URINALYSIS/URINE CHEMISTRIES

The kidney regulates the internal environment of the body by controlling electrolyte and water balance; and establishes the threshold for resaborption of needed substances such as glucose. In addition, unwanted toxic metabolic by-products such as urea are excreted. Thus, the kidney reacts to and thereby reflects the physiologic status of the body. As a result, it is ideal for indirect examination of the body's internal environment.

Urine Composition: Reflects the body's hydration status, dietary intake and basal metabolic rate. During disease states, increased levels of substances in the blood may appear in the urine, the most important being protein, sugar, ketones, bilirubin (bile), urobilinogen, and hemoglobin. In addition to various amounts of hormones, casts, crystals, and bacteria.

Daily Urine Output: Ranges from 750 to 2000 ml. Amounts greater or less than this are usually considered abnormal. The amount of urine excreted during the day is 3 to 4 times that excreted during the evening. Therefore, any quantitative test must take the time interval into account.

Purpose: The routine urinalysis is a valuable tool used as a screening procedure, or to verify or disprove a diagnostic hypothesis. It is used to check for: 1) diabetes mellitus; 2) acid-base, fluid, or electrolyte disturbance; 3) inflammation or infection. Information on renal tubular function is obtained from the specific gravity, or osmolality.

SPECIMEN COLLECTION

TIMING: A random urine specimen is usually adequate for qualitative examination. However, the first A.M. voided urine is best since it has the most uniform volume and concentration.

Urine must be examined while fresh. Decomposition sets in rapidly, altering test results. Therefore, it is critical that the specimen be handled correctly. A clean-catch, midstream urine
should be examined within 30 to 60 minutes of voiding. If urine is not going to be examined within this time, it must be preserved to avoid bacterial overgrowth and decomposition. Urine decomposes at room temperature. The resultant bacterial overgrowth and glucose utilization may yield a false-negative test for glucosuria. In addition, the production of ammonia by urea-splitting organisms may raise the pH of the specimen and dissolve any casts which may be present.

Preservation: The best method for preserving a urine specimen is refrigeration, which will not distort formed elements such as casts.

**SPECIMEN PREPARATION**

Routine chemical analysis of the urine should be performed on the supernatant portion of the centrifuged specimen, because uroepithelial cells, erythrocytes, and leukocytes, can give a false-positive sulfosalicylic acid test for protein.

Centrifuge 10 to 15 ml of urine at 2000 RPM for 3-5 minutes. Pour the supernatant into a separate tube for dipstick and SSA.

**SPECIMEN EXAMINATION**

Routine Urinalysis should include:

1. Physical Characteristics
   a. Color
   b. Clarity
   c. Odor
   d. Foam
   e. Specific Gravity
   f. Osmolality

2. Chemical Characteristics
   a. Urinary pH
   b. Protein and Sulfosalicylic Acid (SSA)
   c. Bence-Jones Protein
   d. Glucose
   e. Ketones
   f. Occult Blood
   g. Bilirubin and Urobilinogen
h. Nitrite

3. Microscopic Examination
   a. Erythrocytes
   b. Leukocytes
   c. Epithelial Cells
   d. Casts
   e. Crystals
   f. Bacteria

Physical Characteristics

Color: The amber color of urine is due to the presence of the yellow pigment, urochrome. The color of urine changes, and is affected by its concentration, constituents; food pigments, dyes, blood, etc. The color also changes in many disease states because of the presence of pigments which are not normally present.

Clarity: Normal urine is clear or transparent. It may be cloudy due to the presence of amorphous phosphates and carbonates if the urine is alkaline. This will disappear if the urine is acidified. Turbidity can also be seen in urinary tract infections. This is usually due to the alkalinity rather than to the actual number of leukocytes or bacteria present.

Odor: The odor of fresh urine is due to the presence of volatile acids. Urine that has been standing develops an ammoniacal odor due to decomposition of urea within the specimen. The urine of diabetics may have a fruity odor due to the presence of acetone; and may be foul-smelling in those with a UTI (mainly with coliform bacillus).

