into the bile. Therefore, sufficient amounts are returned to the blood to elevate blood levels and cause significant bilirubinuria.

In obstructive biliary tract disease, biliary stasis interferes with the normal excretion of conjugated bilirubin via the intestinal tract. This causes a buildup in the bloodstream with resulting bilirubinuria. Since bilirubin may often appear in the urine before other signs of liver dysfunction (jaundice, clinical illness) are apparent, bilirubinuria is an important diagnostic sign of liver disease and a bilirubin test should be part of every routine urinalysis.

An increase in the amount of unconjugated bilirubin in the circulation will not change the amount of bilirubin excreted in the urine. (Unconjugated bilirubin is not found in the urine because it is not water-soluble and, therefore, cannot be excreted by the kidneys.) This increase of unconjugated bilirubin in the circulation occurs in hemolytic anemias because the greater release of hemoglobin leads to greater production of albumin-bound bilirubin. However, a normal, non-diseased liver can conjugate all the excess bilirubin and excrete the entire amount into the biliary tract.

Bilirubin is an unstable compound that disappears from urine on standing, especially if exposed to light. It is very important that urine be tested for bilirubin as soon after excretion as possible. The instability and reactivity of bilirubin and its derivatives are shown in Table VII.

Table VII. Bilirubin in Urine

<table>
<thead>
<tr>
<th>Glucuronide – Bilirubin – Glucuronide</th>
<th>Soluble and Reactive</th>
</tr>
</thead>
<tbody>
<tr>
<td>On Standing:</td>
<td>Hydrolysis: Glucuronide + Free Bilirubin</td>
</tr>
<tr>
<td>Glucuronide + Glucuronide -&gt; Biliverdin</td>
<td>Oxidation: Glucuronide + Biliverdin</td>
</tr>
</tbody>
</table>

Method of Determination

The bilirubin reagent area on Siemens Multistix 10 SG Reagent Strips for Urinalysis and all other Siemens urinalysis test strips, including the CLINITEST Atlas reagent, is the simplest test for the determination of bilirubin. The reagent area is impregnated with stabilized, diazotized 2, 4-dichloroaniline which reacts with bilirubin in urine to form a brownish-to-purple-colored azobilirubin compound. The urinalysis test strip is dipped into fresh, uncentrifuged urine, tapped to remove excess urine, and, after a 30-second wait, compared to the color chart on the urinalysis test strip bottle. The results are interpreted as negative or small (+), moderate (++), or large (+++) amounts of bilirubin. The test has a sensitivity of 0.4 to 0.8 mg/dL bilirubin.
All positive reactions, or atypical reactions, need to be confirmed by using Siemens Icotest® Reagent Tablets. Icotest tablets have a sensitivity of 0.05-0.1 mg/dL and are more specific for urinary bilirubin than results obtained with any of the urinalysis test strips.

Icotest Reagent Tablets contain 2, 4-dichlorobenzzenediazonium tetrachlorozincate, sodium bicarbonate, and sulfoisalicylic acid.

The procedure is:

1. Place 10 drops of urine on one special test mat. If bilirubin is present in the specimen, it will be adsorbed onto the mat surface.

2. Place an Icotest Reagent Tablet on the moistened area of the mat.

3. Flow two drops of water over the tablet.

4. When elevated amounts of bilirubin are present in the urine specimen, a blue to purple color forms on the mat within 60 seconds. The rapidity of the formation of the color and the intensity of the color development are proportional to the amount of bilirubin in the urine. Normal amounts of bilirubin in the urine give a negative result. The smallest concentration of bilirubin reliably detected by this method is 0.05 to 0.1 mg/dL. An orange to red color may indicate the presence of Pyridium metabolites or azo dyes from other drugs.

These convenient, more specific tests have replaced the older oxidation procedures.

**Urobilinogen**

Conjugated bilirubin, excreted by the liver into the bile, is excreted into the intestinal tract through the bile duct. Bacterial action in the intestinal tract converts the bilirubin to a group of compounds known as urobilinogen. It is estimated that as much as 50% of the urobilinogen formed in the intestines is reabsorbed into the portal circulation and re-excreted by the liver. Small amounts are normally excreted in the urine, but the major excretion is in the feces.

**Expected Values**

Normally, between 1 and 4 mg (1 to 4 Ehrlich units) of urobilinogen is excreted in urine in a 24-hour period. The concentration of urobilinogen in a random normal urine is 0.1 to 1.0 Ehrlich unit/dl (1 EU/dL=1 mg/dL).

**Clinical Significance**

Urinary urobilinogen is increased by any condition that causes an increase in the production of bilirubin and by any disease that prevents the liver from normally removing the re-absorbed urobilinogen from the portal circulation. It is also increased whenever there is excessive destruction of red blood cells, as in hemolytic anemias, pernicious anemia, and malaria, as well as with infectious hepatitis, toxic hepatitis, portal cirrhosis, or congestive heart failure.

Determination of urinary urobilinogen is a useful procedure in routine urinalysis since it serves as a guide in detecting and differentiating liver disease, hemolytic disease, and biliary obstruction. Sequential determinations also assist in evaluating progress of the disease and response to therapy.

Urine urobilinogen is decreased or absent when normal amounts of bilirubin are not excreted into the intestinal tract. This usually indicates partial or complete obstruction of the bile ducts, such as may occur in cholelithiasis, severe inflammatory disease, or neoplastic disease. Also, during antibiotic therapy, suppression of normal intestinal flora may prevent conversion of bilirubin to urobilinogen, leading to an absence of urobilinogen in urine.

More comprehensive information is obtained when the physician can correlate test results for both bilirubinuria and urobilinogenuria. As indicated in Table VIII, the two findings, considered together, provide more helpful information for differential diagnosis than either finding alone.

**Table VIII. Urobilinogen and Bilirubin Tests**

<table>
<thead>
<tr>
<th></th>
<th>Healthy</th>
<th>Hemolytic Disease</th>
<th>Hepatic Disease</th>
<th>Biliary Obstruction</th>
</tr>
</thead>
<tbody>
<tr>
<td>Urine Urobilinogen</td>
<td>Normal</td>
<td>Increased</td>
<td>Increased</td>
<td>Low or Absent</td>
</tr>
<tr>
<td>Urine Bilirubin</td>
<td>Negative</td>
<td>Negative</td>
<td>Positive or Negative</td>
<td>Positive</td>
</tr>
</tbody>
</table>

**Method of Determination**

The urobilinogen reagent area of Siemens Multistix 10 SG Reagent Strips for Urinalysis and all other Siemens urinalysis test strips, including the CLINITEK Atlas reagent, is impregnated with para-diethylaminobenzaldehyde and an acid buffer solution. It reacts with urinary urobilinogen, porphobilinogen, and para-aminosalicylic acid to form colored compounds. A freshly voided urine specimen is necessary for the test, preferably a sample collected over a two-hour period in the early afternoon when urinary urobilinogen excretion is thought to be at the highest rate for the day. The strip is dipped into fresh, uncentrifuged urine, collected without preservatives. It is then removed and, after exactly 60 seconds, the color reaction is compared to the color chart.

The five color blocks provided on the Siemens urinalysis color chart range from peach to pink, representing 0.2, 1, 2, 4, and 8 mg/dL. The first two color blocks, 0.2 and 1 mg/dL, are within the normal range of values for urobilinogen. The remaining three color blocks indicate elevated values. This test will detect the absence of urobilinogen.

Formaldehyde, which may be used as a preservative, can inhibit this reaction, causing falsely lowered results. No other substances are known to clearly inhibit the reaction. Drugs containing azo dyes will have a masking effect on the urobilinogen area.
**pH**
The kidneys and the lungs are the two major organs that regulate the acid-base balance of the body. The lungs excrete carbon dioxide, while the kidneys regulate the excretion of the non-volatile acids produced by the normal metabolic processes of the tissues.

Urine's acidity is due primarily to acid phosphates, with only a minor portion contributed by organic acids such as pyruvic, lactic, and citric acids. These acids are excreted in the urine as salts, primarily sodium, potassium, calcium, and ammonium salts. The kidney regulates the selective excretion of the various cations in order to maintain normal acid-base balance. This is accomplished primarily through the reabsorption of a variable amount of sodium ion by the tubules and the tubular secretion of hydrogen and ammonium ions in exchange. The acidity of urine increases, as the amount of sodium retained by the body increases.

**Expected Values**
Urine pH is a measure of its hydrogen ion concentration. A pH below 7 indicates acid urine. A pH above 7 indicates alkaline urine. Normal kidneys are capable of producing urine that can vary from a pH of 4.5 to slightly higher than 8.0. Freshly voided urine from people not on special diets is acidic and has a pH of about 6.0.

**Clinical Significance**
Kidney stone formation depends significantly on the pH of urine. Phosphate and calcium carbonate stones develop in alkaline urine, while uric acid, cystine, and calcium oxalate stones precipitate in acid urine.

**Acidic Urine**
People on high protein diets may excrete urine with a pH lower than 6.0. Certain medications such as ammonium chloride and mandelic acid may also produce acidic urine. People with acidosis and/or uncontrolled diabetes mellitus also excrete highly acidic urine.

**Alkaline Urine**
Alkaline urine is frequently excreted after meals as a normal response to the secretion of HCl in the gastric juice. It also occurs in individuals consuming diets high in vegetables, milk and other dairy products. Certain medicines, such as sodium bicarbonate, potassium citrate, and acetazolamide, also induce the formation of alkaline urine.

Highly alkaline urine may be indicative of a urinary tract infection or possible bacterial contamination of an old specimen with urea-splitting organisms. Antibiotics (such as neomycin, kanamycin, and streptomycin) are highly effective in the treatment of urinary tract infections in alkaline reaction.

Salicylate excretion is also enhanced by alkalinity.

**Kidney Tubular Acidosis**
Kidney tubular acidosis is a specific disease of the kidneys in which the kidney tubules are unable to adequately excrete hydrogen ions although severe systemic acidosis is present within the body. The urine pH of these people usually remains approximately neutral and never falls below pH 6.0. A similar defect in hydrogen ion excretion occurs in the Fanconi syndrome.

**Method of Determination**
The accurate measurement of urinary pH can only be done on freshly voided specimens, as urine may become alkaline upon standing due to loss of carbon dioxide and the conversion of urea into ammonia by certain bacterial organisms. Urine samples that will not be tested within one hour should be refrigerated.

For routine analysis, urinary pH may be measured with urinalysis test strips and a color chart. The Siemens urinalysis test strips are dipped into the urine specimen, and the color change is compared to a standardized color chart on the bottle label, which shows the pH values ranging from 5 through 8.5. When more exact determinations are needed, a pH meter should be used.

Since urine pH is almost always measured as a part of the more complete urinalysis, it is advantageous to use a urinalysis test strip with multiple parameters, such as Siemens Multistix 10 SG Reagent Strips for Urinalysis, that simultaneously measures pH and checks the urine for several other components. The pH portion of these strips is impregnated with two separate indicators, methyl red and bromthymol blue. These chemicals provide a wide spectrum of color changes, from orange to green to blue, in the pH range of 5 to 8.5.

