Physiology of the Kidney and Formation of Urine

The kidney is a highly discriminating organ, which maintains the internal environment by selectively excreting or retaining various substances according to specific body needs. Approximately 1,200 mililiters of blood flow through the kidneys each minute. This represents about one-fourth of the 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 the low molecular-weight components of the plasma. They filter through the capillary walls and the closely adhering membrane of Bowman's capsule into Bowman's Space from where the plasma ultra-filtrate passes into the tubule where reabsorption of some substances, secretion of others, and the concentration of urine occur. Many components of the plasma filtrate 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 reabsorbed 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 a mechanism for conserving body water. Urine formed by the three physiological processes that are by glomerular filtration, tubular reabsorption, and tubular secretion, is collected by the collecting duct and passes into bladder through ureters and then comes out through urethra.

The Composition of Urine

Normal Urine Constituents	Abnormal Urine Constituents
- Water (about 95% of urine)	- Glucose
- Urea	- Protein
- Creatinine	- Bile pigments
- Uric acid	- Blood cells
- Electrolytes	- Cast parasites and Bacterial microbes

The Factors Affecting The composition of Urine

- Diet and nutritional status
- Condition of body metabolism
- Ability of kidney function
- Level of contamination with pathogenic microorganisms (bacteria) or even non-pathogenic microflora

Renal Clearance and Renal Threshold

Renal Clearance

Renal Clearance value indicates the degree to which a substance is removed from the blood by excretion in the urine. Clearance is usually defined as the blood volume that contains the quantity of a substance excreted in the urine per minute. About 120 ml of glomerular filtrate is produced per minute. The rate at which the glomerular filtrate is formed is known as the

glomerular filtration rate (GFR). Creatinine is a substance present in the filtrate, which is not reabsorbed (however, this is some tubular secretion of creatinine). Therefore the clearance of creatinine from the plasma is 120 ml per minute. Hence creatinine clearance is used clinically to give an approximate indication of glomerular filtrate rate and, therefore, as a test of kidney function.
When the filtration rate falls, the concentration of creatinine in the plasma rises. The creatinine clearance test express the volume of blood containing the amount of creatinine excreted by the kidney in one minute.

The creatinine clearance (Ccr) is calculated by collecting a 24 hr urine specimen, and a blood sample as well within the urine collection time. Creatinine is then determined in both urine and serum, and the creatinine clearance calculated in milliliters per minute (ml / minute)

$$\text{Ccr ml / minute} = \frac{U \times V}{S}$$

Where U= Urine Creatinine Concentration in mol/l
V= Volume of urine in ml per 24 hrs
S= Serum Creatinine Concentration in mol/l

Normal Range:

The normal Ccr value usually ranges between 110 – 140 ml / minute.

Renal Threshold

The renal threshold of a substance refers to the highest concentration of a substance, which is present in the blood before it is found in the urine. A substance such as glucose is a high threshold substance, because it is completely absorbed from the glomerular filtrate and is only found in the urine, when the blood glucose level is markedly raised. Urea and creatinine, however, are always present in the urine independent of the blood level because very little, if any, of these substance is reabsorbed.

Collection And Preservation Of Urine Specimen

Objectives

It is expected that the information presented in this chapter will enable the student to:

- Identify factors affecting the quality of a specimen.
- List the basic rules of urine collection.
- Describe types of urine specimens.
- Identify the commonly used preservatives and know the advantages and disadvantages of their use.

Collection of Urine Specimen

In order to make Urinalysis reliable the urine must be properly collected. Improper collection may invalidate the results of the laboratory procedures, no matter how carefully and skillfully the tests are performed.

Urine Containers

There are many types of containers used for collecting urine. Before specimens are collected, the containers must be cleaned and thoroughly dried. Disposable containers of plastic or coated paper are available in many sizes and are provided with lids to reduce bacterial and

other types of contamination. Special polyethlene bags are available for collection of urine from infants and children who are not toilet trained. URIN-TEK disposable collection system is available for use in collecting, storing, transporting, and testing specimens of urine. The system consists of a flat-bottomed paper collection cup, a 15 ml plastic tube with a plastic snap-cap and self-adhesive identification label. Disposable tube holders are available for handling ten tubes at a time. The patient voids directly into the paper cup, transfers the specimen to the URIN-TEK tube, and covers it with the plastic cap to prevent contamination or spillage. After the label is filled out and attached, the specimen is ready to be transported and analyzed. Specific gravity can be run directly in the URIN-TEK tube if a colorimetric strip or a urinometer is used. By using the convenient reagent strips, many chemical tests can be performed directly in the URIN-TEK tube, making additional laboratory glassware unnecessary. This procedure also decreases the risk of identification errors because transferring and relabeling of the specimens is not necessary. Large, wide-mouthed plastic or glass containers with screw cap tops are used for cumulative collection of urine over a long period of time. These bottles should be kept refrigerated. When urine is to be cultured for bacterial content, the specimen must be obtained under septic condition and collected in a sterile glass container or a sterile disposable plastic container. In either case, the receptacle should be 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

A freshly voided urine specimen is adequate for most urinalysis except the microbiological culture. The patient should be instructed to void directly into a clean, dry container, or a clean, dry bedpan so that the specimen can be transferred to an appropriate container. Specimens from infants and young children can be collected in a disposable collection apparatus. If a urine specimen is likely to be contaminated with vaginal discharge or menstrual blood, this period has to be avoided and the patient must be informed to bring a clean-voided specimen. All specimens should be immediately covered and taken to the laboratory.

Types of Specimen

1) First Morning Specimen - a specimen obtained during the first urination of the day.
    - Most concentrated
    - Bladder incubated

    Best for:
    - Nitrite
    - Protein
    - Microscopic examination
2) Random Specimen - a specimen obtained at any time during examination.
    - Most convenient
    - Most common

    Good for:
    - Chemical Screen
    - Microscopic examination
3) Second-voided Specimen - In this case first morning specimen is discarded and the second specimen is collected and tested.

    Such type of specimen is good for:
    - Reflection of blood glucose.

- Keeping of formed elements intact.

4) Postprandial: a specimen obtained 2 hours after meal. Good for glucose.
5) 24- Hour specimen - a specimen obtained within 24 hours.
    - Necessary for quantitative tests, especially for quantitative determination of protein.

    Procedure for Collection of 24 hour Urine Specimen

    (i) Inform or direct the patient to completely empty his bladder and discard his urine exactly at the beginning of the 24 hour time collection (let say at 6:00 a.m.).
    (ii) Collect all urine voided during the following 24 hours, including that voided exactly at the end of the 24 hour period in a container (at 6:00 a.m.) of the following (second) day.
    (iii) All the urine collected must be preserved.

    The container should be labeled with:
    - The test order
    - The patient's name
    - Time of collection
    - The preservative added

6) Mid- stream Specimen - a specimen obtained from the middle part of the first urine.
    - It is commonly used for routine urinalysis.
    - It is also important for bacteriological urine culture.

7) Clean Catch Urine Specimen
    Used for microbial culture and routine urinalysis. When specimens are collected for bacteriological examination they should be collected by the 'clean catch' method or by catheterization into sterilized container. Catheterization is the process of passing a tube through the urethra to the bladder for the withdrawal of urine (it may introduce urinary tract infection).

    The best method is properly collected 'clean catch' urine, which is collected as follows:
    (i) The genital area should be cleaned with soap and water and rinsed well. This is to keep off bacteria on the skin from contaminating the urine specimen.
    (ii) The patient should urinate a small amount and this is discarded.
    (iii) The urine that comes next, the mid-stream specimen, should be collected into a sterile container of 30 to 50 ml. After obtaining the specimen the patient continues to urinate and this is discarded.

Sources of Errors in the Collection of Urine

1) Bacteriologically or chemically contaminated specimen.
2) Wrong type/amount of preservative.
3) Partial loss of specimen or inclusion of two-morning specimen in the 24 hr collection.
4) Inadequate mixing of specimen before examination.
5) Careless measuring of the 24 hr volume.