Foam: Nephrotic proteinuria and bilirubinuria alter the surface tension of the urine. Foam is produced by agitation and is characteristically yellow with excessive bilirubin excretion, and white with proteinuria.

Specific Gravity: Estimates the concentration of urine. It is not as accurate as urine osmolality. Specific gravity is defined as the weight of a given volume of solution compared to an equal
volume of distilled water. It is proportional to the number and weight of particles present. In normal urine where the primary solutes are sodium and potassium salts, ammonium, and urea, the specific gravity varies predictably with the osmolality. When larger molecules, such as glucose, and radiocontrast material, are present, there will be a disproportionate increase in specific gravity (sometimes exceeding 1.040), when the osmolality is similar to that of plasma. Specific gravity is increased with glucosuria, radiocontrast material, nephrotic syndrome with massive proteinuria, and carbenicillin.

**Osmolality:** Most accurate measurement of urine concentration. It is dependent upon the number of particles in solution and is determined by the freezing point or vapor pressure depression. Variations in urine osmolality (Uosm) are mediated by osmoreceptors in the hypothalamus which influence the secretion of antidiuretic hormone (ADH). After a water load there is a reduction in plasma osmolality. This is sensed by the osmoreceptors which decrease ADH release. As a result, a decreased amount of water is reabsorbed in the collecting duct and the urine becomes dilute (Uosm < Posm). Uosm can fall as low as 50 mosmol/kg and increase to 1200 mosmol/kg.

**Chemical Characteristics**

**Urinary pH:** Reflects the degree of urine acidification and acid-base balance. Useful in differentiating and diagnosing a variety of acid-base disorders. The pH can range from 4.5 to 8.0 but usually falls within 5.0 to 6.5. Urine pH > 7.5 suggests a UTI with an urea-splitting organism; the generation of ammonia raises the pH.

Urine pH has an important effect upon stone formation:
- **Low pH (<5.5)** favors the formation of uric acid and calcium oxalate stones.
- **High pH (>7.5)** favors the formation of calcium phosphate and calcium carbonate stones.

In addition, urine pH also affects the urine sediment; alkaline urine favors dissolution of casts.

**Protein and Sulfosalicylic Acid (SSA):** The supernatant should be analyzed since false-positive results can be obtained using an unspun urine with hematuria, pyuria, or uricosuria. Two methods are used; the dipstick is most sensitive to albumin, while SSA
measures all proteins including low molecular weight proteins.

SSA TEST: Approximately 7.5 ml of 3% SSA is added to 2.5 ml of supernatant urine. The tube is inverted once for complete mixing. Any precipitate is indicative of proteinuria and is abnormal. False-positives include some radiocontrast materials, massive amounts of penicillin, and tolbutamide metabolites.

Under normal circumstances both tests correlate fairly well. When the dipstick is negative and the SSA is positive this is often due to immunoglobulin light chain excretion. It is most commonly seen in multiple myeloma, but can also be seen in lymphoma, leukemia, macroglobulinemia, osteogenic sarcoma, and amyloidosis. Once proteinuria has been discovered, it must be put in the appropriate context, i.e., correlated with the urine concentration. Trace proteinuria in a dilute urine is more significant than in a concentrated urine, i.e., it may become 3+ when the urine becomes concentrated.

Glucose: Glucosuria occurs whenever the glomerular filtrate contains more glucose than the tubules are able to reabsorb, i.e., when the renal threshold is exceeded. It can occur in two types of conditions; diabetes mellitus when the blood glucose level exceeds 160-180 mg/dl; and when there is a renal tubular defect causing decreased reabsorption. The latter is usually seen in Fanconi's Syndrome, cystinosis, and heavy metal poisoning.

Ketones: Whenever there is a decrease in carbohydrate intake or metabolism, fatty acids are utilized and 3 ketone bodies are formed; acetoacetic acid (AcAc), acetone, and B-hydroxybutyric acid (BHB). All 3 ketones are excreted in the urine the following relative proportions: 20% AcAc, 2% acetone, and 78% BHB. Current tests for ketones detect only acetone and AcAc. When lactic acidosis is also present, as much as 90% can exist as BHB, and yield a false-negative urine test for ketones. To convert BHB to acetone add a few drops of hydrogen peroxide and then retest.