**Specific Gravity**
The specific gravity of urine indicates the relative proportions of dissolved solid components to the total volume of the specimen. The measurement is necessary to interpret most routine urinalysis tests. The measurement reflects the relative degree of concentration or dilution of the specimen, which, under normal circumstances, correlates to the concentrating and diluting abilities of the kidney.

**Expected Values**
Urine specific gravity ranges from 1.003 to 1.030, but usually falls between 1.010 and 1.025. Specific gravity is highest in the first-morning specimen, with levels typically greater than 1.020. A specific gravity of 1.025 or above in any random urine specimen indicates normal concentrating ability.

**Clinical Significance**

**Low Specific Gravity**
Diabetes insipidus is a disease characterized by large volumes of urine with low specific gravity. The disease, which is caused by impaired functioning of the anti-diuretic hormone, is the most obvious and severe example of the loss of effective concentrating ability. Specific gravity in these cases usually reads between 1.001 and 1.003.
Low specific gravity may also occur in people with glomerulonephritis, pyelonephritis, and various kidney anomalies. In these cases, tubular damage renders the kidney incapable of concentrating urine.

**High Specific Gravity**
Specific gravity is high in people with adrenal insufficiency, hepatic disease, and congestive cardiac failure. It is also elevated in cases where there has been excessive loss of water, as with sweating, fever, vomiting, and diarrhea.

Abnormally high amounts of some of the urinary constituents such as glucose, or the presence of X-ray contrast media may increase the specific gravity as measured by some procedures, such as the Total Solids (TS) Meter and the urinometer. However, the colorimetric strip test is not affected by such materials and thus gives a more clinically relevant specific gravity.

**Fixed Specific Gravity**
Urine with a fixed low specific gravity (approximately 1.010), which varies little from specimen to specimen, is known as isostenuric. This condition is indicative of severe kidney damage with disturbance of both the concentrating and diluting abilities of the kidney.

**Method of Determination**
Specific gravity indicates the density of the urine by measuring the total solids in urine. It is a number derived from the ratio of the weight of a given volume of urine to the weight of the same volume of water, under standardized conditions.

\[
\text{Specific Gravity} = \frac{\text{Weight of Urine}}{\text{Weight of Water}}
\]

Water has a specific gravity of 1.000. Since urine is a solution of minerals, salts, and organic compounds in water, the specific gravity is greater than 1.000.

**Colorimetric Method**
Dip-and-read strips are the most common method of estimating urine specific gravity. The chemical reaction of the specific gravity reagent area, on Siemens urinalysis test strips, involves three primary ingredients, which are impregnated into the reagent paper:

- A polyelectrolyte: polymethylvinyl ether/maleic acid, partially neutralized
- An indicator: bromthymol blue
- Buffers

The principle of the reaction is based on a pKₐ change of certain pre-treated polyelectrolytes in relation to the ionic concentration. In the specific gravity reagent area of the urinalysis test strips with multiple parameters, the polyelectrolyte (polymethylvinyl ether/maleic acid) is sensitive to the number of ions in the urine specimen. When the concentration of the electrolytes increases (high specific gravity) in the urine, the pKₐ of the polyelectrolyte in the urinalysis test strip is decreased. Thus, the pH decreases. The bromthymol blue indicator changes color from blue-green to green to yellow-green, indicating the pH change caused by increasing ionic strength (increasing specific gravity) and is empirically related to specific gravity values (Figure 5).

**Figure 5. Principle of Multistix 10 SG Reagent Strips**

Siemens Multistix 10 SG Reagent Strips for Urinalysis were not the first indirect method for measuring specific gravity, but they were the first method in a disposable, convenient urinalysis test strip format, and the first to be directly combined with the existing strips used in the clinical laboratory.

**Refractive Index**
Another indirect method of measuring specific gravity is refractometry. The American Optical® Total Solids (TS) Meter measures the refractive index of the solution. The refractive index varies with, but is not identical to, the specific gravity of urine.

Although the TS Meter measures the refractive index of a solution, scale readings have been calibrated in terms of specific gravity, refractive index, and total solids. The TS Meter requires daily calibration. The device, which compensates for temperatures between 60 and 100 degrees Fahrenheit, is scaled from 1.000 to 1.035 in increments of 0.001.
The CLINITEK Atlas Automated Urine Chemistry Analyzer uses a fiber optic refractive index method. Light is transmitted through a specially shaped fiber optic onto which the sample is dispensed; the amount of light passing through the fiber optic is constantly measured at one end. The closer the sample’s refractive index is to that of the fiber optic, the more light is lost from the optic. Since refractive index is proportional to the specific gravity, the light measured at the end of the fiber optic loop is linearly related to the specific gravity of the sample. Specific gravity results can be reported in 0.001 increments and are linear through 1.045 when compared to the TS Meter.

Urinometer
Probably the most widely known direct method for measuring specific gravity is to use the urinometer. The method requires a large urine volume, is cumbersome to perform, and may be the least accurate method, since it is often difficult to read, and calibration is often not maintained. However, it has served the laboratory well for many years.

The urinometer is a weighted, bulb-shaped instrument that has a cylindrical stem marked with a scale calibrated in specific gravity readings. This instrument floats in a cylinder containing urine. The depth to which it sinks in the urine indicates the specific gravity, which is read on the urinometer scale at the meniscus on the floating stem.

The device is calibrated to read 1.000 in distilled water at a specific temperature, which is indicated on each instrument. There is a change in the specific gravity of 0.001 for every change of 3°C above or below this temperature. Corrections are also recommended when glucose or protein is present. For every 1,000 mg/dL of glucose or protein, 0.003 should be subtracted from urinometer reading.

Falling Drop
This is a direct method of measuring specific gravity. The procedure is specific and, therefore, more accurate than refractometry and more precise than urinometers. These instruments use silicone-base oil with a controlled value of specific gravity and viscosity in a specially designed column. This column was developed to measure the time required for a precisely measured specimen drop to fall a distance defined by two optical gates (lamp-photo-transistor pairs) mounted one above the other in a temperature-controlled column filled with water-immiscible oil. The light beams from the lamps travel through the column oil and strike the phototransistors located on the opposite wall of the column. The specific gravity column is equipped with an overflow system that permits sample urine to drain continuously into a liquid waste container without losing any of the column fluid.

A drop of urine dispensed into the column of oil by the pipette will break the beams of light as it falls through the oil. Breaking the upper beam starts an electronic timer. Breaking the lower beam stops the timer. The falling time is measured electronically and computed into specific gravity units (Figure 6).

Osmolality
Urine osmolality is important for understanding the concentrating ability of the kidney.

It is useful in:

a) Determining the differential diagnosis of hyper- or hyponatraemia,
b) Differentiating pre-renal from renal kidney failure
c) For identifying and diagnosing diabetes insipidus.

Expected Values
Normal urine osmolality ranges between 50-1400 mOsm/kg water, and averages about 500-800 mOsm/kg. A random urine osmolality should average between 300-900 mOsm/kg.

Clinical Significance
A higher-than-normal result may indicate inappropriate ADH secretion, dehydration, glycosuria, adrenal insufficiency, or high protein diet.

A lower-than-normal result may indicate diabetes insipidus, excess hydration, acute renal insufficiency, or glomerulonephritis.

Method of Determination
There are two methods used in the laboratory to measure osmolality of urine: freezing-point depression and vapor-pressure depression.

Confirmatory Testing
The following list contains the most common test(s) used to confirm results when test results are questionable or inconsistent with expected results.

<table>
<thead>
<tr>
<th>Test</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glucose</td>
<td>Serum glucose</td>
</tr>
<tr>
<td>Bilirubin</td>
<td>Ictotest Reagent Tablet plus serum bilirubin and liver enzymes</td>
</tr>
<tr>
<td>Ketones</td>
<td>Serum B-hydroxybutyric acid and electrolytes</td>
</tr>
<tr>
<td>Specific Gravity</td>
<td>Creatinine, osmolality</td>
</tr>
<tr>
<td>Blood</td>
<td>Standardized microscopic examination</td>
</tr>
</tbody>
</table>
Chapter 7: Clinical Significance of Urinalysis

Urinalysis is a fast, simple, inexpensive, and reliable tool for ruling in, or ruling out, many medical conditions and diseases related to carbohydrate metabolism, urinary tract health, kidney and liver function, acid-base balance, and many other medical conditions.\(^1\)

Clinically relevant information can be obtained when analyzing the combined results of certain test parameters available on the urinalysis test strips. As with all laboratory tests, definitive diagnostic or therapeutic decisions should not be based on any single result or method.

**Urinary Tract Infections (UTIs)**

UTIs are among the most common medical problem encountered in primary care practice.

- 40-50% of women will have at least one UTI diagnosed in their lifetime\(^9\)
- 25% of all identified infections among the elderly population are UTIs. These represent the second most common form of infection\(^1\)
- 30% of infections recur in 3 months, 60% recur within 1 year, and 80% recur within 2 years\(^1\)
- 6.7 million physician office visits result in a UTI diagnosis and 2.6 million ER visits result in a UTI diagnosis\(^5\)

Some urinary tract infections do not produce symptoms and, if undetected for a long time, can cause damage to the kidneys or urinary system. This situation, called asymptomatic bacteriuria, is most common in young women, pregnant women, and people with diabetes.

In combination, the following tests were found to be a better predictor of the presence or absence of UTIs, than any one parameter alone.\(^6\)

- **Nitrite** – Detects nitrate-reducing, gram-negative bacteria
- **Leukocyte** – Detects leukocyte esterase found in white blood cells
- **Blood** - May indicate damage to the urinary tract
- **pH** - Typically high or alkaline if UTI is present

Urinalysis test strips are an effective “rule-out” tool for people with suspected UTI. A key advantage of combining the results of leukocyte and nitrite is that if both tests are negative, very few UTIs will be missed.
Kidney Disease
Kidney disease is recognized as a public health problem.

In the U.S.,

- 1 in 9 adults (or 20 million persons) have chronic kidney disease
- More than 20 million adults are at increased risk and may be unaware
- Incidence of end-stage renal disease (ESRD) is increasing by 6% per year

The presence of continuous proteinuria, and/or the presence of cellular casts observed by microscopic examination of the urine sediment, may indicate that a patient’s kidneys are not functioning properly. In addition, if blood is also found in the urine, this may give further clues regarding compromised kidney function.

- **Protein** – Detects the presence of proteinuria which may be caused by kidney malfunction
- **Albumin** – A common type of urine protein
- **Leukocyte** – Indicates urinary tract infections that can lead to kidney disorders
- **Blood** – Detects blood in urine which may indicate damage to the kidney
- **P:C Ratio & A:C Ratio** – Correct for varying urine concentration, which improves the accuracy of result interpretation, without a timed or 24-hour urine collection

A single urinalysis test strip can measure both the protein and creatinine, at the same time, to correct for varying urine concentration. The P:C and A:C ratios in a first-morning or random, untimed “spot” urine specimen is effective for the clinical evaluation of people at an increased risk of developing chronic kidney disease.

The P:C and A:C ratios are recognized by the National Kidney Foundation and the American Diabetes Association as diagnostic indicators of the presence of kidney disease. These tests, along with testing for the presence of blood, help physicians detect early stages of kidney disease in people who are at risk for kidney damage, enabling fast initiation of therapy to slow or stop the progression of kidney damage.