Preservation of Urine Specimen

Urine should be examined immediately as much as possible after it is passed, because some urinary components are unstable. If urine specimen cannot be examined immediately, it must be refrigerated or preserved by using different chemical preservatives. The maximum time that urinary contents to be maintained in urine specimen is one hour. Long standing of urine at room temperature can cause:

- Growth of bacteria
- Break down of urea to ammonia by bacteria leading to an increase in the pH of the urine and this may cause the precipitation of calcium and phosphates.
- Oxidation of urobilingen to urobilin.
- Destruction of glucose by bacteria.
- Lysis of RBCs, WBCs and casts.
- Precipitation of urates crystals in acidic urine while calcium and phosphates precipitates in alkaline
- Oxidation of bilirubin to biliverdine

Method of Preservation of Urine Specimen

1) Physical Method
   - ✓ Refrigeration
   - ✓ Freezing
2) Chemical Method

   Use of chemical preservatives such as :
   - Thymol, Toluene, Formaldehyde, Hydrochloric acid (HCl),
   - Chloroform
   - Boric acid, Chlorhexidine
   - Sodium carbonate

Methods	Advantages	Disadvantage
Refrigeration (2-6^{o}C)	No chemical Interference	Use for a short period of time (3-6 hours). For prolonged periods additonal preservatives must be used
Freezing	For specimen transport	May destroy formed elements
Toluene (Till it forms thin layer over the urine)	Preserves acetone, Reducing Substances, protein	Flammable
Thymol (small crystal 5 mm diametre/100ml urine)	Preserves most consitiuents	Can cause false positives for proteins

Chloroform (1 tablet/60 ml urine)	Preserves urine aldosterone level	Settles to the bottom of the urine containers
Formaldehyde (1 drop/30 ml urine)	Preserves formed elements	Interfers with glucose evaluation
HCL (1 drop/15 ml urine)	Stablizes steroides, catecolamines	Formed elements are destroyed,
Boric acid	Preserves chemicals and formed elements	Precipitate uric acid
Sodium Carbonate	Preserves porphyrines and urobilinogen	Interfers with other urine constituents

Type of Examination in Routine Urinalysis

Specimen evaluation

- Specimen is evaluated in terms of acceptability e.g, labelling, proper specimen, proper preservatives, visible signs of contamination, any transportation delays etc.

Physical Examination of Urine

- Volume
- Color
- Odor
- Appearance
- pH
- Specific gravity

Chemical Examination of Urine

- Glucose
- Protein
- Ketones
- Bilirubin
- Urobilinogen
- Blood
- Nitrite
- Leukocyte Esterase
- Indican
- Melanin

Microscopic Examination of Urine

- RBCs
- WBCs
- Epithelial cells
- Casts
- Bacteria
- Yeasts
- Parasites
- Crystals
- Artifacts

Categories of Urine Tests

According to their degree of accuracy urine tests are grouped into three broad categories:

- Screening tests
- Qualitative tests
- Quantitative test

Screening tests tell only whether a substance is present or absent, and the results are reported as positive or negative. They are done on random specimen.

Qualitative tests give rough estimate of the amount of substance present. They are also called semi-quantitative tests. The results of qualitative tests can be graded as negative, trace, +1, +2, +3 or +4.

Quantitative tests determine accurately the amount of the substances to be tested. However, since they are time consuming, they are not included in routine urinalysis. Most common quantitative tests performed in urinalysis laboratory are those for sugar and for protein. The results of a quantitative test are usually reported in milligrams per deciliter, gram per deciliter, and per liter. For quantitative test, a complete 24-hour urine specimen is needed. An appropriate preservative should be added to the container or the specimen should be stored in refrigerator.

Physical Examination of Urine

Objective

At the end of this chapter, the student shall be able to carry out physical examination of urine such as odor, volume, color, transparency, foam, specific gravity, pH of urine and interpret the result of the investigation so that to identify further the necessary type of examination (chemical or microscopic or both).

Introduction

Physical examination of urine is the first part of routine urinalysis. It is the simplest procedure of all urine examination, but this simplicity does not mean that anyone can do it without any background knowledge and experience. Physical examination of urine usually gives hint for the subsequent urinalysis. For example, white turbid urine sample may suggest to the technician the presence of Leukocytes (pus cells) and/or Epithelial cells in microscopic examination, and in chemical examination, with positive result of Nitrite.

Volume

Normally, 600 – 2000 ml of urine is voided per 24 hr. Volume of urine excreted is related to:

- Individual fluid intake
- Body temperature
- Climate
- Individual's health status
- Abnormally higher amount (greater than 2000 ml/24) or very low amount i.e. less than 600 ml/24 occur mostly due to some pathological conditions.

Test Procedure

For the measurement of the volume of urine, the patient should collect 24 hr urine specimen. The laboratory technician supplies the urine container, and it should be

- Clean and dry.
- Brown colored to avoid direct sunlight contact with the collected urine and interaction of sunlight with the chemicals.
- Contains appropriate preservative for the desired urine chemical test, or that is kept after each urine collection within refrigerator.
- Labeled on the wall, that indicates
    - Name of patient
    - Collection time and date
    - Type of chemical test ordered
    - Preservative used

* Using graduated cylinder does measurement of urine volume. The amount is recorded in terms of ml/24 hr.

Clinical Significance

The Measurement of the volume of urine indicates the evaluation of fluid balance and kidney function. When an individual excretes more than 2000 ml of urine/24 hr, consistently (for long period) it is called Polyuria. It may occur due to:

- Diabetic mellitus
- Diabetic insipidus
- Certain tumors of brain and spinal cord
- Acromegaly
- Myxedema
- Some type of tubular necrosis(improper function of urine tubules)

Any increased amount of urine volume, even if for short period, is called Diuresis. It is usually due to excessive fluid intake. Excretion of constantly small amount of urine, i.e. below 400 ml of urine/24 hr is called Oliguria. It may occur due to:

- ✓ Dehydration or poor blood supply to kidney that may be due to prolonged vomiting, diarrhea, etc.
- ✓ Obstruction of some area of the urinary tract/system (mechanical)
- ✓ Cardiac insufficiency
- ✓ Various renal diseases such as glomerulonephritis, etc.
- ✓ Fasting
- ✓ Excessive salt intake etc.

Complete absence of urine excretion, is called Anuria. It is less than 100 ml of urine per 24 hr. It may occur due to:

- ✓ Complete urinary tract obstruction
- ✓ Acute renal failure
- ✓ Acute glomerulonephritis
- ✓ Hemolytic transfusion reaction, etc

Polyuria may result physiologically after consumption of

- ✓ Intravenous glucose or saline
- ✓ Coffee, alcohol, tea, caffeine

- ✓ Pharmacological agent, such as thiazides and other diuretics

Odor

Normally fresh voided urine from healthy individuals has faint aromatic odor, which comes from volatile acids, normally found in urine, mostly, ammonia.

Test Procedure

The test is conducted by smelling of urine and the result is based on the perception of the technician.

Clinical Significance

Abnormal urine odor may result from aging of urine, disease and diet.

- If the urine specimen is old, i.e. after collection, left on the bench without preservative for more than 2 hrs, it will have ammonical (pungent) odor. The ammonical odor result is due to break down and conversion of urea in the urine into ammonia by the action of bacteria.
- Cystinuria and homocystinuria (type of amino acids, voided from abnormal metabolism) have sulfurous odor.
- Oasthouse urine disease has a smell associated with the smell of a brewery (yeast).
- Tyrosenemia is characterized by cabbage like or “fishy” urine odor.
- The presence of ketone bodies in the urine that may be due to diabetes mellitus, vomiting, starvation, strenuous exercise, characterized by “sweet fruity” odor.
- Butyric / hexanoic acidemia produce a urine odor resembling that of sweat.
- Urine of infants, which has inherited amino acid metabolism disorder, smells like “burnt sugar” or maple, hence the name, “maple sugar urine disease”.
- Also due to some food stuff such as asparagus, characteristic, urine odor is produced, which has no clinical significance.

Foam

Normally when urine specimen is voided in a container, it produces small amount of white foam. But during certain abnormal physiological and metabolic conditions, the color and amount of foam may be changed. For example, when there is high bile pigment in the urine, the amount of foam increases, and the color of foam becomes yellowish. This may indicate the presence of bilirubin in the urine. But the presence of yellowish foam should not be taken as a confirmatory test for the presence of bilirubin in urine. Chemical analysis of urine for billirubin should be done.

Color

Normally color of urine may vary within a day; in the morning it has dark yellow color, while in the afternoon or evening, the color ranges from light yellow to colorless. Normal urine color varies from straw (light yellow color) to dark amber (dark yellow).

- Light yellow indicate that the urine is more diluted, and has low specific gravity. Such exceptional condition occurs in case of diabetic mellitus. In this condition the color of urine is mostly light yellow, but because of having high glucose content, its specific gravity is high.
- On the other hand, dark amber (dark yellow) color mostly indicates that the urine is concentrated, and has high specific gravity. This type of urine is seen normally in the first morning urination.

