Bio& 242: Unit 2 / Lab 2
Urinalysis Lab

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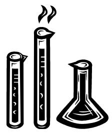


Urinalysis can reveal diseases such as diabetes mellitus, various forms of glomerulonephritis, and chronic urinary tract infections.

The most cost-effective device used to screen urine is a paper or plastic dipstick. This microchemistry system has been available for many years and allows qualitative and semi-quantitative analysis within one minute by simple but careful observation. The color change occurring on each segment of the strip is compared to a color chart to obtain results. Microscopic urinalysis requires a light microscope and a centrifuged urine sample.

METHODS OF URINE COLLECTION

1. Random collection taken at any time of day with no precautions regarding contamination. The sample may be dilute, isotonic, or hypertonic and may contain white cells, bacteria, and squamous epithelium as contaminants. In females, the specimen may cont contain vaginal contaminants such as trichomonads, yeast, and during menses, red cells.
2. Early morning collection of the sample before ingestion of any fluid. This is usually hypertonic and reflects the ability of the kidney to concentrate urine during dehydration which occurs overnight. If all fluid ingestion has been avoided since 6 p.m. the previous day, the specific gravity usually exceeds 1.022 in healthy individuals.
3. Clean-catch, midstream urine specimen collected after cleansing the external urethral meatus. A cotton sponge soaked with benzalkonium hydrochloride is useful and non-irritating for this purpose. A midstream urine is one in which the first half of the bladder urine is discarded and the collection vessel is introduced into the urinary stream to catch the last half. The first half of the stream serves to flush contaminating cells and microbes from the outer urethra prior to collection. This sounds easy, but it isn't (try it yourself before criticizing the patient).
4. Catherization of the bladder through the urethra for urine collection is carried out only in special circumstances, i.e., in a comatose or confused patient. This procedure risks introducing infection and traumatizing the urethra and bladder, thus producing iatrogenic infection or hematuria.
5. Suprapubic transabdominal needle aspiration of the bladder. When done under ideal conditions, this provides the purest sampling of bladder urine. This is a good method for infants and small children.

<u>MACROSCOPIC URINALYSIS</u>

The first part of a urinalysis is direct visual observation. Normal, fresh urine is pale to dark yellow or amber in color and clear. Normal urine volume is 750 to 2000 ml/24hr.

Turbidity or cloudiness may be caused by excessive cellular material or protein in the urine or may develop from crystallization or precipitation of salts upon standing at room temperature or in the refrigerator. Clearing of the specimen after addition of a small amount of acid indicates that precipitation of salts is the probable cause of turbidity.

A red or red-brown color could be from a food dye, eating fresh beets, a drug, or the presence of either hemoglobin or myoglobin. If the sample contained many red blood cells, it may be cloudy as well as red.

URINE DIPSTICK CHEMICAL ANALYSIS

(Record your urine dipstick results on the Urinalysis Results Form provided by your instructor)

pH:

The glomerular filtrate of blood plasma is usually acidified by renal tubules and collecting ducts from a pH of 7.4 to about 6 in the final urine. However, depending on the acid-base status, urinary pH may range from as low as 4.5 to as high as 8.0. The change to the acid side of 7.4 is accomplished in the distal convoluted tubule and the collecting duct. In general, people with high protein (meat) diets will have a urine pH in the acid range and a person who is a vegetarian will have a urine pH in the alkaline range.

Specific Gravity (sp gr):

Specific gravity (which is directly proportional to urine osmolality which measures solute concentration) measures urine density, or the ability of the kidney to concentrate or dilute the urine over that of plasma. Dipsticks are available that also measure specific gravity in approximations. Most laboratories measure specific gravity with a refractometer. In this SFCC lab we will use a urinometer/hydrometer to measure specific gravity as described by your instructor.

Specific gravity between 1.001 and 1.030 on a random sample should be considered normal if kidney function is normal. Since the sp gr of the glomerular filtrate in Bowman's space ranges from 1.007 to 1.010, any measurement below this range indicates hydration and any measurement above it indicates relative dehydration. Dilute urine may result when a person drinks excessive amounts of fluids, uses diuretics, or suffers from diabetes insipidus or has chronic renal failure. High specific gravity may be due to limited fluid intake, fever, or inflammation of the kidneys such as in pyelonephritis. Excessively concentrated urine may precipitate or crystallize and form renal calculi (kidney stones).

If sp gr is not > 1.022 after a 12 hour period without food or water, renal concentrating ability is impaired and the patient either has generalized renal impairment or nephrogenic diabetes insipidus. In end-stage renal disease, sp gr tends to become 1.007 to 1.010.

Any urine having a specific gravity over 1.035 may be contaminated or possibly contains very high levels of glucose or other dissolved substances.