The P:C ratio differs from the A:C ratio in its sensitivity to urine proteins. The P:C ratio test detects higher levels of protein than the A:C ratio, and is therefore more appropriately used with the broader population. The A:C ratio detects very low levels of albuminuria (microalbuminuria), and is therefore most appropriate for testing people with diabetes.

Siemens has a portfolio of unique tests for the early detection and management of kidney disease targeted towards specific groups – the general patient population, people at risk for kidney disease, people with diabetes, and people with confirmed kidney disease (See Figure 7).

**Figure 7. Detecting and Managing Kidney Disease; Which Siemens Test Products to Use?**

<table>
<thead>
<tr>
<th>General Population</th>
<th>Multistix 10G / Multistix 8G Strip</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Traditional Dip-and-Read Strip</td>
</tr>
<tr>
<td>People Who Have One or More Kidney Disease Risk Factors (Other than Diabetes)</td>
<td>Multistix PRO+ Strips</td>
</tr>
<tr>
<td></td>
<td>Protein-to-Creatinine Ratio (Semi-Quantitative) *Available in the U.S. and Japan</td>
</tr>
<tr>
<td>People with Diabetes</td>
<td>CLINITEK Microalbumin Strips</td>
</tr>
<tr>
<td></td>
<td>Albumin-to-Creatinine Ratio (Semi-Quantitative)</td>
</tr>
<tr>
<td></td>
<td>DCA Microalbumin/Creatinine Test</td>
</tr>
<tr>
<td>People with Confirmed Kidney Disease</td>
<td>DCA Microalbumin/Creatinine Test</td>
</tr>
<tr>
<td></td>
<td>Albumin-to-Creatinine Ratio (Quantitative)</td>
</tr>
</tbody>
</table>

NOTE: Although only CLINITEK Microalbumin strips appear in the diagram, Microbustix® test strips can also be used to obtain a semi-quantitative albumin-to-creatinine ratio visually.

Diabetes
In the U.S.,

- 18.2 million people (6.3% of the population) have diabetes
- An additional 20 million Americans have pre-diabetes
- 5.2 million people are unaware that they have the disease
- 5-10% of Americans have type 1 diabetes

The following tests provide useful information regarding diabetes when performing a routine examination and/or managing people with confirmed diabetes.

- **Glucose** – May detect unsuspected diabetes
- **Ketones** – May detect early ketoacidosis in confirmed diabetics
- **A:C Ratio (Microalbuminuria)** – May detect early kidney damage associated with diabetes
- **Nitrite** – Detects nitrate-reducing (usually gram-negative) bacteria
- **Leukocyte** – Detects leukocyte esterase found in white blood cells
The ketone test helps assess the severity of diabetes and avoid progressive diabetic ketosis, which can eventually lead to coma and even death.

The American Diabetes Association recommends the annual measurement of microalbuminuria in all people with type 2 diabetes, and in people with type 1 diabetes with at least 5 years disease duration to aid in the early detection of kidney disease.

Nitrite and leukocyte tests help detect urinary tract infections, which are relatively common complications of diabetes.

**Kidney Stones**
There are over 1 million cases of kidney stones reported in the U.S. annually.12

**Blood** – May indicate damage to the kidney

**pH** – Used to determine type of stone

**Specific Gravity and/or Creatinine** – Provides a relative indication of urine concentration or dilution

Kidney stone formation depends significantly on the pH of urine. Phosphate and calcium carbonate stones develop in alkaline urine, while uric acid, cystine, and calcium oxalate stones precipitate in acid urine.

**Pregnancy-Related Disorders**
The following tests may be useful in managing women during pregnancy:

**Glucose** – May indicate gestational diabetes

**Protein** – May indicate pre-eclampsia during pregnancy

Urinary testing for glycosuria during pregnancy is routinely performed to diagnose gestational diabetes, which accounts for 33% of all pregnancy-related diabetes.13

Urinary protein tests are done to aid in the diagnosis of pre-eclampsia, a condition of hypertension and proteinuria that occurs in pregnancy and affects about 4% of all pregnancies (mother and unborn), and may advance rapidly with few other symptoms.14

**Urinary Cancers: Bladder or Kidney**
In the U.S., there are 31,000 cases of kidney cancer diagnosed each year and 53,000 cases of bladder cancer diagnosed each year. In early stages of disease, bladder and/or kidney cancers do not cause any signs or symptoms. The first most common sign of disease is the presence of blood in the urine.15

**Blood** – May indicate damage to the kidney or urinary tract

Although there are many benign reasons for the presence of blood in urine, finding unexpected and unexplained blood in the urine requires follow-up to determine the cause and rule out the presence of cancer.

**Liver Disease or Damage**
In the U.S., 400,000 persons have chronic liver disease, which is the seventh leading disease-related cause of death. The following tests, when analyzed in combination, provide more useful information regarding liver function than any one test finding alone:16

**Bilirubin** – May indicate abnormalities affecting the liver or biliary system

**Urobilinogen** – Serves as an aide in detecting and differentiating liver disease, hemolytic disease, and biliary obstruction

**Specific Gravity** – Provides a relative indication of whether other test results are affected by urine concentration or dilution

Urinary bilirubin reaches significant levels in disease processes that increase the amount of conjugated bilirubin in the bloodstream. This often occurs before other signs of liver dysfunction (jaundice, clinical illness) are apparent (see Table IX).

**Table IX. Urobilinogen and Bilirubin Tests**

<table>
<thead>
<tr>
<th>Test</th>
<th>Healthy</th>
<th>Hemolytic Disease</th>
<th>Hepatic Disease</th>
<th>Biliary Obstruction</th>
</tr>
</thead>
<tbody>
<tr>
<td>Urine Urobilinogen</td>
<td>Normal</td>
<td>Increased</td>
<td>Increased</td>
<td>Low or Absent</td>
</tr>
<tr>
<td>Urine Bilirubin</td>
<td>Negative</td>
<td>Negative</td>
<td>Positive or Negative</td>
<td>Positive</td>
</tr>
</tbody>
</table>

Urinary urobilinogen can also indicate liver problems, as well as certain types of anemia. Consequently, when urobilinogen and bilirubin results are considered together, they provide more helpful information for differential diagnosis than either finding alone.

**Sexually Transmitted Diseases**
Sexually transmitted diseases (STDs) are infections that spread from one person to another during sexual relations. Chlamydia and gonorrhea are bacteria that most commonly cause STDs. In cases of urethritis caused by an STD, such as chlamydia or gonorrhea, Siemens urinalysis test strips may show a positive leukocyte result, even if the routine culture is negative, due to the difficulty in culturing bacteria.

**Leukocyte Esterase** – Elevated test results may indicate detection of bacteria responsible for STDs
**Eating Disorders**
In the U.S., 8 million people have an eating disorder and 90% are women.

- 1 in 20 women suffer from anorexia
- 2 in 100 women suffer from bulimia

Eating disorders represent the highest mortality rate than any other mental illness.

The following tests, when analyzed in combination, provide useful information regarding proper hydration that may be compromised for people diagnosed with eating disorders, such as anorexia nervosa and bulimia nervosa.

**Ketones** – Detect the presence of ketones in urine that may indicate starvation or vomiting

**Specific Gravity** – A high value may indicate excessive vomiting

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**Chapter 8: Instrumentation**

**Overview**
Technological advances have led to the development of instrumentation and data management systems that automate the performance and reporting of urinalysis testing. These analyzers are common in busy laboratories and many physician offices because they help standardize testing and improve laboratory efficiency.

Because the systems read every test the same way every time, under the same set of conditions, they eliminate operator-to-operator variability, standardize the reading of timed tests, and eliminate the possibility of transcription errors. And with read rates as fast as seven seconds per strip, productivity is significantly improved. In most cases, these systems also interface with the Laboratory Information System (LIS) for data management and reporting.

The most sophisticated instruments are fully automated systems capable of integration with automated sample processing systems. Some systems even automate the microscopic examination of urine by standardizing the way the sample is processed and examined and then performing an analysis of the suspended particles.

**Urine Chemistry Analyzers**
Siemens offers a broad range of urinalysis analyzers that can be used in:

- Point-of-care settings such as doctor offices, clinics, and hospital wards
  or
- Laboratory settings such as hospitals or private laboratories.

**Point of Care Urine Chemistry Analyzers**
Urinalysis systems for the point of care include the CLINITEK Status® Analyzer and Clinitek® 50 Urine Chemistry Analyzer.
CLINITEK Status Analyzer (Urinalysis and Rapid Immunoassay Tests)

The Siemens CLINITEK Status Analyzer (Figure 8), introduced in 2003, offers physician offices and
smaller laboratories the reliability and consistency of state-of-the-art instrument-based testing
capability, affordably. The system is capable of performing popular point-of-care rapid tests (i.e.,
hCG pregnancy test) as well as urine testing via Siemens portfolio of Multistix, CLINITEK, and
other popular Siemens urinalysis test strips.

The CLINITEK Status analyzer performs the following functions:

- Urinalysis test results are generated in 60 seconds
- Offers easy operation via a large touchscreen interface
- Urine color and clarity can be added to the test result
- Patient and operator information, such as name or ID, can be recorded as part of
  the patient test result
- Provides a printed record to insert into the patient’s file using paper or label rolls
- Provides results for the following tests: Glucose, Bilirubin, Ketone, Specific Gravity, Blood, pH, Protein, Albumin, Creatinine, Urobilinogen, Nitrite, Leukocytes, A.C and P.C ratios
- Provides results for hCG pregnancy
- Stores up to 200 patient results which can be recalled for onscreen viewing or
  transferred to a PC or LIS
- Operates on line power or with an optional battery pack for alternate site operation

CLINITEK 50 Urine Chemistry Analyzer

The Siemens CLINITEK 50 Urine Chemistry Analyzer (Figure 9), introduced in 1996, offers physician
offices and smaller laboratories the reliability and consistency of convenient instrument-based testing.

The CLINITEK 50 performs the following functions:

- Analyzes the urinalysis test strip and gives printed results that can be inserted into the patient’s file
- Provides results for the following tests: Glucose, Bilirubin, Ketone, Specific Gravity, Blood, pH, Protein, Albumin, Creatinine, Urobilinogen, Nitrite, Leukocytes, A.C and P.C ratios
- Automatically notes the urine color and distinguishes between hemolyzed
  and intact red blood cells
- Operates on line power or with an optional battery pack for alternate site operation
- Stores up to 50 patient results

NOTE: Production of the CLINITEK 50 Urine Chemistry Analyzer was discontinued in 2004.
Siemens will continue to service and support discontinued products for 7 years after manufacturing ceases.

Laboratory Urine Chemistry Analyzers

Urinalysis systems for the laboratory include the CLINITEK Advantus™,
CLINITEK® 500, CLINITEK AUW, and the CLINITEK Atlas.

CLINITEK Advantus™ Urine Chemistry Analyzer

The Siemens CLINITEK Advantus Urine Chemistry Analyzer (Figure 11), introduced in 2007, is a semi-
automated, benchtop analyzer with a throughput of up to 500 specimens per hour.