- Normal urine color results from three pigments. They are:
  - ✓ Urochrome, responsible for yellow color formation. This pigment is found in high proportion than the other two.
  - ✓ Uroerythrin, responsible for red color formation.
  - ✓ Urobilin, – responsible for the orange-yellow color formation. Thus, normal urine gets its color from a combination of the above-mentioned three pigments.

Procedure of the Test

Urine color is recorded, after looking at freshly voided urine specimen. If the urine sample color is not recorded within 30 minutes after collection, chemical changes will occur in it, and so its color will change, and will result in false report.

Clinical Implication

By observing the color of freshly voided urine, an experienced laboratory technician can forecast the possible findings in the chemical and microscopic examination of urine. Depending up on the constituents of urine, the abnormal color of urine varies as follows:

- ✓ Pale to colorless urine may indicate:
  - o Large fluid intake
  - o Diabetic mellitus
  - o Diabetic insipidus
  - o Alcohol consumption
  - o Nervousness
- ✓ Dark yellow or brown red urine may indicate:
  - o Concentrated urine
  - o Decreased fluid consumption
  - o Dehydration
  - o Fever
  - o Certain urinary tract medication (eg. phenazophyridine)
  - o Yellow brown or “beer brown” color may indicate the presence of bilirubin.

This is also confirmed:

- By looking at the yellow foam or green foam by shaking the sample.
- By letting it to stand for more than 30 minutes and looking at the change of color into green, because of oxidation of bilirubin into biliverdin.
- Due to bilirubin crystals, as mentioned in urine segment, the urine samples have opalescent appearance.
- By doing chemical tests for bilirubin.

- ✓ Clear red may indicate presence of Hemoglobinuria (presence of hemoglobin in the urine). This hemoglobinuria may result from:
  - Incompatible blood transfusion.
  - Increased red blood cell destruction (intravascular haemolysis) due to different hemoparasites, e.g. Malaria.
  - Glucose – 6 - phosphate dehydrogenase deficiency.
  - Certain infections or disease.

- ✓ Cloudy red / smoky red color may indicate hematuria (presence of red blood cell in the urine).It differs from clear red by the presence of RBC rather than Hb alone. It is important to differentiate hemoglobinuria from hematuria, because the cause of this abnormal urine differs. On standing the red cell in hematuria may hemolize and settle and so the urine becomes clear red (hemoglobin in urine). To differentiate this the definition of specific gravity is important. Hematuria has high specific gravity than helmoglobinuria.
- ✓ Dark brown colored urine may contain porphyrines, melanin, homogenstic acid, which is associated with an abnormal metabolism of tyrosine. Milky urine may contain fat, cystine crystals, and many WBC or amorphous phosphates. Dark reddish color may indicate myoglobin (muscle Hgb), usually associated with extensive muscle injury, hemoglobinuria and porphyrine.

Interfering Factors

It is usually important to consider, that on standing of urine for more than 30 minutes, the urobilinogen that is found in urine will oxidize and change to urobilin. Thus due to this process, the color of urine becomes dark. Therefore, the physical examination of urine should be done immediately after the delivery of urine to the laboratory.

Other interfering factors that result in abnormal urine color formation are certain foodstuff, and medications.

- Food stuf, such as beets will give white red color.
- Drugs such as Vitamin B12 and riboflavin will give bright yellow color to urine.
- Rifampicin will give red color to urine.
- Iron salt will give dark color to urine.
- Sulfonamides will give rusty yellow or brownish color.

Therefore, when abnormal colored urine is observed, it is important to ask the patient, what kind of food he consumed in the last 36-24 hrs, and also whether he used drugs or not. If so, it is important to know what food and what drug he used.

Appearance (Transparency)

Fresh voided urine specimen is normally clear and transparent. On long standing, due to chemical changes that occur in normal constituents of urine through time, as described in the introduction part of this lecture note, it becomes turbid.

Procedure of the Test

- ✓ Appearance (transparency) of urine can be measured only by observation of fresh voided urine specimen.
- ✓ Degree of cloudiness of the urine is described by using common terms, starting by clear to turbid i.e. clear, hazy, cloudy, very cloudy and turbid.

Clinical Implications

- Freshly voided urine specimen appearance may indicate the presence of some abnormal constituents in it.
- Causes of turbid urine, as it is freshly voided includes:
    - White blood cells (pus cells) that occur due to UTI

- Kidney stones
- RBC's
- Yeast cells,
- High number of bacteria cells
- High number of epithelial cells
- Fat droplets in urine, which give opalescent appearance (rare condition).
- Amorphous urates, in case of gout and leukemia.
- High number of mucus trades. All the above findings are confirmed by urine microscopic examination.

Interfering Factors

High consumption of foodstuff that contains urates and phosphates may produce cloudy urine. This is because of the precipitation of urates and phosphates in the form of amorphus urate and phosphates respectively. Semen, or vaginal discharge mixed with urine is other common causes of urine turbidity. Urine specimen, stood for long period in the bench, will become hazy or cloudy due to precipitation of crystals, mucus trades etc., which normally occur in urine. The settlements of crystal and mucus trades seen in urine sample are to be preserved in refrigerator. Amorphous urates have "Brice red" precipitation, while amorphous phosphates have white precipitations.

pH

A test that determine acidity, neutrality or alkalinity of a solution.

- pH 7 indicates neutrality.
- pH < 7 indicate acidity.
- pH > 7 indicate alkalinity. Normally, freshly voided urine pH range from 5-7 in healthy individuals, and average is pH 6.

Procedure of the Test

pH of urine can be measured by using different techniques, such as by using:

- Litmus paper
- Nitrazine paper
- Dipstick
- Glass electrode

These different pH-measuring techniques vary in their sensitivity and reading techniques.

Litmus Paper

In this technique pH measurement takes place by using blue, and red litmus paper. The procedure is:

- ✓ Collect a freshly voided well mixed urine.
- ✓ Tear small blue litmus paper.
- ✓ Dip the paper, in the urine and remove immediately.
- ✓ Look for color change of blue litmus paper. If the blue colors of paper change to red, it indicates the acidity of the urine.
- ✓ Tear small red litmus paper.
- ✓ Dip the paper, in the urine and remove it immediately.
- ✓ Look for color change of red litmus paper.

If the red litmus paper change to blue, it indicates that the urine is alkaline. The blue and red litmus paper technique is a less sensitive method. This is because it indicates only the alkalinity or acidity of urine; it does not tell the exact quantity or figure of pH.

Nitrazine Paper

This is also a paper that changes its color from yellow (for acidic urine) to blue (for alkaline urine). The paper is impregnated with sodium dinitrophenolazo-naphthal disulphonate chemical. This chemical is responsible for the color change in acidic and alkaline urine. Unlike litmus paper, the color change is matched with reference color chart, and based on the value of color change on the reference color chart; the pH of the urine is recorded.

Procedure

The procedure of the test is:

- Tear small nitrazine paper
- Dip the paper in well mixed freshly voided urine sample and remove immediately
- Compare the color change with that of reference color chart.
- Record the value of color change form reference color chart.

Reference color chart -value range from 3 to 4 (for yellow color) to pH 9 (that is for deep blue color). The result of urine pH is usually reported by saying acidic or alkaline and by indicating the figure.

Urine Dipstick Method

This is a reagent strip test impregnated with chemicals called methyl red and bromethymol blue. These impregnated chemicals depending up on the concentration of hydrogen ion in the urine change their color from yellow (acid) to blue (alkaline). The color change is interpreted by comparing the reference color chart supplies with the reagent strip. pH indicator- strip is usually manufactured together with other tests for urine constituents.

Procedure

The procedure of the test is:

- Dip the reagent strip in the well mixed freshly voided urine and remove immediately.
- Remove the excess urine from the strip, by taping the strip at the edge of urine container.
- According to the time mentioned by the manufacturer to read the result, wait for full color development in the strip.
- Then read the color change by comparing within the reference color chart and report the result.
- The reference color chart value, range from 5 for acidic urine (yellow color) to 9 for alkaline urine (blue color).

Glass Electrode

This is a very sensitive pH measurement technique. The measurement is done by using small electronic instrument that has electronic pH value indicator and glass electrode. This instrument operates on battery or electricity.