Occult Blood: Positive in hematuria, hemoglobinuria, and myoglobinuria. The dipstick detects free hemoglobin. Intact RBCs are lysed by the test pad and hemoglobin is then detected.

Bilirubin and Urobilinogen: Bilirubinuria indicates either hepatocellular disease, or intra or extrahepatic biliary obstruction. Since unconjugated bilirubin is bound to albumin, it cannot pass through the glomerulus and does not appear in the urine. Conjugated bilirubin is freely filtered by the glomerulus.
Bilirubinuria usually occurs when the serum level of conjugated bilirubin reaches approximately 2 mg/dl.
Urobilinogen appears in the urine in the presence of hepatocellular disease and hemolysis.

**Nitrite:** Indirect test for bacteriuria. Positive when UTI caused by E. Coli, Enterobacter, Citrobacter, Klebsiella, Proteus sp.

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**Microscopic Examination**

After separating the supernatant, resuspend the sediment at the bottom by GENTLY flicking the side of the tube. Pipette the resuspended sediment onto 3 glass slides for: microscopic analysis (coverslip), Hansel stain- air dry, and Gram stain- air dry. Scan the coverslip slide with the low power field (100x) specifically searching for casts, cells, white blood cell clumps, crystals. When these objects are encountered, the objective should be changed to the high power field (400x). If needed, the Hansel stain may be used to detect eosinophiluria, present in interstitial nephritis; and a gram stain used to detect bacteriuria.

**Cells:**

**Erythrocytes:** Normal urine contains no more than 2-3 RBC/HPF. RBCs can originate from any site in the urinary tract. Cell morphology may be very helpful in determining the site of bleeding. In extraglomerular lesions, RBCs usually have a fairly uniform shape. In contrast, glomerular lesions usually yield dysmorphic RBCs in which the cell membranes have been traumatized during passage through the glomerular capillary. One may see blebs, budding or segmental loss of membrane structure. Cells may be confused with yeast. However, yeasts are ovoid, not round; appear in chains, and show budding.

**Leukocytes:** Normal urine contains no more than 3-4 WBC/HPF. WBCs are slightly larger than RBCs and are distinguished by their granular cytoplasm and multilobed nuclei. When present in increased numbers indicative of infection at some site in the urinary tract. The presence of WBC casts localize the infection to the kidney. Isolated pyuria is also seen in interstitial nephritis (may be accompanied by eosinophiluria), and kidney transplant rejection. Pyuria is also seen in acute glomerulonephritis, and the nephrotic syndrome but proteinuria, hematuria, and RBC casts usually predominate.
Epithelial Cells: Can originate from the proximal convoluted tubule to the urethra.

Renal tubular epithelial cells: Normally see a few cells per slide which indicates normal cellular turnover. Increased numbers of cells, sheets, or casts of cells indicate tubular damage as seen in pyelonephritis, acute tubular necrosis, salicylate intoxication and kidney transplant rejection. However, they may also be seen in glomerulonephritis and the nephrotic syndrome.

Transitional epithelial cells: Originate from the bladder. Large numbers suggest inflammation or tumor.

Squamous epithelial cells: Originate from the urethra and vagina. Large numbers suggest vaginal contamination rendering the specimen inappropriate for culture.

Casts: Are formed within the nephron. Under normal conditions, a mucoprotein (Tamm-Horsfall protein) is secreted by the tubular cells in the thick ascending limb of the loop of Henle. When conditions favor protein precipitation (acid pH, tubular stasis, proteinuria), any cells within the lumen will be trapped in the cast resulting in a cellular cast.

Hyaline casts: When the tubular lumen is clear, the cast will be composed primarily of mucoprotein and is called a hyaline cast. They are not indicative of renal disease and can be seen in increased numbers in effective circulating volume depletion.