The CLINITEK Advantus offers:

- Ability to consolidate microscopy results
- Automatic Quality Control prompting and lockout capability
- One-touch switching between different urinalysis strip types
- Storage of 500 patient and 200 control results
CLINITEK 500 Urine Chemistry Analyzer

The CLINITEK 500 Urine Chemistry Analyzer (Figure 12), introduced in 1997, is a semi-automated, bench-top analyzer with a throughput of up to 500 specimens per hour. It is flexible enough to meet the needs of medium-to-large-volume testing laboratories and increases productivity in laboratories where urine chemistry is performed. The analyzer will perform automatic color determinations and detection of non-hemolyzed trace blood on every sample.

The CLINITEK 500 performs the following basic functions:

• Automatic urinalysis test strip detection for operator-paced sample processing

• Tests one sample every seven seconds

• Automatic calibration

• Confirmatory and microscopic sieve functions

• User interface with a touch-screen display

• Bar-code reader improves laboratory efficiency through rapid entry of sample identification, color, and clarity values

• The instrument uses Multistix, Clinitek, and other popular Siemens brands of urinalysis test strips and can provide results for: Glucose, Bilirubin, Ketone, Specific Gravity, Blood, pH, Protein:Albumin, Creatinine, Urobilinogen, Nitrite, Leukocytes, P:C, Ratio, and Color

NOTE: Production of the CLINITEK 500 Urine Chemistry Analyzer will be discontinued in 2008. Siemens will continue to service and support discontinued products for 7 years after manufacturing ceases.

CLINITEK Atlas® Automated Urine Chemistry Analyzer

Siemens CLINITEK Atlas Automated Urine Chemistry Analyzer is a fully automated, modular, precision bench-top analyzer with true walk-away functionality. It is designed to meet the urinalysis needs of laboratories whose testing volume requires the productivity gained from high-throughput and walk-away automation.

The CLINITEK Atlas analyzer is available in two stand-alone configurations, offering separate sample-handling designs that accommodate different batch sizes and walk-away times.

• **CLINITEK Atlas Analyzer with Carousel Sample Handler** (Figure 12) uses a circular sample tray holding 50 tubes, for up to 13 minutes walk-away time

• **CLINITEK Atlas Analyzer with Rack Sample Handler** (Figure 13) uses linear sample racks holding 10 tubes each for a loading capacity up to 200 tubes and walk-away time up to 53 minutes

The CLINITEK Atlas instrument also features a modular design that facilitates adaptability to sample automation tracks. The system uses a unique, on-board Reagent Pak consisting of a roll of 490 urinalysis test strips affixed to a clear plastic support.
The CLINITEK Atlas instrument performs the following basic functions:

- Tests one sample every 16 seconds
- Utilizes a Reagent Pak in a unique continuous-roll format that minimizes reagent handling and simplifies loading
- Automatically pipettes samples onto the reagent areas of urinalysis test strips
- Runs 12 tests, including specific gravity, color, and clarity, and the following nine chemistries: glucose, bilirubin, ketone, occult blood, pH, protein, urobilinogen, nitrite, leukocytes
- Stores 1000 patient results and 200 controls or calibration results
- Transmits results to printer, LIS, and/or instrument display
- Offers a wide range of options for customized testing and reporting

ADVIA® Urinalysis WorkCell (AUW)*system

The Siemens ADVIA Urinalysis WorkCell system (Figure 14) is a fully automated and integrated urine chemistry and sedimentation solution for high volume laboratories.

The ADVIA Urinalysis WorkCell system offers:

- Complete walkaway testing
- Fully integrated urine chemistry and sediment analysis
- Linked by a track to automate workflow management
- Throughput of over 90 samples per hour

*Available in the U.S., Canada and Puerto Rico

NOTE: Production of the AUW system with the UF-100 was discontinued in June 2008. Siemens will continue to support and service discontinued products for 7 years.

CLINITEK Automated Workcell System (AUWi)*

The Siemens CLINITEK AUWi system (Figure 15) is a fully automated and integrated urine chemistry and sedimentation solution for high volume laboratories.

The CLINITEK AUWi system offers:

- Complete walkaway testing capability
- Fully integrated urine chemistry and sediment analysis by a common sample track and powerful workflow management software
- Continuous load-and-go processing with no centrifugation, and reduced off-line dilution
- New UF-100i enhancements include improved bacteria detection, anti-carryover function, and ability to cross-check with urine chemistry leukocyte and nitrite results

*Available in the U.S., Canada and Puerto Rico

Figure 14. ADVIA Urinalysis WorkCell (AUW)

Figure 15. CLINITEK Automated Workcell System (AUWi)
Chapter 9: Microscopic Examination

Overview
Qualitative or quantitative evaluation of urine sediment provides adequate information for the majority of diagnostic and clinical needs. However, further examination may be required in evaluating the course and progression of kidney disease.

NOTE: Some laboratories have reduced the number of microscopic urine sediment examinations performed by first evaluating the urine appearance and urinalysis test strip results. These clinicians have determined that urine specimens may not require further testing unless an abnormal result is obtained for one or more of several urinalysis test strip parameters such as blood, protein, leukocytes, nitrite, or clarity. This concept is often referred to as a “screen” or “sieve.” This procedure is intended to eliminate the performance of labor-intensive microscopic examinations on specimens that tend to have a low yield of abnormal microscopic findings. Of course, clinicians must ultimately determine whether this testing protocol is appropriate for their particular laboratory situation and patient population.

By detecting and measuring the number of formed elements (urine sediment) found in the urine (cells, casts, crystals, bacteria, parasites, and artifacts), microscopic examination of urine sediment can provide the following information:

1. Evidence of kidney disease as opposed to lower urinary tract infection.

2. Indication of the type and activity of a kidney lesion or disease condition.

It is vital that a standardized procedure be used to prepare the sample for examination to ensure consistency between operators, and from one institution to another.

The microscopic results and urine chemistry results should be checked against each other. Discrepancies should be explained before reports are issued.

Specimen Requirements
While the first-morning specimen is usually the preferred specimen, other specimens can be used. The urine specimen should be examined as soon as possible (less than two hours after collection), as the formed elements (cells, casts, etc.) begin to lyse upon standing. If the specimen cannot be examined within two hours, it must be refrigerated or otherwise preserved. Refrigeration may cause the precipitation of amorphous urates or phosphates (acid or alkaline urines, respectively) that can make it hard to see formed elements in the microscopic examination. Warming of the urine specimen to room temperature will re-dissolve the amorphous urates. The amorphous phosphates require the addition of a weak acid (acetic acid) for them to go back into solution.

Standardized Microscopic Procedure
Only cloudy urine specimens or specimens that show positive strip results for blood (RBCs), white blood cells (WBCs), nitrite, or protein are the most likely to show anything significant with the microscopic examination. However, determining the criteria for microscopic examination needs to be made by the individual laboratory and the patient’s physician.

Once a urine specimen has been identified as requiring a microscopic examination, the entire test procedure needs to be standardized. One way this is achieved is by using a complete test system that has special test slides that come complete with cover slips and a grid pattern on the slide so that the number of cells/μL of urine can be reported. These standardized systems may add costs to the operation, but the quality of results improves dramatically.

The following is an example of a standardized procedure.

1. Start with the same volume of fresh urine to be concentrated (12 mL).

2. Centrifuge for a constant time (5 minutes), at a constant relative centrifugal force (RCF)* of 400 G.

3. Decant or aspirate the supernatant to leave a standard volume of concentrate (0.5 mL).

4. Examine a standardized volume of urine in a standardized slide chamber with a set depth, first under low power and then high power magnification.

5. Report the number of elements/μL of urine, based on a factor established for the specific test system.

Microscopic Techniques

Brightfield Illumination
Most microscopic examinations of the urine sediment are made under what is called Brightfield Illumination. This standard examination should start with examination under “low power” magnification (10x objective and 10x eyepiece [100-x]), with contrast achieved by varying the opening of the iris diaphragm. This permits the observation of larger elements such as casts, especially hyaline casts, mucus, and some cells. The specimen is then examined with a 40x objective and 10x eye piece (400x or high power).

Phase Contrast Microscopy
This type of microscopy is particularly useful in identifying hyaline casts, mucus, cells, and bacteria that may be difficult to see using Brightfield Illumination.
Interference Contrast Illumination
This method is not widely used, but it offers the benefits of phase contrast, plus showing the elements in 3D.

*RCF = 11.18 x 10^3 x radius in centimeters of the centrifuge head x the revolutions per minute (RPM)*

Polarized Light
Plain or compensated polarized light is ideal for assisting in the identification of crystals, starch, fat, and fibers.

Staining
A variety of stains can be used to assist in the identification of various elements. These stains can include supravital stains (Sternheimer-Malbin stain or toluidine blue) or lipid stains such as the Sudan group. Gram stains can be used to help to identify bacteria.

With supravital stains:
- Leukocytes may appear unstained initially, or the nucleus may appear red-blue with red cytoplasm after a prolonged period of time
- Hyaline casts stain bright blue, while finely granular casts stain bright red
- Coarsely granular casts and “waxy” casts stain reddish violet
- RBC casts are outlined in shades of pink, while hemoglobin casts appear rust-brown
- Miscellaneous substances, such as oval fat bodies and yeast, will not stain
- Trichomonas will not stain or may appear bluish

Automated Urine Microscopy
There are instrument systems on the market that automate sediment analysis of formed elements. These systems work on principles of flow imaging or flow cytometry to identify and quantify cells and other formed elements. Certain specimens with casts, crystals, or a large number of formed elements are appropriately flagged for visual review and/or the microscope.

Expected Findings
Normal sediment is not free of cells or casts, but contains a limited number of formed elements. While a precise definition of a normal sediment is difficult to obtain, the presence of one or two blood cells, one or two leukocytes, and a few epithelial cells is not necessarily abnormal. An occasional hyaline cast may also be a normal finding. The urine of mature females may normally contain large numbers of squamous epithelial cells from the vaginal walls.

Clinical Utility

Red Blood Cells
More than two to three red blood cells per high power field is an abnormal condition, as the presence of RBCs can indicate a variety of kidney and systemic diseases, including trauma to the kidney.

They may also appear following:
- Traumatic catheterization
- Passage of stones
- Contamination from menstrual blood
- Strenuous exercise

Hematuria occurs with pyelonephritis, tuberculosis of the genitourinary tract, cystitis, prostatitis, kidney calculi, kidney tumors, and other malignancies of the urinary tract, and hemorrhagic diseases, such as hemophilia.

It is important to note that RBCs tend to lyse or dissolve in alkaline or dilute urine.

White Blood Cells
The presence of large numbers of white cells or leukocytes (pyuria) usually indicates bacterial infection in the urinary tract. Pyuria may also be seen in acute glomerulonephritis. The cells are segmented neutrophils or polys. Large numbers of mononuclear cells (lymphs) in a patient with a kidney transplant may indicate early tissue rejection.

Epithelial Cells
Squamous epithelial cells frequently appear in normal urine. Large numbers of kidney epithelial cells, which are common in the urine of people with acute necrosis and necrotizing papillitis, may indicate active tubular degeneration.