Procedure

The procedure is to dip the glass rods in the freshly voided mixed urine specimen, and then look for the figure in the instrument to find out the value of pH.

Clinical Significance

As indicated in the chapter one, one of the functions of renal system is to regulate pH of blood i.e. keep pH of blood at 7.4 + 0.05. This is done by absorption or release of hydrogen ion, especially at distal convoluted tubules of the nephron, depending on the pH of blood, i.e. hydrogen ion absorbed from surrounding blood capillaries of nephrone when pH is acidic

(below 7.35), and release from nephron to the surrounding blood vessels when pH of blood is alkaline (above 7.45). pH measurement of urine, like other physical tests of urine, may indicate the on- going process in body, mostly about the renal system.
Normal pH of urine is 5-6.

* Persistent alkaline urine (pH > 6) may be caused by:
  - UTI
  - Renal failure
  - Vomiting
  - Anorexia nervosa
  - Alkalosis (metabolic or respiratory e.g. due to accumulation CO2 in our body.
  - Alkalizing drugs i.e. during intake of drugs such as streptomycin, kanamycin etc. eg. for UTI.
  - It should also important to bear in mind that certain vegetables, citrus fruits, and milk products also may cause alkaline urine, which is not pathological
* Persistent acid urine (pH < 6) may be caused by:
  - Diarrhea
  - Malabsorption syndromes
  - Diabetic ketoacidosis
  - Dehydration
  - Fever
  - Starvation
  - And also certain drugs such as – Phenacetic
  - Here it is important to bear in mind that high protein diet may also result in acidic urine, but this is not a pathological condition.
  - pH measurement is also important in the management of renal stone patients, who are being treated for renal calculi and who are frequently given diets or medications to change the pH of the urine so that kidney stone will not form.
  - Calcium phosphates, calcium carbonate, and magnesium phosphate stones develop in alkaline urine. In such instances the urine must be kept acidic (i.e. either by diet such as meat, or medication).
  - Uric acid, cystine, and calcium oxalate stones are precipitated in acidic urine. Therefore, as part of treatment, the urine should be kept alkaline (either by diet eg. leguminous plants, citrus fruits and most vegetables or by medication).

Interfering Factors

If urine specimen is left on the bench for more than 2 hours, bacteria will grow in it and by converting urea into ammonia, the pH will become alkaline. This is false alkaline urine, and indicates the specimen in not- fresh urine.

Specific Gravity of Urine

Specific gravity is defined as the ratio of the weight of a fixed volume of solution to that of the same volume of water at a specified temperature, usually 20^{o} C (in some books 25^{o}C). The

specific gravity of urine has been used for years as measure of the total amount of material dissolved in it (total solids), and thus of the concentrating and excretory power of the kidneys.

Measurement of Specific Gravity

The following methods are used to test the specific gravity of urine:

- Urinometer
- Refractometer
- Reagent strip
- Weighing technique

Specimen: It should be the first urine passed at the beginning of the day with the patient having taken no fluid for 10 hours. The testing of random urine specimen has little clinical value.

The Urinometer

The specific gravity of a urine specimen is often measured with a urinometer. The urinometer is a glass float weighted with mercury, with an air bulb above the weight and a graduated stem on the top . It is weighted to float at the 1.000 graduations in distilled water when placed in a glass urinometer cylinder or appropriate sized test tube. It is important that the cylinder, or test tube, be of the correct size so that the urinometer can float freely. The specific gravity of the urine is read directly from the graduated scale in the urinometer stem. The scale of the urinometer is calibrated from 1.000-1.060 with each division being equal to 0.001.

Calibration

To obtain the correct specific gravity readings in urine, the urinometer must be weighted to read exactly 1.000 in distilled water. The reading on the urinometer scale should be exactly 1.000. If it is not, a correction must be applied to all values obtained for urine specimens with the urinometer. For Example, Suppose the urinometer reads 1.002 in distilled water. The specific gravity of water is 1.000. Therefore the urinometer correction is 0.002 must be subtracted from the subsequent relative specific gravity. If a urine specimen has an apparent specific gravity of 0.037, this value minus 0.002 results in the corrected specific gravity of 1.035 for the urine specimen.

Temperature Correction

The specific gravity of a solution is dependent on temperature. Most urinometers are calibrated for use at 15 ^{o}C. For each 3^{o}C difference 0.001 must be added if above, or subtracted if below than the calibration temperature. For example, if the specific gravity of the urine is 1.022 at 23^{o}C, and the urinometer has been calibrated at 20^{o}C, the correct reading is 1.022+0.001= 1.023.However, significant error will result if the reading is taken on the urine specimen that has been refrigerated. Instead of applying this correction, the urine specimen should be allowed to warm up to room temperature before its specific gravity is determined.

Correction for abnormal Dissolved Substances

The specific gravity increases by 0.004 for every 1% glucose in urine and 0.003 for every 1% protein in solution. Therefore subtract 0.004 from the specific gravity reading for every 1% glucose in urine. And subtract 0.003 from the specific gravity reading for every 1% protein in the urine. It is not usual however for the Laboratory Technician to correct specific gravity readings for the presence of sugar or protein when laboratory results are reported. Instead, the clinician will be aware that the specific gravity is elevated because of the presence of sugar or protein and takes this into account in the assessment of kidney function.

Procedure for Using the Urinometer

1) Fill the urinometer cylinder or test tube to about 1 from the top with well mixed urine being careful so as not to cause it to foam.

2) Float the urinometer in the by rotting it rapidly to prevent its touching the bottom or side of the cylinder.
3) When it comes to rest, read the graduation on the stem of the urinometer at the level of the lower part of the meniscus. When the reading is taken, the urinometer must not be touching the sides of the container.
4) Record the reading.
5) If the quantity of the urine is too small to float the urinometer, the urine must be diluted with distilled water. The specific gravity is read and the last two digits of the specific gravity are multiplied by the amount of the dilution. This method is also used if the urine specific gravity is greater than the calibration on the urinometer.

Sample Calculation

If the urine is diluted 1:2 (one part of urine and two parts of water), the last two digits of the urinometer reading are multiplied by the dilution factor. If the reading of the specific gravity is 1.021, the last digit 0.021 is multiplied by the dilution factor 2 (0.021 x 2 = 0.042) and added to 1.000 (1.000 + 0.042 = 1.042). Hence the corrected specific gravity is 1.042.

Sources of Error:

- Temperature differences
- Proteinuria
- Glycosuria
- X-ray contrast media, it increases urine specific gravity
- Chemical preservatives

Urinometer Controls:

The following solutions can be used to check urinometers:

Solutions	**Specific gravity**
Pure water	1.000
Sodium chloride solution (2.5 g/dl)	1.018
" " " (5 g/dl)	1.035
" " " (7.5 g/dl)	1.051

Refractometer

It is an instrument, which reads the refractive index of the urine. The refractive index measurement depends on the number of dissolved particles in the urine. The higher the concentration of the particles the greater the refractive index, and so the specific gravity.

Reagent Strip Test of the Specific Gravity of Urine

A test area to determine specific gravity in urine can be found in the multiple test strip of Ames called N-multistix. The reagent test area responds to the concentration of ions in the urine. It contains certain pretreated polyelectrolytes. The pKa of which changes depending on the ionic concentration of the urine .The indicator bromothymol blue is used to detect the change. Colors ranges from deep blue when the urine is of low specific gravity through green to yellow-green when the urine is of high ionic concentration.

Chemical Analysis of Urine

Introduction

Chemical analysis of urine is an important procedure, which is impotant in the detection of many diseases. Urine contains normal chemical compositions. But in abnormal (pathological) conditions its composition varies in kind and quantities. So the chemical changes of urine can indicate disease at the early stage. The composition of urine varies because it is the principal route for soluble waste material from body metabolism. Its composiotion therefore depends greatly on how much and what specific waste material is to be excreted. Urea, creatinine, uric acid, ammonium salts, chlorides, sulphates and phosphates of sodium, potassium, calcium and magnesiumn are the normal composition of urine. They are excreted through the urine as a final body metabolism. Glucose, protein, ketone bodies, bilirubin , bile salts etc. are the abnormal constituents of urine. Normaly these substances do not appear in the urine in detectable amount . So their appearance in the urine shows the pathological condition. For example, glucose does not appear in the urine in detectable amount . But during diabetes mellitus it appears in the urine. Protein also appears in the urine during renal disease. Generally the chemical examination of urine helps to investigate the health condition of individual.