Protein:

Dipstick screening for protein: Normally, only small plasma proteins filtered at the glomerulus are reabsorbed by the renal tubule. However, a small amount of filtered plasma proteins and protein secreted by the nephron   Normal total protein excretion does not usually exceed 150 mg/24 hours or 10 mg/100 ml in any single specimen. More than 150 mg/day is defined as proteinuria. Proteinuria > 3.5 gm/24 hours is severe and known as nephrotic syndrome.

Dipsticks detect protein by production of color with an indicator dye, Bromphenol blue, which is most sensitive to albumin.

Trace positive results (which represent a slightly hazy appearance in urine) are equivalent to 10 mg/100 ml or about 150 mg/24 hours (the upper limit of normal). 1+ corresponds to about 200-500 mg/24 hours, a 2+ to 0.5-1.5 gm/24 hours, a 3+ to 2-5 gm/24 hours, and a 4+ represents 7 gm/24 hours or greater.

Glucose:

Less than 0.1% of glucose normally filtered by the glomerulus appears in urine (< 130 mg/24 hr). Glycosuria (excess sugar in urine) generally means diabetes mellitus. Dipsticks employing the glucose oxidase reaction for screening are specific for glucose but can miss other reducing sugars such as galactose and fructose. For this reason, most newborn and infant urines are routinely screened for reducing sugars by methods other than glucose oxidase (such as the Clinitest, a modified Benedict's copper reduction test).

Ketones:

Ketones (acetone, aceotacetic acid, beta-hydroxybutyric acid) resulting from either diabetic ketosis or some other form of calorie deprivation (starvation), are easily detected using urine dipsticks. Ketonuria may result from starvation or diets low in carbohydrates since the body is forced to use fat stores. Ketonuria and glycosuria together, is generally diagnostic for diabetes mellitus.

Nitrite:

A positive nitrite test indicates that bacteria may be present in significant numbers in urine. Gram negative rods such as E. coli are more likely to give a positive test. Presence of bacteria in urine (bacteriuria) may be confirmed by observing the urine microscopically.

Leukocyte Esterase:

A positive leukocyte esterase test results from the presence of white blood cells either as whole cells or as lysed cells. Pyuria can be detected even if the urine sample contains damaged or lysed WBC's. A negative leukocyte esterase test means that an infection is unlikely and that, without additional evidence of urinary tract infection, microscopic exam and/or urine culture need not be done to rule out significant bacteriuria.

Blood / Hemoglobin:

The urine dipstick can detect whole red blood cells as well as lysed RBC's. A positive result for blood may be verified by observing the urine specimen under the microscope.

MICROSCOPIC URINALYSIS

Methodology

A sample of well-mixed urine (usually 10-15 ml) is centrifuged in a test tube at relatively low speed (about 2-3,000 rpm) for 5-10 minutes until a moderately cohesive button is produced at the bottom of the tube. The supernate is decanted and a volume of 0.2 to 0.5 ml is left inside the tube. The sediment is resuspended in the remaining supernate by flicking the bottom of the tube several times. A drop of resuspended sediment is poured onto a glass slide and coverslipped. A drop of methylene blue stain may be added if staining of the cells is desired.

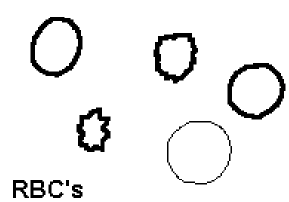
Examination

The sediment is first examined under low power to identify most crystals, casts, squamous cells, and other large objects. The numbers of casts seen are usually reported as number of each type found per low power field (LPF). Example: 5-10 hyaline casts/L casts/LPF. Since the number of elements found in each field may vary considerably from one field to another, several fields are averaged.

Next, examination is carried out at high power to identify crystals, cells, and bacteria. The various types of cells are usually described as the number of each type found per average high power field (HPF). Example: 1-5 WBC/HPF.

Red Blood Cells

Hematuria is the presence of abnormal numbers of red cells in urine due to: glomerular damage, tumors which erode the urinary tract anywhere along its length, kidney trauma, urinary tract stones, upper and lower urinary tract infections, nephrotoxins, and physical stress. Red cells may also contaminate the urine from the vagina in menstruating women or from trauma produced by bladder catherization. Theoretically, no red cells should be found, but some find their way into the urine even in very healthy individuals. However, if one or more red cells can be found in every high power field, and if contamination can be ruled out, the specimen is probably abnormal.