Figure 16 shows the origin of various epithelial cells that may appear in the urine. However, due to the osmotic, pH, and traumatic changes the cells undergo during passage through the genitourinary system, they rarely retain their original shape.
Crystals
The type and quantity of crystalline precipitate vary with the pH of the urine. Amorphous material is of little importance. Crystals in normal urine are formed as the specimen cools.

Crystals in abnormal urine include cystine, leucine and tyrosine, and cholesterol. Table X lists some crystals found in urine sediment and the physical characteristics associated with them.

Table X. Crystals Found In Urine Sediment

<table>
<thead>
<tr>
<th>Name</th>
<th>Color</th>
<th>Shape</th>
</tr>
</thead>
<tbody>
<tr>
<td>Amorphous Urates</td>
<td>Brick-Red</td>
<td>Granules</td>
</tr>
<tr>
<td>Uric Acid</td>
<td>Yellow-Brown</td>
<td>Polymorphous – Whetstones, Rosettes or Prisms, Rhombohedral Prisms, Hexagonal Plate</td>
</tr>
<tr>
<td>Sodium Urate</td>
<td>Colorless to Yellow</td>
<td>Fan of Slender Prisms</td>
</tr>
<tr>
<td>Cystine (Rare)</td>
<td>Colorless, Highly Refractive</td>
<td>Flat, Hexagonal Plates with Well-Defined Edges; Singly or in Clusters</td>
</tr>
<tr>
<td>Cholesterol (Rare)</td>
<td>Colorless</td>
<td>“Broken Window Panes” with Notched Corners; Flat Panes</td>
</tr>
<tr>
<td>Leucine (Rare)</td>
<td>Yellow or Brown, Highly Refractive</td>
<td>Spheroids with Striaations; Pure Form Hexagonal</td>
</tr>
<tr>
<td>Tyrosine (Rare)</td>
<td>Colorless or Yellow</td>
<td>Fine, Silky Needles in Sheaves or Rosettes</td>
</tr>
<tr>
<td>Bilirubin</td>
<td>Reddish, Brown</td>
<td>Cubes, Rhombic Plates, Amorphous Needles</td>
</tr>
<tr>
<td>Calcium Oxalate</td>
<td>Colorless</td>
<td>Octahedral, Dumbbells, Often Small – Use High Power</td>
</tr>
<tr>
<td>Hippuric Acid</td>
<td>Colorless</td>
<td>Rhombic Plates, Four-Sided Prisms</td>
</tr>
</tbody>
</table>

| Acid, Neutral, or Slightly Alkaline Urine       |
| Octahedral, Dumbbells, etc. – Use High Power   |

| Alkaline, Neutral, or Slightly Acid Urine      |
| Rhombic Plates, Four-Sided Prisms             |

Bacteria
Normal urine contains no bacteria. If a proper and careful technique is used to obtain the specimen, and if the specimen is protected from contaminants before the examination, the presence of bacteria in significant numbers may indicate urinary tract infection. The presence of leukocytes helps to differentiate between contamination and infection.

Yeast
Yeast cells (Candida albicans) may be indicative of urinary candidiasis, especially in people with diabetes mellitus. Frequently, yeast appears as a contaminant in the urine of females with vaginal candidiasis.

Parasites
The majority of parasites observed in urine are contaminants from fecal or vaginal material. A urinary tract parasite infestation may be associated with the presence of red blood cells, e.g., Schistosoma haematobium.

Spermatozoa
Spermatozoa are frequently seen in the urine following nocturnal emissions or sexual intercourse.

Casts
Cast formation usually occurs in the distal convoluted tubule of the nephron. Casts may also occur in the ascending loop of Henle or the collecting duct. Requirements for cast formation are an acid condition, high salt concentration, reduced urine flow, and protein (Figure 17).

Casts are named according to the inclusions contained in them (red blood cell cast, white blood cell cast, etc.) Red blood cell casts indicate the presence of an acute inflammatory or vascular disorder in the glomerulus, causing kidney hematuria. They should always be regarded as pathological and may be the only manifestation of acute lomerulonephritis, kidney infarction, collagen disease, or kidney involvement in subacute bacterial endocarditis.

White blood cell casts may be found in the urine of people with acute glomerulonephritis, nephrotic syndrome, or pyelonephritis.

Since pyelonephritis may remain completely asymptomatic even though it is progressively destroying kidney tissue, careful examination of the urinary sediment for leukocyte casts is important. In some cases, it may be the only significant laboratory finding in an asymptomatic situation.

Epithelial cell casts are formed by fused desquamated tubular cells. Since the tubule is a living membrane, it is always replacing itself. Thus, the finding of an occasional kidney epithelial cell or clump is not unusual. However, in any disease producing damage to the tubular epithelium, the appearance of many epithelial casts may indicate excessive desquamation such as may occur in nephrosis, eclampsia, amyloidosis, and in the presence of poisoning with heavy metals and a variety of other toxins.
Hyaline casts, formed of the gel of Tamm-Horsfall protein, may imply damage to the glomerular capillary membrane, permitting leakage of proteins through the glomerular filter. Such damage may be permanent or transient as a result of fever or the effects of posture (orthostatic, lordotic), emotional stress, or strenuous exercise. If a person exercises strenuously, the blood flow to the kidneys is reduced, as it is re-directed to the muscles. During this period, hyaline casts may be formed. When the exercise is over and the blood flow returns to normal, there is a “flushing out” of these casts into the urine. This “shower of casts” is usually non-pathological.

When describing granular casts, the terms “coarsely granular” and “finely granular” are used to indicate the degree of degeneration that has occurred in the cellular inclusion, i.e., whether the cells have been broken down into coarse or fine particles. While an occasional granular cast may be found in normal individuals, numbers beyond occasional may indicate pyelonephritis. Granular casts are also found in chronic lead intoxication.

Waxy and fatty casts are associated with tubular inflammation and degeneration. The broad, waxy cast is formed in the collecting tubules when the urine flow through them is reduced. Both waxy and fatty casts are found in chronic kidney disease.

Identification of Specific Elements in the Sediment

Red Blood Cells (Erythrocytes)

Red blood cells usually look like pale, light-refractive, bi-concave discs when viewed under high power magnification. They have no nuclei. Red blood cells seen in fresh, unstained sediment are pale in color (Figure 18).

Figure 18. Erythrocytes

In urine that is not fresh, they are colorless “shadow cells.” In concentrated urine, the red blood cells may be small and crenated. And in dilute urine, they are often large and swollen, and sometimes rupture to produce “ghost” cells.

Red blood cells must be differentiated from yeast cells, urate crystals, and oil droplets. Yeast cells are usually ovoid and frequently show budding. Ammonium biurate crystals occur in large quantities and in a great range of sizes. Mineral oil droplets also vary greatly in their size and are more refractile and spherical.

White Blood Cells (Leukocytes)

The predominant type of leukocyte (Figure 19) appearing in the urine is the polymorphonuclear leukocyte. These leukocytes have segmented nuclei, are usually granular, and are approximately 1x as large as red blood cells. Certain neutrophils are larger than the usual leukocytes, and their cytoplasmic granules show Brownian movement. These cells are called granular motility or “glitter” cells. Originally they were thought to be pathognomonic of pyelonephritis but are now thought to be a result of hypotonic urine. With white blood cells in the sediment, the urine should give a positive chemical test for leukocyte esterase.

Figure 19. Leukocytes

Epithelial Cells

Kidney tubular epithelial cells are round and slightly larger than leukocytes. Each contains a single large nucleus.

Bladder epithelial cells are larger than kidney tubular epithelial cells. They range in shape from flat to cuboidal or columnar.

Squamous epithelial cells are large flat cells with single small nuclei and a large cytoplasm. The majority of these cells are contaminants from the vagina or vulva, but some originate in the urethra (Figure 20).

Figure 20. Squamous Epithelial Cells and Erythrocytes
Casts
The appearance, size, and inclusion of a cast will offer incontrovertible evidence of the condition of at least one nephron of one kidney just prior to passage of the urine. Practically all casts have a hyaline matrix that may or may not contain inclusions such as desquamated cells from the lining of tubules, white blood cells, or red blood cells. Casts are classified according to the contained material.

Red blood cell casts form in three stages:

1. Presence of free red blood cells
2. Degenerating cells within a protein matrix
3. Homogeneous blood casts (Figure 21).

Figure 21. Formation of Red Blood Cell Cast

Any disease that alters the integrity of the glomerulus will alter the composition of the urine. Disease of injury to the glomerulus usually results in a leakage of RBCs and protein. An actual photograph of a red blood cell cast is shown in Figure 22.

Figure 22. Red Blood Cell Cast

White cell casts are usually composed of many leukocytes in a cylindrical encasement and indicate kidney origin. Figure 23 shows the formation of a white blood cell cast.

Figure 23. Formation of White Blood Cell Cast in Pyelonephritis

A typical white blood cell cast seen in the urine sediment is shown in Figure 24.

Figure 24. White Blood Cell Cast
Coarse granular casts contain homogeneous, coarsely granular material. They are clear, colorless, and appear very dense. Coarse granular casts may represent the initial stages of degeneration of epithelial cell casts. These casts further degenerate into fine granular casts and terminate as waxy casts or fatty casts.

Fine granular casts are differentiated from coarse granular casts by the presence of fine granular material. Figure 25 shows several typical granular casts.

Figure 25. Granular Casts

Waxy casts are composed of a homogeneous, yellowish material. They are relatively broad, have a highly refractile outline, and appear very brittle. They are irregularly shaped, show characteristic clefts, and occasionally may have a “corkscrew” appearance (Figure 26).

Figure 26. Waxy Cast

Broad casts (kidney failure casts) are two to six times as wide as ordinary casts. They are usually waxy, granular, or cellular. They are thought to appear in the collecting tubules as a result of markedly decreased urinary output, presumably due to severe kidney disease (Figure 27).

![Figure 27. Broad Cast (Waxy)](image)

Epithelial cell casts are formed by fused desquamated tubular cells. The degeneration of the discrete cellular casts into coarsely and finely granular material is purely a function of age and permits the inference that there has been stasis in the nephron (Figure 28).

Figure 28. Epithelial Cell Cast

Hyaline casts are pale, colorless, occasionally refractile “cylinders.” They are best seen when the intensity of the light is sharply reduced. These casts are formed from the gel of proteins that have presumably traversed the glomerular capillary membrane (Figure 29).

Figure 29. Hyaline Casts
Crystals
A variety of crystals may appear in the urine. These can be identified by their specific appearances and solubility characteristics.

While most crystals are non-pathological, some do indicate pathology. Cystine crystals (Figure 30) indicate cystinuria, a condition in which cystine stones form in the kidney and cystinosis, an innate metabolic disorder in which cystine crystals are found in the urine, reticuloendothelial system, spleen, and eyes.

Figure 30. Cystine Crystals

Uric acid crystals may appear in the urine in a variety of shapes and colors. They may appear as a result of pathology or metabolism. Uric acid may appear as needles, hexagonal shapes, rosette shapes, “whetstone form,” or as rhombic plates. The crystals may appear colorless, yellow, or brown. Increased uric acid denotes increased purine metabolism. Uric acid crystals may be found in cases of fever, leukemia, some kidney tubular diseases, and gout. Figure 31 shows uric acid crystals.