Objective:

Give basic knowledge to the students on how to perform chemical tests and show the various types of method used to determine the chemical
constituents of urine.

Determination of Urinary Sugar (Glucose)

Introduction

Glucose, a monosaccharide, is the principal sugar in blood, serving the tissues as a major metabolic fuel. It is mainly the end- product of carbohydrate digestion, which provides energy for life process. When body requires energy glucose oxidized to pyruvate and then to acetyl-CoA and enter cycle Krebs (tricarboxilic acid,TCA,cycle). Along these metabolic processes it gives energy in the form of adenosine triphosphate (ATP). ATP is very important energetic organic compound used for proper body function. When glucose is not required for the body's immediate energy needs, it is converted to glycogen and stored in liver and muscles by the metabolic process called glycogenesis. When there is an excess glucose in the blood (specially after carbohydrate meal), it can be also converted to fats. Glucose first oxidized to acetyl-

CoA through glycolysis. The formed excess acetyl-CoA and then converted to fats to be stored in the tissue. When it is required to maintain the blood glucose level, particularly during starvation, glycogen is converted to glucose by glycogenolysis. For maintaining the blood glucose level, it can be synthesized from non-carbohydrate precursors like amino acids, glycerol, lactate and etc. by the metabolic process, which is called gluconeogensis. The blood glucose level is controlled by a hormone, insulin, which is produced by the beta-islets of Langerhans of the pancreas. Insulin lowers the content of the glucose in the blood and increases its utilization and storage in the liver and muscle as glycogen. The absence or lower production of insulin resulted in Diabetes mellitus, which is characterized by an elevated blood glucose levels (hyperglycemia) and accompanying glycosuria and may be accompanied by changes in fat metabolism. Glucose is the sugar most commonly found in the urine, although other sugars , such as lactose, fructose , galactose, and pentose, may be found under certain condition. Normally, urine does not contain a sufficient amount of sugar to react with any of the popular enzyme or reducing tests. When sugar appears in the urine, it shows the abnormality caused by disease diabets mellitus.Hence urine sugar tests are extremly usefull in monitoring the treatment of diabetes.

Clinical Significance

The presence of detectable amount of glucose in the urine is known as glycosuria. Normally almost all the glucose, which passes from the blood into the glomerular filtrate, is reabsorbed back into the circulation by the kidney tubules (proximal convoluted tubules). Usually less than 15 - 20 mg/dl (0.8 mmol) is excreted in the urine. But this amount cannot be detected by the routine laboratory tests. The term glycosuria is usually used to describe the presence of more than the normal amount (15- 20 mg /dl) of glucose in the urine.
The occurrence of glucose in the urine is not normal if more than 15 - 20 mg/dl. The blood glucose concentration normally lies between 65 and 110 mg/dl. After a meal it may increase to 120 - 160 mg/dl. If the blood glucose concentration becomes too high (usually greater than 170 - 180 mg/dl), the excess glucose will not be reabsorbed into the blood and glucose start appearing in urine. The lowest blood glucose concentration that will result in glycosuria is termed as the renal threshold. The most common condition in which the renal threshold for glucose exceeds is diabetes mellitus.

Causes of Glycosuria

- Physiological
- Pathological Physiological

Sometimes under physiological situations, glycosuria can occur

a. After large ingestion of carbohydrates
b. Anything that stimulates sympathetic nervous system such as excitement, stress etc.
c. 15 to 20% cases of pregnancy may be associated with physiological glycosuria.
d. Renal Glycosuria:

In some persons, glycosuria is found when blood glucose is in normal range. This is known as renal glycosuria. This is again due to lowered renal threshold. Usually this is a benign condition.

Pathological Glycosuria

A. Diabetes mellitus

The most common condition for glycosuria is diabetes mellitus, a metabolic disorder due to deficiencies of insulin. Glucose is not properly metabolized and blood glucose concentration rises, and when it is in range of 170 - 180 mg /dl , glucose starts appearing in urine.

B. Glycosuria due to other endocrine disorders

Deranged function of a number of endocrine disorders can cause hyperglycemia and this may result in glycosuria, e.g. - Hyperthyroidism - Hyperadrenalism - Hyperpitutarism - Some diseases of pancreas

Types of Urinary Sugar (Glucose)Tests

ƒ Test for urine sugar is used to detect diabetes mellitus and also used to monitor the effectiveness of diabetic control. ƒ There are various tests for glucose which may be applied to urine. The most frequently used are : a. Non specific reduction tests based on the reduction of certain metal ions by glucose; b. Enzymatic tests based on the action of glucose oxidase on glucose.

Non- Specific Tests for Glucose

These tests are based on the ability of glucose to act as reducing substances. Tests that are based on the reducing ability of glucose, are not specific for glucose. In these tests, glucose is acting as a reducing agent, and any compound with a free aldehyde or ketone group will give the same reaction. Hence Glucose is not the only reducing substance that may be found in urine. Urine contine nonglucose reducing substance (NGRS) such as: uric acid, creatinine, galactose, fructose, lactose, pentose, levulose, homogentisic acid, ascorbic acid, chloroform, and formaldehyde. Commonly used non-specific tests for urinary sugar are Benedict's Qualitative Test and the Clinitest Tablet Test.

A. Benedict's Qualitative Test

Benedict is a very sensetive copper reduction test and may give positive reactions with non-specific non-glucose reducing substances normally present in urine. Since glucose is the reducing agent, it is oxidized to gluconic acid. The positive reaction is indicated by a color change. It is a qualitative test in which the degree of color formation is proportional to the amount of reducing substance present in the
specimen and the results are graded as negative, trace 1+, 2+, 3+, and 4+.

Principle

When boiled in an alkaline copper sulphate solution, glucose and other reducing substances reduce (convert) the blue copper (II) in Benedict's qualitative reagent to copper (I) oxide (Cu_2O), which is orange to red in color. A positive reaction is graded as a change in color ranging from blue to green, yellow, orange and finally red.
The overall reaction is:

Cupric ions +	reducing sugar	alkali	Cuprous ions +	Oxidized sugar
(CuSO4)	(eg. glucose)	heat	(Cu2O)	(e.g. gluconic acid)
(Blue)			(Orange-red)	

The copper (II) ions are supplied in Benedict's qualitative reagent in the form of copper sulphate (CuS04). In the presence of a strong alkali this is converted to copper (I) oxide (Cu2O). The heat is supplied by means of a boiling-water (100OC) bath. The tubes are brought back to room temperature, and the results are read when convenient.

Procedure:

1. Measure 8 to 10 drops or 0.5 ml of well-mixed urine in a test tube.
2. Add 5 ml of Benedict's qualitative reagent. Mix well.
3. Place in boiling-water bath for exactly 5 minutes (or boil in naked flame for exactly 2 minutes.
4. Remove from the boiling-water bath and immediately cool to room temperature in a cold water bath (about 10 minutes).
5. Observe the color change.

A positive reaction depends on the presence of a fine yellow, orange, or brick red precipitate. The test is then graded on the basis of the color of the mixed solution.

Grade results according to the following criteria:

Negative: No change in the blue color of the reagent or the occurrence of a white or green precipitate from phosphates in the urine.

Trace: Slight amount of yellow precipitate with a greenish blue to bluish green mixed solution. (This represents less than 500mg/dl of sugar). + : Moderate amount of yellow precipitate with green, often referred to as apple green, mixed solution. (Approximately 500mg/dl of sugar). ++: Large amount of yellow precipitate with a yellowish green, often called muddy green mixed solution. (Appr. 750mg/dl of sugar). +++: Large amount of yellow precipitate with green yellow, or muddy orange, mixed solution. Some blue color remains in supernatant. (Appr. 1000mg/dl of sugar) ++++: Large amount of yellow to red precipitate with reddish yellow to red mixed solution. No blue remains in the supernatant. (Appr. 2000mg/dl)

B. Clinitest Tablet Test

Principle:

This is a qualitative, non-specific test for sugar. The principle of clinitest is essentially the same as that of Benedict's Qualitative Test. The clinitest tablet may be thought of as a solid form of Benedict's Qualitative reagent. In addition, the clinitest tablet contains anhydrous sodium hydroxide, which results in moderate boiling when added to dilute urine gives heat in its reaction with citric acid. In other words, the heat for the reaction is also supplied in the tablet, making a boiling water bath unnecessary. The reaction for clinitest is analogous to Benedict's reaction. Results are also graded as negative, trace, 1+, 2+, 3+, or 4+ by comparison with a permanent color chart supplied with the tablets. Colors are comparable to those described for Benedict's Qualitative Test.