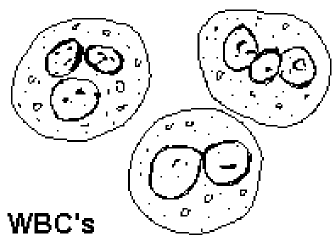


RBC's may appear normally shaped, swollen by dilute urine (in fact, only cell ghosts and free hemoglobin may remain), or crenated by concentrated urine. Both swollen, partly hemolyzed RBC's and crenated RBC's are sometimes difficult to distinguish from WBC's in the urine. In

addition, red cell ghosts may simulate yeast. The presence of dysmorphic RBC's in urine suggests a glomerular disease such as a glomerulonephritis. Dysmorphic RBC's have odd shapes as a consequence of being distorted via passage through the abnormal glomerular structure.

White Blood Cells

Pyuria refers to the presence of abnormal numbers of leukocytes that may appear with infection in either the upper or lower urinary tract or with acute glomerulonephritis. Usually, the WBC's are granulocytes. White cells from the vagina, especially in the presence of vaginal and cervical infections, or the external urethral meatus in men and women may contaminate the urine.



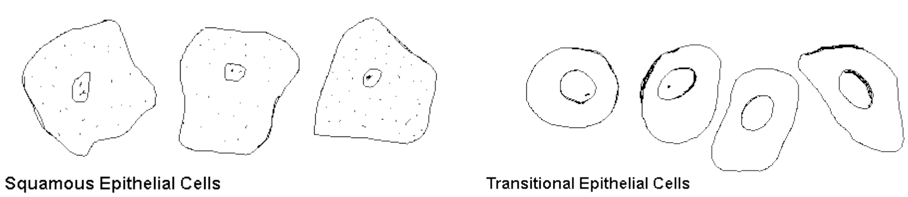
If two or more leukocytes per each high power field appear in non-contaminated urine, the specimen is probably abnormal. Leukocytes have lobed nuclei and granular cytoplasm.

Epithelial Cells

Renal tubular epithelial cells, usually larger than granulocytes, contain a large round or oval nucleus and normally slough into the urine in small numbers. However, with nephrotic syndrome and in conditions leading to tubular degeneration, the number sloughed is increased.

Transitional epithelial cells from the renal pelvis, ureter, or bladder have more regular cell borders, larger nuclei, and smaller overall size than squamous epithelium. Renal tubular epithelial cells are smaller and rounder than transitional epithelium, and their nucleus occupies more of the total cell volume.

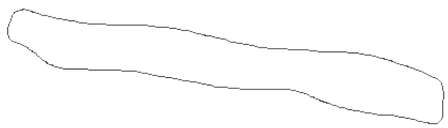
Squamous epithelial cells from the skin surface or from the outer urethra can appear in urine.



Their significance is that they represent possible contamination of the specimen with skin flora.

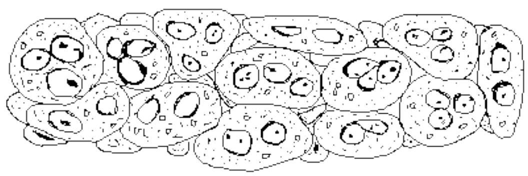
Casts:

Urinary casts are formed only in the distal convoluted tubule (DCT) or the collecting duct (distal nephron). The proximal convoluted tubule (PCT) and loop of Henle are not locations for cast formation. Hyaline casts are composed primarily of a mucoprotein secreted by tubule cells. Even with glomerular injury causing increased glomerular permeability to plasma proteins with resulting proteinuria, most matrix or "glue" that cements urinary casts together is mucoprotein, although albumin and some globulins are also incorporated.



Hyaline Cast

Red Blood Cell Cast



White Blood Cell Cast

Granular Cast

Waxy Cast

The factors which favor protein cast formation are low flow rate, high salt concentration, and low pH, all of which favor protein denaturation and precipitation, particularly that of the mucoprotein. Protein casts with long, thin tails formed at the junction of Henle's loop and the distal convoluted tubule are called cylindroids. Hyaline casts can be seen even in healthy patients.

Red blood cells may stick together and form red blood cell casts. Such casts are indicative of glomerulonephritis, with leakage of RBC's from glomeruli, or severe tubular damage.

White blood cell casts are most typical for acute pyelonephritis, but they may also be present with glomerulonephritis. Their presence indicates inflammation of the kidney, because such casts will not form except in the kidney.

When cellular casts remain in the nephron for some time before they are flushed into the bladder urine, the cells may degenerate to become a coarsely granular cast, later a finely granular cast, and ultimately, a waxy cast. Granular and waxy casts are be believed to derive from renal tubular cell casts. Broad casts are believed to emanate from damaged and dilated tubules and are therefore seen in end-stage chronic renal disease.

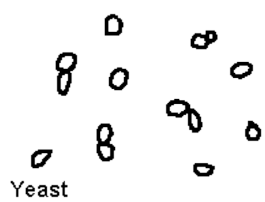
Bacteria

Bacteria are common in urine specimens because of the abundant normal microbial flora of the vagina or external urethral meatus and because of their ability to rapidly multiply in urine standing at room temperature. Therefore, microbial organisms found in all but the most scrupulously collected urines should be interpreted in view of clinical symptoms.