Figure 31. Uric Acid Crystals

Leucine and tyrosine are abnormal crystals occasionally seen in urine of people with liver problems. When there are severe liver problems, these amino acids are not metabolized. Tyrosine crystals appear as colorless fine needles and are usually grouped in clusters (Figure 32).

Figure 32. Tyrosine

Bacteria
Bacteria may be seen in the sediment as a result of either urinary tract infection or contamination of the specimen. The two causes cannot usually be distinguished by examination of the specimen, although the presence of large numbers of leukocytes, a positive nitrite test, and/or a positive leukocyte esterase test is suggestive of urinary tract infection. Bacilli are more easily recognized than cocci, which may be mistaken for amorphous crystals. A culture on a clean-catch specimen should be performed when in doubt (Figure 33).

Figure 33. Bacteria

Yeast
Yeast cells may be seen in the urinary sediment. They are sometimes confused with red blood cells. They differ by being ovoid (rather than round), colorless, and variable in size. They may also frequently show budding. If in doubt, the addition of acetic acid to the sediment on the slide will lyse red blood cells but leave yeast cells intact. Large numbers of yeast with hyphae are suggestive of vaginitis (Figure 34).
Parasites
Trichomonas vaginalis is the most frequently seen parasite in urine. It is a unicellular organism with anterior flagella and an undulating membrane. The parasites may resemble flattened, ovoid epithelial cells, but are usually recognized by their swimming motions through the sediment, the movements of their flagella, and the characteristic undulating membrane (Figure 35).

Figure 35. Trichomonas Vaginalis

Spermatozoa
Spermatozoa have oval bodies with long delicate tails. They may be mobile or stationary (Figure 36).

Figure 36. Spermatozoa

Contaminants and Artifacts
Cotton threads, hair, starch granules, wood and wool fibers, and other contaminants must be recognized to ascertain that these substances do not represent any significant finding in the urinary sediment. Cotton fibers and starch granules are shown in Figures 37 and 38.

Figure 37. Cotton Fibers

Figure 38. Starch Granules
**Appendix I**

### Tables of Average Normal Values for Urine Determinations*

<table>
<thead>
<tr>
<th>Test</th>
<th>Average Normal Value</th>
<th>Type of Specimen</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>A: Routine</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Albumin Qualitative</td>
<td>Negative 15-150 mg/24 hour**</td>
<td>Random 24 hour</td>
</tr>
<tr>
<td>Quantitative</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Bilirubin</td>
<td>Negative</td>
<td>Random</td>
</tr>
<tr>
<td>Blood, occult</td>
<td>Negative</td>
<td>Random</td>
</tr>
<tr>
<td>Glucose Qualitative</td>
<td>Negative 130 mg/24 hour</td>
<td>Random 24 hour</td>
</tr>
<tr>
<td>Quantitative</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hemoglobin</td>
<td>Negative</td>
<td>Random</td>
</tr>
<tr>
<td>Ketones</td>
<td>Negative</td>
<td>Random</td>
</tr>
<tr>
<td>pH</td>
<td>4.6-8.0</td>
<td>Random</td>
</tr>
<tr>
<td>Protein Qualitative</td>
<td>Negative 40-150 mg/24 hour</td>
<td>Random 24 hour</td>
</tr>
<tr>
<td>Quantitative Bence Jones</td>
<td>Negative</td>
<td>Random</td>
</tr>
<tr>
<td>Specific Gravity</td>
<td>1.001-1.030</td>
<td>Random</td>
</tr>
<tr>
<td>Sugars</td>
<td>Negative</td>
<td>Random</td>
</tr>
<tr>
<td>Urobilinojen Semi-quantitative</td>
<td>0.3-1.0 Ehrlich units 0.5-4.0 mg/24 hour</td>
<td>2 hour afternoon</td>
</tr>
<tr>
<td>Quantitative</td>
<td>24 hour, collected in dark bottle with 5g Na2CO3, refrigerated</td>
<td></td>
</tr>
<tr>
<td>Volume - Adults</td>
<td>600-1600 mL/24 hour</td>
<td>24 hour</td>
</tr>
</tbody>
</table>


** B: Non-routine

<table>
<thead>
<tr>
<th>Test</th>
<th>Average Normal Value</th>
<th>Type of Specimen</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aldosterone</td>
<td>2-26 mu-g/24 hour</td>
<td>24 hour, refrigerated</td>
</tr>
<tr>
<td>Amino acid nitrogen</td>
<td>100-290 mg/24 hour</td>
<td>24 hour, refrigerated</td>
</tr>
<tr>
<td>Ammonia nitrogen</td>
<td>20-70 mEq/24 hour</td>
<td>24 hour, refrigerated</td>
</tr>
<tr>
<td>Calcium Sulfawitch</td>
<td>Positive 1+ 100-240 mg/24 hour on average diet</td>
<td>Random 24 hour</td>
</tr>
<tr>
<td>Quantitative</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Catecholamines</td>
<td>&lt;100 mg/24 hour</td>
<td>24 hour, preserve with 1 mL concentrated H2SO4</td>
</tr>
<tr>
<td>Chloride</td>
<td>140-250 mEq/24 hour</td>
<td>24 hour</td>
</tr>
<tr>
<td>Concentration test</td>
<td>Specific gravity of 1.025 or higher</td>
<td>Fluids for the day prior to test</td>
</tr>
<tr>
<td>Coproporphyrin</td>
<td>3-20 mu/g/100 Mm/L/dL Adults: 50-160 mu-g/24 hour Children: 0-80 mu-g/24 hour</td>
<td>Random 24 hour preserve with 5 g NA2CO3</td>
</tr>
<tr>
<td>Creatine</td>
<td>Male: 1.0-2.0 g/24 hour Female: 0-100 mg/24 hour Higher in children</td>
<td>24 hour</td>
</tr>
<tr>
<td>Creatinine</td>
<td>Male: 1.0-2.0 g/24 hour Female: 0.8-1.8 g/24 hour</td>
<td>24 hour</td>
</tr>
<tr>
<td>Dilution test</td>
<td>Specific gravity of 1.001 to 1.003</td>
<td>After, 1,200 mL water load</td>
</tr>
<tr>
<td>Estrogens (total)</td>
<td>Male: 5-18 mu-g/24 hour Female: 0-100 mu-g/24 hour</td>
<td>24 hour, refrigerated</td>
</tr>
<tr>
<td>17-hydroxyrostosteroids</td>
<td>Male: 5.5-14.5 mg/24 hour Female: 5-13 mg/24 hour</td>
<td>24 hour, tranquilizers interfere</td>
</tr>
<tr>
<td>17-ketosteroids</td>
<td>Male: 8-15 mg/24 hour Female: 6-11 mg/24 hour Children: 5 mg/24 hour</td>
<td>24 hour, tranquilizers interfere</td>
</tr>
<tr>
<td>Lead</td>
<td>&lt; mu-g/24 hour</td>
<td>24 hour, collected in lead-free bottle</td>
</tr>
<tr>
<td>Osmolality</td>
<td>500-800 mOsm/kg water</td>
<td>Random</td>
</tr>
<tr>
<td>Phenylpyruvic acid</td>
<td>Negative</td>
<td>Random</td>
</tr>
</tbody>
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### Appendix II

#### Siemens Urinalysis Test menu

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*Not available in all markets.*

### Glossary

**Accuracy** – The correctness of a test result. Freedom from error or how close a test result is to the “true” or actual value.

**Acute Glomerulonephritis (AGN), Postinfectious Glomerulonephritis** – The acute form of glomerulonephritis that may occur one to six weeks after a streptococcal (usually Group A, B-hemolytic) infection. Characterized by hematuria, oliguria, edema, and proteinuria with red blood cell and/or granular casts found in the microscopic examination of the urine. **Acute Tubular Necrosis** – Sudden failure of functioning of the kidney tubules. Commonly caused by an interruption of the blood supply to the tubules, resulting in ischemia.

**AER (Albumin Excretion Rate)** – AER increases by 10-30% per year during the evolution of diabetic nephropathy. The coefficient of variation for AER in a given subject is 30-40% despite a laboratory measurement error of less than 5%. It follows that no test on a single urine specimen can have definitive clinical value, whether it is semi-quantitative, ACR, or AER. For this reason, it is appropriate to use frequent measurements for assessment of treatment response.

**Albumin-to-Creatinine Ratio (A:C Ratio, ACR)** – Because of the wide variations in urine volume and concentration that occur during the day, the measurement of both the albumin and creatinine, with a ratio of the two results, permits the “correction” of the results for these variations.

**Albuminuria** – The presence of albumin in the urine at a level above what is considered normal. The amount that is reliably detected by most urine protein test strips is a level of >300 mg/l (30 mg/dl).

**Aldosterone** – A mineral corticosteroid hormone released from the adrenal medulla that stimulates active absorption of sodium ion in exchange for excretion of the potassium ion by the tubular cells (the sodium/potassium pump).

**Amorphous Material** – Crystalline material seen in the urine sediment as granules, without shape or form.

**Anti-Diuretic Hormone (ADH)** – A pituitary hormone that decreases the production of urine by increasing the re-absorption of water by the kidney tubules. It is also called vasopressin.

**Ascorbic Acid (Vitamin C)** – A strong reducing agent. Ascorbic acid acts as an interfering substance (resulting in a delayed or reduced reaction) in some strip tests that utilize the release of oxygen and subsequent oxidation of a chromogen.

**Bence-Jones Protein** – A light chain immunoglobulin that is seen in high amounts in the urine of about 50% of people with multiple myeloma. It may also be present in some other malignancies. It coagulates at temperatures of 45°C to 55°C and re-dissolves completely on boiling.

**Benedict’s Test** – Copper reduction test for reducing substances, based on the reduction of cupric (copper II) ions to cuprous (copper I) ions in the presence of alkali and heat.

**Bilirubin** – Vivid yellow compound that is a major by-product of normal red blood cell destruction. See also conjugated bilirubin and free (unconjugated) bilirubin.

**Bladder (Urinary)** – Muscular membranous sac that stores urine for discharge through the urethra. It is located in the pelvis, connected anteriorly with the two ureters to the kidneys and posteriorly with the urethra.

**Bowman’s Capsule (Glomerular Capsule)** – Cup-shaped end of the kidney nephron that contains the glomerulus. Blood filtrate, which comes through the glomerulus, is collected into this sac where it then progresses through the tubules to the renal pelvis as urine is formed.
Brightfield Microscopy – Illumination system on a microscope that uses ordinary light. This is the most common light source with a clinical microscope.

Calculus (Stone) – A stone or deposit of mineral salts formed and found in the urinary tract. A variety of different salts can precipitate out of the urine and produce these stones.

Casts – Structures that result from the solidification of Tamm-Horsfall mucoprotein in the lumen of the renal tubules. They form a mold or cast of the tubule and trap other material that may be present when they form. Several types exist, ranging from hyaline (pure Tamm-Horsfall mucoprotein) to those containing cells, to granular, waxy, and fatty casts. They represent a biopsy of the kidney and are usually clinically important.