Procedure

Follow the directions supplied with the Clinitest tablets.

1. Place 5 drops of urine in a test tube and add 10 drops of distilled water.
2. Add one clinitest tablet.
3. Watch while boiling takes place, but do not shake.

4. Wait 15 seconds after boiling stops; then shake the tube gently and compare the color of the solution with the color scale.
5. Grade the results as negative, trace, 1+, 2+, 3+, or 4+. The results correspond to the following concentrations (mg/dl): trace, 250mg; 1+, 500mg; 2+, 750mg; 3+, 1000mg; and 4+, 2000mg.
6. Watch the solution carefully while it is boiling. If it passes through orange to a dark shade of greenish brown, the sugar concentration is more than 2000mg/dl and the result should be recorded as 4+ without reference to the color scale.

Contents of the tablet

Clinitest tablet contains copper sulphate, citric acid, sodium carbonate, and anhydrous sodium hydroxide.

Precautions

Observe the precautions in the literature supplied with clinitest tablets. The bottle must be kept tightly closed at all times to prevent absorption of moisture and must be kept in a cool, dry place, away from direct heat and sunlight.

Sensitivity

Clinitest reagent tablets will detect as little as 250mg of sugar in 100ml of urine.

Specific (Enzymatic) Tests

Enzymatic tests are specific tests for glucose. They are reagent strips (dipsticks), which are impregnated with enzymes glucose oxidases. Glucose oxidase catalyzes only the oxidation glucose to gluconic acid and hydrogen peroxide. The principle of all enzymatic, which is based on the uses of glucose oxidase, is the same. They differ only on the uses of different type of chromogen (a color indicator).

A. Clinistix Reagent Strip Test

Principle

This is a specific test for glucose based on the use of the enzyme glucose oxidase, which is impregnated on a dip strip. In this test glucose oxidase oxidizes glucose to gluconic acid and at the same time reduces atmospheric oxygen to hydrogen peroxide. The hydrogen peroxide formed , in the presence of the enzyme peroxidase, oxidizes the reduced form of o-toluidine(a chromogen) to oxidized form of the indicator, wich produces a color change proportional to the amount of glucose in the urine. Other sugars are not substrates for the enzyme do not react with this test.

A positive reaction is seen as a change of color from red to blue, depending on the amount of glucose present in the urine.

The overall reaction is :

$$\text{Glucose} + 0_2(\text{air}) \xrightarrow{\text{glucose oxidase}} \text{Gluconic acid} + \text{Hydrogen Peroxide } (H_2O_2)$$

$$H_2O_2 + \underset{\text{(red)}}{\text{o-tolidine}} \xrightarrow{\text{peroxidase}} \underset{\text{(blue)}}{\text{Oxidized o-tolidine}} + H2O$$

Contents of the reagent strip

The clinistix, reagent strip contains glucose oxidase,peroxidase,and 0-toluidine.

Procedure

Follow the directions supplied with the strips.

1. Rapidly dip the test end of the strip in the urine.

2. Read the results after exactly 10 seconds, looking for the presence of a purple color.
3. Record the results as positive or negative.

If the test area remains red, the result is negative. A positive result is indicated by the appearance of a purple color in the test area.

Sensitivity:

Clinistix is more sensitive to the presence of glucose than Benedict's Test or the Clinitest tablets and will detect 100mg/dl of glucose or less in the urine.

Precautions:

ƒ Observe the precautions in the literature supplied with the clinistix strips. The test area must be completely moistened, but excessive contact with the specimen will dissolve the reagents from the strip. The result must be read within 10 seconds.Falsely positive results may be obtained.

ƒ Large concentrations of ascorbic acid (vitamin C) cause false negative results or results that are delayed for 2 minutes or so, while bleach or peroxide may cause falsely positive reactions.

B. Tes - Tape Test for Glucose

Principle

Tes-Tape is also a screening test, specific for glucose. The principle of the test and the reaction are virtually identical to those of clinistix; the tests differ in the oxidation -reduction indicator employed, and the material the reagents are impregnated on. In Tes-Tape the reagents are impregnated on a tear strip of special paper, and the indicator is yellow in its reduced form and green to blue in its oxidized form. Therefore, a positive reaction is the appearance of a green to blue color.

Sensitivity

Like Clinistix, Tes-Tape is more sensitive to the presence of glucose than the Benedict's and clinitest methods and will detect 100mg/dl of glucose or less.

Contents of the test strip

Tes-Tape is impregnated with glucose oxidase, peroxidase, and an oxidation-reduction indicator in its reduced form.

Precaution

Observe the precaution in the literature with the product. Procedure

Follow the manufacture direction:

1. Tear off approximately 1 and 1/2 inch.
2. Dip part of the tape into the urine specimen; remove it immediately.
3. Wait for 30 seconds; then observe the appearance of any green color.
4. Record the result as positive or negative. If the test area remains yellow after 30 seconds, the result is negative. If any green color is present at this time, the result is positive.

C. Diastix Reagent Strip for Glucose

Principle

Diastix is a specific test for glucose based on the use of glucose oxidase, which is impregnated on the reagent strip. The chemical reaction is the same as for clinistix, the difference being the chromogen system used to indicate the presence of glucose. The reagent area contains glucose oxidase, peroxidase, a blue background dye, and potassium iodide as the chromogen. In a

positive reaction oxidation of potassium iodide results in the formation of free iodide, which blends with the blue background dye to give shades of green through brown. (The Boeringer dip-strip Test is also based on the same principle). As with clinistix, large amounts of ascorbic acid may give falsely negative or delayed results for glucose. This suppression is not as great as with clinistix, but it may cause problems. Bleach and hydrogen peroxide may cause falsely positive reactions, as with Clinistix.
Diastix has the advantage of being suitable as a screening test for the presence of glucose in the urine, and giving a rough estimate of the amount of glucose present. It detects as little as 100 mg of glucose per 100 ml of urine. However, urine specimens from pediatric patients must be subjected to a non-specific test for urinary sugar (Clinitest or Benedict's test) in addition to the specific glucose screening test in order to detect the presence of sugars other than glucose.

Diastix is incorporated in the glucose test area in various other multiple- reagent strips produced by the manufacturer, Ames Co. these other tests include: Combistix, Ketodiastix, Labstix, Multistix, Uristix and the like. Procedure
Follow the directions supplied with the reagent strip. 1. Dip the reagent area of the strip briefly into the specimen. 2. Compare the test area with the colour chart after 10 seconds to see whether the reaction is positive or negative for glucose. 3. Compare the test area with the color chart at 30 seconds, for a semiquantitative result, and report the results as indicated on the chart.

Sensitivity

Diastix reagent strip detects as little as 100mg of glucose in 100 ml of urine.

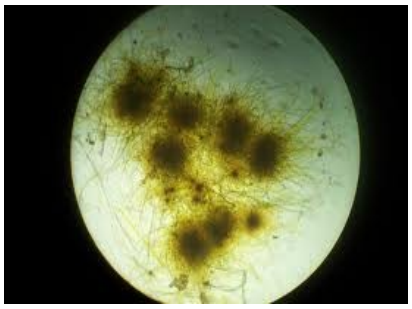
Identification of some reducing sugars

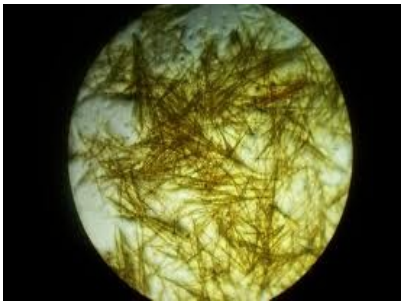
- Clinistix – for glucose
  - A reagent paper strip impregnated with glucose oxidase system which turns purple in presence of glucose
- Seliwanoff's – for fructose
  - Contains Resorcinol and conc. Hcl
  - Fructose when boiled with seliwanoff reagent forms red color
- Bials Test – for pentoses
  - Contains Orcinol and conc. Hcl
  - Pentoses (ribose, arabinose, xylose) when boiled with bials reagent forms green color
- Methylamine – for lactose and maltose
  - When heated with methylamine hydrochloride in alkaline medium at 56^{o}C, lactose & maltose turns red.
- Osazone – glucose, fructose and lactose
  - Some sugars when boiled with phenylhydrazine hydrochloride, forms yellow crystalline structures called osazone
  - They form easily and have needle-like or rod-like structures
  - Osazones formed from glucose and fructose are called phenylglucosazone
  - Osazones prepared from lactose are feathery and are referred to as phenyllactosazone
  - Phenyllactosazone crystals are more difficult to form than phenylglucosazone and are soluble in water