RBC casts: ALWAYS PATHOLOGIC! Indicates damage to glomerular basement membrane. Present in glomerulonephritis, and vasculitis; and have been reported in renal infarction, severe pyelonephritis, and interstitial nephritis.

WBC casts: Found in pyelonephritis, and interstitial nephritis. Also seen in acute glomerulonephritis, and the nephrotic syndrome although proteinuria and RBC casts usually predominate. Cells may degenerate and form granular casts.

Granular casts and waxy casts: Represent successive stages in cellular cast degeneration. Waxy casts are thought to occur in nephrons with lengthened transit time from markedly diminished flow, as seen in renal failure. Granular casts have also been reported in normal subjects after exercise.

Epithelial cell casts: Damaged renal tubular cells sloughed into the tubular lumen. Usually arranged in parallel rows and indicate desquamation of an entire nephron segment.

Broad casts: "Renal failure casts". Wide casts formed in the distal portion of the nephron segment. Indicate severe stasis and large numbers of nonfunctioning nephrons. Appear in chronic
renal failure and in severe acute renal failure.

Fatty casts: Usually seen when there is heavy proteinuria, but also seen with ethylene glycol and mercury intoxication. Free floating fat droplets "lipiduria" may be present in the urine and can be identified by a maltese-cross pattern under polarized light. Fat droplets may also appear as vacuoles within degenerating cells (oval fat bodies), or as fatty casts.

Crystals: Formation of crystals is determined by the pH of the urine, the concentration of the reactants, and the presence of inhibitors of crystallization. Uric acid crystals and amorphous urates are formed in an acidic urine (pH <5.5). Calcium phosphate crystals are formed in an alkalotic urine (pH >7.0). Calcium oxalate and cystine crystals are independent of pH.

Cystine crystals: Hexagonal shape and diagnostic of cystinuria.

Calcium oxalate crystals: Envelope appearance. When these crystals occur in the setting of acute renal failure, high anion gap metabolic acidosis, and a plasma osmolar gap >20 (measured Posm-calculated Posm); think increased oxalate production from ethylene glycol ingestion.

**URINE CHEMISTRIES AND FORMULAS**

Proteinuria: Estimation of proteinuria from a random urine specimen. More useful than urine dipstick, can estimate severity and follow the course more closely. Does not vary with urine concentration (like the dipstick) as it affects both parameters and does not change the ratio. Correlates closely with total protein excretion in grams/day per 1.73 m2 body surface area.

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\text{Uprotein/ Ucreatinine} < 0.2 \quad (\text{< 200 mg/day total protein excretion})
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Fractional excretion of sodium (FENa): Calculated from a random urine specimen. Used primarily in acute renal failure, much less useful in patients with normal renal function.
FENa (%) = \frac{\text{quantity of Na excreted}}{\text{quantity of Na filtered}} \times 100

= \frac{\text{UNa} \times \text{PCr}}{\text{PNa} \times \text{Ucr}} \times 100

FENa < 1 in hypovolemic states, radiocontrast nephropathy

> 2 in acute tubular necrosis

Fractional excretion of chloride (FECl): Useful in a patient that seems to be volume depleted but has an elevated UNa, or a FENa >1. This most often occurs in the setting of metabolic alkalosis. Urinary excretion of bicarbonate promotes sodium excretion (NaHCO3) and can elevate the UNa even in the setting of volume depletion. A hint is that the UCl will remain low (<20).

Urine electrolytes:

**Urinary sodium** (UNa): Most frequently used in the differential diagnosis of acute renal failure, and to determine effective extracellular fluid (ECF) volume status.

UNa < 20: Hypovolemic states
- Decreased effective arterial volume (congestive heart failure, nephrotic syndrome, liver failure, hepatorenal syndrome, burns.
  (Kidney is able to conserve sodium suggesting intact tubular function)

UNa > 20:
- No renal disease: Osmotic diuresis (glucose, mannitol, urea)
  - Diuretics
  - Primary adrenal insufficiency
  - Metabolic alkalosis with bicarbonaturia

Intrinsic renal disease: Acute renal failure
- Renal tubular dysfunction
- Chronic renal disease