Catheterized Urine Specimen – Urine specimen obtained by the introduction of a catheter into the urinary bladder by way of the urethra.

Clean-Catch (Midstream) Urine Specimen – Urine specimen collected during the flow of urine. The first portion of the urine flow is discarded and then a portion of the remaining urine is collected. This procedure is used to collect urine that is not contaminated by external cells and bacteria.

Clinical Laboratory Improvement Act of 1967 (CLIA ’67) – A U.S. Government act providing for the licensing of laboratories that accept specimens for testing from across state lines (interstate commerce). These are generally large hospital and reference laboratories. CLIA ’67 has been superseded by CLIA ’88.

Clinical Laboratory Improvement Amendments of 1988 (CLIA ’88) – A U.S. law that applies to facilities that perform tests on specimens derived from the human body. The CLIA ’88 regulations include requirements for quality assurance, quality control, proficiency testing, personnel, and inspections. Laboratories holding a CLIA certificate are regulated according to the complexity of the tests and procedures performed in the laboratory. Tests are categorized as highly complex or moderately complex. Laboratories that only perform simple (waived) tests and procedures are eligible to operate on a CLIA Certificate of Waiver.

Collecting Tubules – The relatively large, straight renal tubules which are located after the distal convoluted tubules of the nephron. They connect individual nephrons at various intervals and funnel urine into the renal pelvis. The site of final concentration of urine is under the control of ADH (vasopressin).

College of American Pathologists (CAP) – This professional organization of pathologists provides services, guidance, and information to its members and consultation to others in the field of clinical laboratory science. It develops programs, publications, and services that span the entire field of pathology. Special attention is given to areas of laboratory improvement, quality control, and laboratory planning and administration. Survey programs on laboratory improvement and quality assurance offer a wide range of inter-laboratory comparison and proficiency programs that meet the requirements set out by CLIA ’88, JCAHO, and most state regulatory agencies.

Compensated Polarized Microscopy – Modification of the normal brightfield microscope in which two crossed polarized filters, plus a first-order red compensator or filter, are inserted to observe the presence and type of birefringence. Especially useful in the identification of crystals in synovial fluid.

Confirmatory Test – A test used to confirm the correctness of a procedure; an alternative method with at least the same or better specificity, based on a different principle, with equal to or better sensitivity than the original test. This may also be called a comparative method.

Conjugated Bilirubin, Bilirubin Glucuronide, Direct Bilirubin – Bilirubin that has been made water-soluble by conjugation with glucuronide in the Kupffer cells of the liver. Once conjugated, increased levels of bilirubin in the blood can be filtered through the kidneys and be found in the urine.

Control Specimen (Solution) – Material or solution with a known concentration of the analyte(s) being measured. It is used for quality control where the test result for the control specimen must be within certain limits in order for the "unknown" values run in the same batch or time frame to be considered reliable and reportable.

Cortex (Renal) – The outer layer of the kidney, made up of the glomerular portion of the nephron and the proximal convoluted tubules.

Creatinine – Derived from the nonenzymatic dehydration of creatine in skeletal muscle. The amount of creatine per unit of muscle mass is constant, and thus the rate of spontaneous breakdown is also constant. As a result, the plasma creatinine concentration is very stable. Creatinine is freely filtered at the glomerulus and is not reabsorbed by the tubules. The concentration of creatinine in the urine can be used to "correct" the level of an analyte by compensating for the variation in the urine concentration.

Creatinine Clearance – An estimate of the glomerular filtration rate of the plasma obtained by measurement of the amount of creatinine in plasma and its rate of excretion into the urine. The formula used for obtaining the creatinine clearance is $(\text{ml/min}) = \frac{UV}{P}$, where $U$ is the urine creatinine (units/L), $V$ is the volume of urine (ml/min), and $P$ is plasma creatinine (units/L).

Crenated (Red Blood Cells) – Red blood cells showing spicules or projections on the surface. The notched, shrunken surface results from a loss of fluid from the red blood cells into the urine, caused by hypertonicity.

Crystalluria – The presence of crystals in the urine sediment.

Crystals, Abnormal – Urinary crystals of metabolic or iatrogenic origin that are generally of pathological significance and require further confirmation and identification.

Crystals, Normal – Urinary crystals that may be found in normal urine specimens of an acid or alkaline pH. Generally they are not pathologic and can be reported on the basis of morphologic appearance.

Cylindroid – Hyaline cast with one end that tapers off into a tail or a point. They are clinically equivalent to, and reported as, hyaline casts.

Cystitis – Inflammatory condition of the urinary bladder and ureters characterized by pain, urgency and frequency of urination, and hematuria. It may be caused by a bacterial infection, calculi or stone.

Diabetes Mellitus – Chronic metabolic syndrome of impaired carbohydrate, fat, and protein metabolism that is secondary to insufficiency of insulin secretion or to the inhibition of insulin activity. It is characterized by increased concentrations of glucose in the blood and urine.

Diabetic Nephropathy – A series of metabolic and structural changes that occur to the kidneys of individuals with diabetes mellitus. The early changes include a thickening of the basement membranes of the glomeruli and progress to end-stage kidney disease. (see the chapter on Diabetic Nephropathy for additional information.)

Diazo Reaction – Coupling of a diazonium with another aromatic ring to produce a colored azo dye.
Direct Bilirubin – See conjugated bilirubin.

Distal-Convoluted Tubule – Convoluted tubules of the nephron, located furthest from the glomerulus, where final re-absorption of sodium (maintaining water and electrolyte balance) and removal of excess acid (maintaining acid-base balance) occurs.

Dysmorphic (Red Blood Cells) – Distorted, irregular, or misshapen red blood cells, indicative of glomerular bleeding, especially when accompanied by proteinuria and red blood cell casts.

Edema – Abnormal accumulation of fluid in interstitial spaces of tissue.

Ehrlich’s Aldehyde Reaction – Reaction of urobinogen, porphobilinogen, and other Ehrlich-reactive compounds with δ-dimethylaminobenzaldehyde in concentrated hydrochloric acid to form a colored aldehyde.

Exogenous – Coming from, or being introduced into, a specimen from outside sources, as in contamination from powder in gloves.

Extravascular Fluids – Body fluid other than blood.

First-Morning Urine Sample – The first urine voided in the morning. It is generally the most concentrated specimen of the day because less fluid or water is ingested during the night while the kidney continues to excrete a constant concentration of solid or dissolved substances. It is usually the preferred sample for testing, due to the increased likelihood of identifying low levels of analytes or formed elements.

Free Bilirubin, Unconjugated Bilirubin – Water-insoluble form of bilirubin that must be carried through the blood as a bilirubin-albumin complex. Because of its insolubility, this form of bilirubin cannot be excreted by the kidney and is not found in urine. The blood level becomes elevated if there is an increased rate of destruction of old red blood cells, as in a hemolytic process. This is also the form of bilirubin that crosses the blood-brain barrier and causes kernicterus in hemolytic disease of the newborn.

Galactosemia – Inherited, autosomal recessive disorder of galactose metabolism. It is characterized by a deficiency of the enzyme galactose-1-phosphate uridyl transferase, which results in increased levels of galactose in blood and urine.

Ghost or Shadow Cells – Red blood cells that have lysed (burst), releasing their cellular fluid, including hemoglobin, leaving only the red cell membrane. Visualization is enhanced by using phase contrast microscopy.

Glitter Cells – Swollen neutrophils with cytoplasmic granules in constant Brownian motion. They are seen in dilute urine and are also known as Sternheimer-Malbin positive cells.

Glomerular Capsule – See Bowman’s capsule.

Glomerular Filtrate – Ultrafiltrate of blood formed as it passes through the glomerular capillaries of the glomerulus into Bowman’s capsule. It is the first step in urine formation and is basically blood plasma without protein or fat.

Glomerular Filtration Rate (GFR) – The rate which filtrate is produced in Bowman’s capsule each minute. Between 1,000 and 1,500 mL of blood pass through the kidneys each minute, creating about 130 mL of filtrate. This is called the glomerular filtration rate.

Glomerulonephritis – Inflammation of the glomerulus of the kidney, characterized by proteinuria, hematuria, decreased urine production, and edema. Includes acute (post-streptococcal) glomerulonephritis, chronic glomerulonephritis, and sub-acute glomerulonephritis.

Glomerulus – Tuft or cluster of blood capillaries found in the renal nephron. Urine formation begins at the glomerulus as an ultrafiltrate, called the glomerular filtrate.

Glucose Oxidase – Enzyme that oxidizes glucose to gluconic acid while reducing atmospheric oxygen to hydrogen peroxide. This enzyme is the basis for most of the urine tests for glucose.

Glucosuria, Glycosuria – Presence of abnormal amounts of glucose in urine.

Gram Stain Reaction – Method of differentially staining microorganisms that retain the violet (purple) color of the primary stain (crystal violet-iodine complex) and are considered Gram “positive.” Examples of these are Staphylococci and Streptococci. Microorganisms having the red-pink color of the counterstain (safranin) are considered Gram “negative.” Examples of these are E. coli and Proteus bacteria. Use of these properties serves to classify or differentiate organisms.

Hematuria – Blood in the urine.

Hemoglobinuria – Presence of free hemoglobin in the urine.

Hemolytic Jaundice, Prehepatic Jaundice – Jaundice that results from the increased destruction of red blood cells in the blood (intra-vascular hemolysis).

Hepatic Jaundice, Hepatocellular Jaundice – Jaundice resulting from conditions that affect the liver cells directly, such as viral or toxic hepatitis.

High Power Examination, High Power Field (HPF) – Usually a 40x magnification microscope objective and a 10x ocular, giving a total magnification of 400x. It is used for a more detailed examination of wet preparations such as the urine sediment.

Hypoalbuminemia – A low level of albumin in the serum, usually due to loss of albumin into the urine. It can also result from poor nutrition or certain pathologic liver conditions.

IGF-1 – Insulin-like growth factor-1, considered to be the main activator of early glomerular and kidney enlargement in diabetes.

Interstitial Cystitis – A condition characterized by chronic inflammation of the lining of the bladder wall. It causes considerable pain and discomfort and is diagnosed generally as a result of not finding a causative agent for the inflammation.

Interstitial Nephritis – Inflammation of the interstitial tissue of the kidney, including the tubules. It may be acute or chronic. Acute interstitial nephritis is an immunologic, adverse reaction to certain drugs, such as sulfonamide or methicillin.

Isosthenuria – Condition where the kidney loses the ability to concentrate or dilute the glomerular filtrate, resulting in the specific gravity becoming fixed at about 1.010.

Jaundice – Yellow-orange discoloration of the skin due to an increase in the concentration of free or conjugated bilirubin in the blood (serum) with accumulation of bilirubin in the body tissues.

Kernicterus – A severe cerebral form of icterus neonatorum associated with hemolytic disease of the newborn.

Ketosis – Increased concentration of ketones in the blood (ketonemia) and urine (ketonuria).

Kidney – One of two glandular bodies in the lumbar region that secrete urine. Each adult kidney is about 4x2x1 inch (10x5x2.5 cm) in size and weighs about 4-6 oz (115-170 g).