- These crystals are examined microscopically using x10 and x40

- <u>Yeast fermentation test – glucose and fructose</u>
  - Some sugars such as fructose, glucose and sucrose when incubated with yeast, yields ethyl alcohol which has a characteristic small and carbon dioxide which can be detected by turning lime water milky
- <u>Paper chromatography – for all sugars</u>







Phenylmaltosazone Phenyllactosazone
Phenylglucosazone

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Determination of Ketone Bodies

Introduction

Ketone bodies, also called Ketones, are a group of three related substances such as, acetone, acetoacetate (acetoacetic acid or diacetic acid), and β -hydroxybutyrate (β -hydroxybutyric acid). Ketone bodies are normal products of fat metabolism. They are normally not detectable in the blood or urine. In normal metabolism, fat is broken down in the tissues to glycerol and fatty acids. The free fatty acids are transported by the plasma albumin to the liver where they are broken down to acetyl coenzyme A (acetyl Co-A) molecules. These condense with oxaloacetate in the Krebs cycle to produce citrate. The citrate is then oxidized to produce heat and energy. Whenever there is inadequate carbohydrate in the diet or a defect in carbohydrate metabolism or absorption, the body metabolizes increasing amounts of fatty acids, which is then converted into excessive amount of acetyl-CoA. The extra acetyl-CoA molecules join up in pairs to form acetoacetic acid. Most of this is reduced to β-hydroxybutyric acid while some is decarboxylated to acetone. Acetoacetic and β-hydroxybutyric acids are transported in the blood to the peripheral tissues to serve as an alternative fuel for cells. In the peripheral tissues

these ketone bodies are reconverted to acetyl- CoA, and oxidized by the tricarboxylic acid cycle to give energy. Acetone is excreted in the urine.

Clinical Significance

When the rate of formation of ketone bodies is greater than the rate of their use, their levels begin to rise in the blood, which is called ketonemia, and eventually in the urine, which is known as ketonuria. These two conditions are seen most often in cases of starvation and diabetes mellitus. Ketone bodies can be seen also in the urine during prolonged vomiting, severe diarrhea, anesthesia, severe liver damage, high fat intake and low carbohydrate diet. The excessive production and accumulation of ketone bodies may lead to ketosis. Its physiological effect is serious because acetoacetic acid and β- hydroxybutyric acid contribute excess hydrogen ions to the blood, resulting in acidosis - a condition that tends to lower the blood pH. If not corrected in time this may result in death.

Another physiological effect of ketone accumulation concerns the substance acetone and acetoacetic acid. Both have been found to be toxic to brain tissue when present in increased amounts in the blood. So this condition can result in permanent brain damage. When ketones accumulate in the blood and urine, they do not occur in equal concentrations. β-hydroxybutric acid is present in the greatest concentration and acetone in the smallest concentrations. However most of the tests for ketonuria are most sensitive to the presence of acetoacetate. There are no simple laboratory tests for β-hydroxybutyric acid. Most tests react with acetone and acetoacetate or both.

Types of Tests for Ketone Bodies

A test for ketone bodies should be done routinely on any urine that is positive for glucose because they appear in the urine of diabetics. Test for ketones should be done with in 2 hours after collection Some of the commonly used tests for ketone bodies are the following:-

- Acetest tablet test,
- Acetone powder test,
- Reagent strip tests (Ex. Ketostix),
- Lang's test,
- Rothera's test.

Principle of the Tests

Both acetone and acetoacetate give a purple color with alkaline sodium nitroprusside. This is the general principle for the tests mentioned above.

Results - Report the test as positive or negative

A. Rothera's Test for Acetone and Acetoacetate

Procedure

1. To 5 ml of fresh urine, add ammonium sulphate crystals until saturated (about 1 g.).
2. Add 2 drops of sodium nitroprusside reagent and mix thoroughly.
3. Overlay with ammonium hydroxide solution (28% full strength).
4. If acetone or acetoacetate is present, a red to purple color will develop at the line of contact. The color may not appear for 10-15 minutes. Disregard any brown or orange colors.
5. Report the test as positive or negative.

Note: Urine collected after a big meal may give a purplish color within 30 seconds but it fades within 3-4 minutes. This is not a positive test.
Preparation of Sodium Nitroprusside Reagent (See Reagent Number15)

B. Lang's Test for Acetone and Acetoacetate

Procedure

1. Pour about 5 ml of fresh urine into a test tube.
2. Add 5 drops of glacial acetic acid and a few drops of saturated solution of sodium nitroprusside mix.
3. Slowly overlay with ammonium hydroxide (28%, full strength)
4. If acetone is present, a purple or reddish purple ring will appear at the meeting place of the two liquids.
5. Report the test as positive or negative.

Preparation of Saturated Sodium Nitroprusside (See Reagent Number 16)

C. Acetest Tablet Test

Procedure

1. Place an acetest tablet on a piece of white paper.
2. Place a drop of urine on the tablet.
3. If acetone or diacetic acid is present, a purple color will develop within 30 seconds. (compare with the color chart that comes with the reagent).
4. Report the test as positive or negative.

Contents of tablet The acetest tablet contains Sodium Nitroprusside (Nitroferricyanide), Glycine, and a strongly Alkaline buffer.

D. Acetone Powder Test

Procedure

1. Place a small amount of acetone powder on a clean sheet of paper.
2. Add three or four drops of urine.
3. A purple color is a positive test for acetone and diacetic acid.

Preparation of Acetone Powder Reagent (See Reagent Number 2)

E. Ketostix, Rreagent Strip Test

Procedure

1. Dip test- end of the strip in urine
2. At 15 seconds compare the color of dipped- end with the color chart.
3. Report as indicated by the color chart.

Contents of Reagent Strip

It contains Sodium Nitroprusside, Glycine, and a strongly Alkaline buffer. In alkaline medium, acetoacetic acid (diacetic acid) and acetone react with nitroprusside in the presence of glycine to form a purple complex.

Gerhardt's Test for Acetoacetate (Diacetic Acid)

Gerhardt's test is specific for acetoacetate (diacetic acid); however it is capable of detecting only large amounts of acetoacetate. The test has been used as a means of determining the severity of ketosis. A positive result indicates severe ketosis, and treatment must be started immediately. For this reason, Gerhardt's Test is performed whenever a positive reaction occurred with Rothera's or Lang's Test.

Principle of the Test

When acetoacetate (diacetic acid) reacts with a ferric chloride ($FeC1_3$) solution, Bordeaux red color is formed.

Procedure

1. To 5ml fresh urine in a test tube, add 10 % ferric chloride solution drop by drop until any precipitate of ferric phosphate dissolves. This generally takes 5 - 10 drops of ferric chloride. Filter if necessary, and add more ferric chloride.

If a red- brown to Bordeaux red (dark red) color appears, it merely indicates the possible presence of acetoacetate since other substances (phenol, salicylates, salicylic acid and sodium bicarbonate) can give a similar color.

To confirm the presence of acetoacetic acid, divide the test solution in half and boil one portion for 5 minutes. If the color disappears or becomes lighter after boiling, acetoacetic acid is present. If the color remains unchanged after boiling, one of the interfering substances is present. Report the result as positive or negative for acetoacetic acid.

Determination of Urinary Protein

Introduction

Protein is a macromolecule, composed of one or more polypeptide chains, each possessing a characteristic amino acid sequence and molecular weight. It has many biologically important functions. Some of the functions are acting as enzyme(e.g trypsin), transport protein (e.g hemoglobin, myoglobin) nutrient and storage protein (e.g ovalbumin (egg), casein (milk), contractile or motile protein (e.g actin, myosin) structural protein (e.g keratin, fibroin, collagen), defense protein (e.g antibodies, fibrinogen), and regulatory protein (e.g insulin, growth hormone).

Test for urinary protein is one of the most important and valuable parts of the routine urinalysis. Albumin is one of the important proteins, which appears in urine during a pathological condition. It often occurs as a symptom of renal disease. Globulins are excreted less frequently. Bence Jones protein is a specific type of globulin excreted in multiple myeloma.

Clinical Significance

The presence of protein in the urine is called Proteinuria. It is one of the most important indicator of renal disease. Its presence in the urine depends on the nature of the clinical and pathological disorder and the severity of the specific disease.