Diagnosis of bacteriuria in a case of suspected urinary tract infection requires culture. A colony count may also be done to see if significant numbers of bacteria are present. Generally, more than 100,000/ml of one organism reflects significant bacteriuria. Multiple organisms reflect contamination. However, the presence of any organism in catheterized specimens should be considered significant.

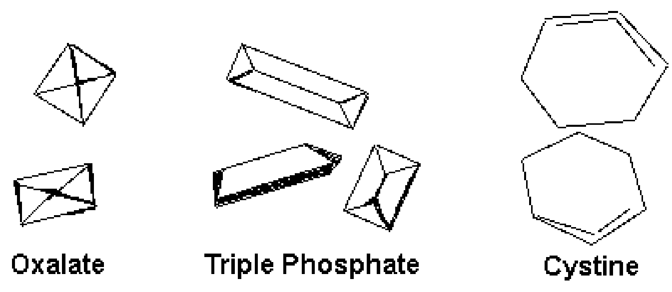
Yeast

Yeast cells may be contaminants or represent a true yeast infection. They are often difficult to distinguish from red cells and amorphous crystals but are distinguished by their tendency to bud. Most often they are *Candida albicans* species, which may colonize bladder, urethra, or vagina.



Crystals:

Common crystals seen even in healthy patients include calcium oxalate and amorphous phosphates.



Miscellaneous:

Unidentifiable objects may find their way into a specimen, particularly those that patients bring from home. “Colored worms” are often cloth fibers from the person’s underwear. Pubic hairs may also appear wormlike.

Spermatozoa can sometimes be seen in both male and female urine specimens.

Summary

To summarize, a properly collected clean-catch, midstream urine after cleansing of the urethral meatus is adequate for complete urinalysis. In fact, these specimens generally suffice even for urine culture. A period of dehydration may precede urine collection if testing of renal concentration is desired, but any specific gravity > 1.022 measured in a randomly collected specimen denotes adequate renal concentration so long as there are no abnormal solutes in the urine.

Another important factor is the interval of time which elapses from collection to examination in the laboratory. Changes which occur with time after collection include: 1) decreased clarity due to crystallization of solutes, 2) rising pH, 3) loss of ketone bodies, 4) loss of bilirubin, 5) dissolution of cells and casts, and 6) overgrowth of contaminating microorganisms. Generally, urinalysis may not reflect the findings of absolutely fresh urine if the sample is > 1 hour old. Therefore, get the urine to the laboratory as quickly as possible.

Terms Used to Describe Abnormal Result From Urinalysis:

Albuminuria: Excessive amount of albumin in the urine. (Greater than 300 mg/d)

Bilirubinuria: Excessive amounts of bilirubin in the urine.

Glucosuria: Excessive glucose in the urine.

Hematuria: Present of Red blood Cells in the urine.

Hemoglobinuria: Presence of free hemoglobin in the urine

Ketonuria: Accumulation of ketone bodies in the urine

Proteinuria: Excess protein in the urine

Pyuria: Pus (collection of white blood cells) in the urine.

Terms Associated with Alterations of Urine Formation or Micturition:

Anuria: Absence or suppression of urine formation

Calculus: A kidney stone

Cystitis: Inflammation of the bladder

Diuresis: The increased formation of urine by the kidney.

Dysuria: Painful or difficult urination.

Incontinence: Inability to control micturition

Polyuria: The excessive production of dilute urine (at least 2.5 liters per day for an adult)

Urethritis: Inflammation of the urethra.

URINALYSIS TEST RESULTS Name:

MICROSCOPIC EXAM: (Only to be used with Student Specimen)

	Normal Values	Results:
WBC's	0 – 5 / hi pwr field	
RBC's	0 – 5 / hi pwr field	
Bacteria	small numbers present	

Epithelial Cells Observed:

Crystals Observed:

Casts Observed:

Urine observations: Normal results expected:		**Student**	**Patient #1**	**Patient #2**	**Patient #3**	**Patient #4**
Color	Lt > Dk Yellow					
Transparency	Clear					
DIP STICK Results:						
Specific Gravity	1.001-1.030					
pH	4.5 – 8.0					
Leukocytes	Negative					
Nitrite	Negative					
Protein	Negative					
Glucose	Negative					
Ketones	Negative					
Urobilinogen	Negative					
Bilirubin	Negative					
Blood	Negative					
Hemoglobin	Negative					

Results:

Indicate possible reasons for any Positive results for your data or the other patient's data above. Use the correct clinical term to describe the positive result observed.