Leukocyte Esterase – An enzyme present in the azurophilic or primary granules of the granulocytic leukocytes. An increased concentration of this enzyme in the urine indicates the presence of white blood cells (pus cells) resulting from a urinary tract infection or other inflammatory process. Urethritis, caused by a sexually transmitted disease, will also result in a positive leukocyte esterase test in urine.
Lipiduria – Presence of fats of biological origin in urine. These may be cholesterol or triglycerides (neutral fats).

Loop of Henle – The U-shaped portion of the nephron between the proximal and distal convoluted tubules. It consists of a thin descending (concentrating) limb and a thick ascending (diluting) limb.

Low Power Examination – Usually a 10x magnification objective and a 10x ocular, which gives a total magnification of 100x. It is used for the initial screening and observation in a microscopic urinalysis. This permits the observation of larger formed elements such as casts, which may be reported as the number/LPF.

Lower Urinary Tract – Portion of the urinary tract excluding the kidneys (i.e., the ureters, bladder, and urethra). Lower urinary tract infections, also known as cystitis, are generally infections of the bladder.

Maltese Cross – White cross on a black background, characteristic of cholesterol ester droplets in urine or other body fluids when viewed with polarized light. These must not be confused with granules of starch.

Medulla (Renal) – A part of the parenchyma of the kidney, beneath the cortex, which includes the renal pyramids and columns. It also includes the loop of Henle and collecting tubules.

Microalbuminuria – The consistent passage of small but abnormal amounts of albumin into the urine. The excretion of 30-300 mg/day or about 5-160 mg/L is considered to be microalbuminuria. Higher levels, detected by most urine protein dipsticks in screening, is called proteinuria. Microalbuminuria is an early indicator of diabetic nephropathy and cardiovascular disease.

NCCLS (Formerly the National Committee for Clinical Laboratory Standards) – The U.S. voluntary consensus standards organization that develops and publishes standards and guidelines for all areas of the clinical laboratory.

Nephritic Syndrome (NS) – A clinical syndrome characterized by massive proteinuria, hypoalbuminemia, edema, and lipiduria. It is a serious kidney condition.

Nephron – The working unit of the kidney, where urine is formed; includes the glomerulus, Bowman’s Capsule, proximal and distal convoluted tubules, and loop of Henle. Each kidney contains about 1-1.2 million nephrons that decrease in number with age.

Nephrotic – A substance that can damage the kidney.

Obstructive Jaundice, Post-Hepatic Jaundice – Jaundice resulting from blockage of the normal outflow of conjugated bilirubin from the liver and gall bladder into the intestine. This blockage may be due to a gall stone or a malignant tumor of the head of the pancreas.

Occult Blood – Blood in a specimen that is not observable by the naked eye. It is detected by a chemical test or microscopic examination.

Osmolality – A measure of the number of solute particles per unit of solvent, or the number of mols of solute per liter of solution.

Oval Fat Bodies; Renal Tubular Fat – Renal epithelial cells (also macrophages or histiocytes) filled with fat droplets (cholesterol or neutral fat), which are associated with the presence of free fat globules and fatty casts.

Peroxidase – An enzyme that catalyzes the release of free oxygen from hydrogen peroxide. The peroxidase-like activity of the heme portion of the hemoglobin molecule is the basis of strip tests for blood.

Phase Contrast Microscopy – A microscope illumination system that uses a special condenser containing an annular diaphragm with a matched absorption ring in the corresponding objective. It is used to give additional contrast in wet preparations and is especially useful for observing urine sediment.

Polarizing Microscope – A microscope illumination system that employs two polarized lenses that are crossed, extinguishing passage of light through the microscope. It is used to detect objects or crystals that bend or polarize light, making them visible when viewed with crossed polarized filters.

Porphobilinogen – A colorless precursor of the porphyrins, which are a group of compounds utilized in the synthesis of hemoglobin.

Precise – The repeatability or reproducibility of a test result. A measure of the closeness of repeat test results on the same sample. A test can be precise but not accurate.

Pre-Eclampsia – A condition that occurs during pregnancy, usually after the 20th week of gestation. The patient develops hypertension with albuminuria/proteinuria and/or edema. If untreated, it can develop into eclampsia, which is often fatal.

Proficiency Testing (PT) – A program, usually sponsored by an accrediting agency, under which samples are sent to a group of laboratories or testing sites for analysis. Test results are compared with other laboratories participating in the program. It is included as a component of a quality assurance program and gives a measure of uniformity of test results among laboratories. Participation in a proficiency program is required under CLIA ’88 for specified analytes for moderate and high-complexity laboratories.

Protein Error-of-pH Indicators – A phenomenon in which, at a fixed pH, certain pH indicators will show one color in the presence of protein and another if protein is absent.

Protein-to-Creatinine Ratio (P:C Ratio) – Corrects for variations in the urine concentration as a means of determining the significance of small amounts of protein in urine. The P:C ratio is useful because urine concentration varies greatly over time; slightly elevated levels of protein may be normal if they are found in a concentrated specimen. Conversely, low levels may be significant if found in dilute urines. The use of a protein to creatinine ratio “corrects” for variations in the urine concentration as a means of determining the significance of small amounts of protein in urine.

Proteinuria – Abnormal amounts of protein in urine, clearly detected by most urine protein test strips at levels above 300 mg/L (30 mg/dL).

Proximal Convoluted Tubules – Convoluted tubules of the nephron closest to the glomerular capsule, where about 80% of the fluid and electrolytes filtered through the glomerulus is re-absorbed.

Pyelonephritis – Acute pyelonephritis is usually the result of an infection that ascends from the lower urinary tract to the kidney. Chronic pyelonephritis develops slowly after bacterial infection of the kidney. It is usually associated with obstruction and may progress to kidney failure.

Pyuria – The presence of white blood cells in the urine; a sign of inflammation or infection.

Quality Assurance (QA) Program – Comprehensive set of policies, procedures, and practices used to ensure that the laboratories reported results are reliable and can be used by the physician to diagnose or treat the patient. QA includes all aspects of the laboratory (technical and non-technical) to prevent errors and ensure the accuracy of test results. It includes pre-analytical, analytical, and post-analytical factors such as specimen collection, record-keeping, calibration and maintenance of equipment, quality control, proficiency testing, and training.

Quality Control (QC) – Part of a quality assurance program utilizing a set of laboratory procedures designed to ensure that a test method is performing properly. QC includes the routine use of control materials, record-keeping, and analyzing them statistically.
Random Voided Urine Sample – A urine specimen obtained at any point of a 24-hour period. To compensate for variations in concentration over the day, a ratio of the result of an analyte (e.g., protein) to the creatinine concentration helps to “correct” for the urine being either very concentrated or dilute.

Refractive Index – A measure of dissolved substances in solution, determined by ratioing the velocity of light in air to the velocity of light in the solution. The refractive index of urine corresponds to the specific gravity of the urine.

Refractometer – An instrument used to measure the refractive index of a solution. In urinalysis, a special relative specific gravity scale is used in the device.

Renal Epithelial Cells, Renal Tubular Epithelium – Epithelial cells originating from the lining of the tubules of the kidney in the region of the proximal to the collecting tubules. Their appearance varies and depends on the actual site of origin. A cytological examination can differentiate the types of cells.

Renal Epithelial Fragment – A fragment of renal epithelial cells, originating from the collecting ducts and consisting of three or more attached renal cells.

Renal Failure Cast – A synonym for waxy cast, indicating serious kidney pathology.

Sensitivity – The minimum detectable level of an analyte in a specimen. Clinically, the proportion of subjects with a positive diagnostic test in a diseased population, expressed as a percent.

Specific Gravity – A measure of the amount of dissolved substances present in a solution. It is a ratio of the density of a solution compared to the density of an equal volume of pure, solute-free water at a constant temperature. It depends on the mass and number of particles in a solution.

Specificity – A statistical expression of the proportion of subjects with a negative diagnostic test in a population without the disease, expressed as a percent; a measure of the false positive rate. Analytically, specificity indicates the degree that a test method measures only the desired substance and is not detecting other unwanted substances.

Staghorn Calculus or Stone – Urinary calculi or stones that fill the kidney collecting system. They are especially characteristic of cystine, struvite (usually from an infection), and uric acid calculi.

Sternheimer-Malbin Stain – An all-purpose supravital stain consisting of crystal violet and safranin O; used in the Brightfield examination of urine sediment and available commercially as KOVA® Stain.

Supravital Stain – A stain that is taken up by cells and other biological objects without killing or destroying the cell.

Tamm-Horsfall Protein – Glycoprotein (mucoprotein) secreted by the renal tubular cells and not derived from the blood plasma. This protein forms the matrix of urinary casts. It is secreted as a lubricant to protect the renal tubular cells from the hostile environment of urine.

Transitional Epithelial Cells, Urothelial Cells – Stratified epithelial cell lining of the urinary tract from the pelvis of the kidney to the base of the bladder (trigone) in females and to the proximal part of the urethra in males. In the urinary tract, urothelial cells originate in structures located between structures lined by squamous and renal epithelial cells.

Trichomonas Vaginalis – A motile protozoan parasite that may infect the vagina, urethra, periurethral glands, bladder, prostate, and mouth. It may be seen in urine sediment, but a wet preparation of direct swabs from the urethra or vagina is more effective for its identification. Special culture methods are also available.

Ultrafiltrate of Plasma – Filtrate of plasma across a membrane where extremely small particles such as proteins are restricted or not filtered. Essentially, the filtrate has the same composition as plasma except that no protein is present.


Urea – The primary end-product of protein and/or amino acid metabolism; the result of amino acid and protein breakdown.

Ureters – The tubes that carry urine from the kidney to the bladder. Each kidney has one ureter.

Urethra – A small tubular structure that drains urine from the bladder. In men, the urethra also serves as a passageway for semen during ejaculation.

Urinalysis (Routine) – The testing of urine with procedures commonly performed in an expeditious, reliable, and cost-effective manner; includes physical, chemical, and microscopic analyses of urine.

Urinary System – Consists of two kidneys and two ureters, plus the bladder and urethra.

Urine Sediment – Any solid matter that is suspended in the voided urine. It includes cells, casts, crystals, and amorphous deposits of chemical compounds.

Urinometer – A specialized hydrometer calibrated to measure the specific gravity of urine at a given temperature. Measurement of specific gravity by urinometer is considered inaccurate and is discouraged by the NCCLS.

Urobilin – An orange-red or orange-brown pigment that is an oxidation product of urobilinogen. It is present in small amounts in normal urine and in increased quantities in urine containing high concentrations of urobilinogen.

Urobilinogen – A group of colorless chromogens formed in the intestine and produced by the reduction of bilirubin by normal bacterial flora. They are normal products of bilirubin metabolism.

Urochrome – A yellow pigment that gives the primary yellow color to normal urine.

Xanthochromia – The faint pink, orange, or yellow color of a body fluid caused by release of hemoglobin from hemolyzed red blood cells. It usually indicates the occurrence of a previous hemorrhage. Strictly speaking, xanthochromia refers to the production of a yellow color; however, the term is applied to pale pink, orange, or yellow when describing the color of spinal fluid specimens.
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Suggested Reading

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