Causes of Proteinuria

1. Increased permeability of the glomerulus

Normally, the glomerular membrane, the initial stage in the formation of urine, is not permeable for protein molecules. If the glomerular membrane is damaged these large protein molecules can pass through, and end up in the urine.

2. A decrease in normal reabsorption in the tubules

Under normal conditions, the small amount of protein (with lower molecular weight), which does filter through the glomerulus, is reabsorbed back into the blood stream. Normal urine, therefore, contains only traces of protein, insufficient for detection by routine laboratory tests. However, the concentration of protein that normally filters into the glomerular filtrate is extremely small, and only 1% of the glomerular filtrate is eliminated from the body as urine;the rest is reabsorbed. Failure to reabsorb any protein from this large volume of glomerular filtrate will result in fairly large amounts of protein in the urine.

Types of Proteinuria

1. Accidental or false proteinuria

Accidental or False Proteinuria occurs when there is a mixture of urine with a proteinous fluid such as pus, blood or vaginal discharge. These can occur in infection of the kidney, bladder or vagina.

2. Physiological or functional proteinuria.

Physiological or functional proteinuria is protein excretion in association with fever, exposure to heat or cold, excessive exercise, emotional stress, and later stage of pregnancy. The underlying physiologic mechanism that induces proteinuria in all of these, is renal vasoconstriction.

3. Postural (orthostatic) proteinuria

Postural or orthostatic proteinuria is excretion of protein by patients, who are standing or sitting for a longtime. The proteinuria is intermittent and disappears when the individual lies down. It can also occur during abnormal curvature of spinal cord.

4. Renal or true proteinuria

Renal or true proteinuria occurs when protein passes from the blood into the urine because of some malfunction in the filtering system, either in the glomerulus or tubules.

Table .2 Proteins in Urine

Protein	**Condition (s)**
Albumin	Strenuous Physical Exercise Emotional Stress Pregnancy Infections Glomerulonephritis Newborns (Firs Week)
Globulins	Glomerulonephritis Tubular Dysfunction
Hemoglobin	Hematuria Hemoglobinuria
Fibrinogen	Severe Renal Disease Nucleoproteins WBCs in Urine Epithelial Cells in Urine
Bence Jones	Multiple Myeloma Leukemia

Tests for Urinary Protein

A. Precipitation or Turbidimetric Tests

Principle: The general principle of these tests is that protein is either precipitated out of the urine specimen by means of a chemical, which is usually a strong acid, or it is coagulated out of solution with heat. These tests include:

- Robert's test

- Heller's test (nitric acid ring test)
- Sulphosalicylic Acid Test (turbidmetric method)
- Heat and Acetic Acid Test (boiling method)
-Quantitative 24 hour Protein Determinations using Esbach's albuminometer

Turbidimetric test based on acid reagents are non-specific since any urine components, which is insoluble in acid, will give a positive result. It requires large volumes (0.5 to 5 ml) and requires either disposable tubes or glass tubes which must be cleaned for re-use. The results of the precipitation tests are read in terms of the amount of precipitate or turbidity that is formed in a test tube (in case of Heat and acetic acid, and Sulphosalicylic acid tests) or in terms of the size of
ring of contact between reagents in case of Robert`s and Heller`s tests. The amount of turbidity or precipitation is roughly proportional to the amount of protein present in the urine specimen, and the results are generally graded as negative, trace, 1+, 2+, 3+, or 4+. Since the result in precipitation tests is determined by the presence of either turbidity or a precipitate, it is important that the urine be free from particles or clear before the test is performed. To clear the urine, it should be filtered or centriguged. The clear filtrate is tested for the presence of protein.

The **non-ring** precipitation is read and interpreted as follows :
Negative - No turbidity, or no increase in turbidity (approximately 5 mg/dL or less)
Trace - Perceptible turbidity (approximately 20 mg /dL).
1+ - Distinct turbidity, but no discrete granulation (approximately 50 mg/dL).
2+ - Turbidity with granulation, but no flocculation (approximately 200 mg/dL).
3+ - Turbidity with granulation and flocculation (approximately 500 mg/dL).
4+ - Clumps of precipitated protein, or solid precipitate (approximately 1000mg/dL or more)

The Ring Test is read as follows:-
Negative - No cloudiness appears at the zone of contact
Trace - Ring is just perceptible against a black background
1+ - Ring is distinct against a black background, can barely be seen when held up to the light.
2+ - Ring is very definite against light, fairly visible when viewed from above
3+ - Ring is heavy against light, distinct cloudiness when viewed from above.
4+ - Ring is thick and dense against light, opaque when viewed from above.
The reading is interpreted as in the case of non-ring precipitation
test.

A. Robert's Test

Principle

The principle of this test is based on the precipitation of protein and formation of white compact ring using concentrated Nitric acid (HNO3).

Procedure

1) Place 3-5 ml of clear urine in a test tube.
2) Place the tip of a 5 or 10 ml pipette containing Robert's Reagent to the bottom of the tube and allow 3 ml of the reagent to lay beneath the urine.
3) If several tests are being done, wipe off the tip of the pipette before inserting it into the next tube.
4) A white ring at the zone of contact indicates a positive test.
5) The ring must be read within 3 minutes after adding the reagent, and with the eyes on the level of the contact ring.
6) Rings that are 1-2 mm above the zone of contact are due to mucin and nucleaoalbumin; rings 1-2 cm above the zone of contact are due to urates, uric acid urea and bile, acids. These are not to be reported positive for protein.
7) Report the result according to the chart given on the above for ring tests.

8) The test may be performed by holding a test tube containing a few ml of Robert's Reagent in an inclined position and allowing the clear urine to run slowly down the side of the tube from a pipette.

Note : If bile is present in the specimen, any colors (red, violet, blue, or green) will be found at the line of contact.

B. Heller's Test

Principle : The same as Robert`s Test

Procedure

1) Perform the test as directed under Robert's test using concentrated nitric acid instead of Rober's Reagent, and read the white ring at the zone of contact in the same manner.
2) If bile is present, any colors (red, violet, blue or green) will be found at the line of contact.
3) Interfering rings listed under Robert's Test also apply to Heller's Test.

Note: Heller's Test is not suitable for routine analysis of proteinuria because of the highly corrosive nature of concentrated nitric acid.

Heller's Reagent: It is **c**oncentrated nitric acid.

C. Sulphosalicylic Acid Test

Principle

This test is based on the precipitation of protein (particularly albumin) by sulphosalicylic acid,

Procedure

1. Place 3ml centrifuged urine in a test tube.
2. Add 3 ml of 20 % sulphosalicylic acid.
3. Mix thoroughly and estimate the amount of turbidity 10 minutes later.
4. Read and record results according to the chart given for non-ring precipition test.

D. Heat and Acetic Acid Test

Principle

The test is based on the precipitation of protein by heat.

Procedure

1. Fill a test tube three-fourth full of clear urine, and gently heat the upper portion of urine for 2 minutes to boil, being careful not to shake the tube more than necessary. The lower portion of urine is not heated so that it can be used as a control for comparing.
   Note: Rotate the tube to prevent cracking.
2. Now turbidity (a white cloud) can arise due either of phosphates, carbonates, or protein.
3. Add 3 drops of 10% acetic acid drop by drop, boiling between each drop.
4. A white cloud that disappeared is due to phosphates or carbonates.
   Persistence or development of turbidity implies proteinuria.
5. Read the test and record results according to the chart for nonring precipition test.

Sensitivity

This method is the most sensitive for small amount of protein and can reliably detect protein concentrations of 2 to 3 mg/dl.

II. Colorimetric Reagent Strip (Dipstick)Tests

The Colorimetric (dipstick) Protein Tests are more specific than Turbidimetric Tests. They require only a drop of urine enough to moisten the reagent area.The Colorimetric reagent strip test is based on the ability of protein to alter the color of some cid-base indicators without

altering the pH. When an indicator, such as tetrabromophenol blue is buffered at pH 3, it is yellow in solutions with out protein but , in the presence of protein, the color will change to green and then blue with increasing protein concentrations. In this case the pH of the urine is held constant by means of a buffer so that any change of color of the indicator will indicate the presence of protein. The tests for urinary protein are all commercial ones, that are available as reagent strip, tests (Dipsticks) either alone or in combination with other tests. Example. albustix, Uristix, N-Multistix, Combur3 or Combur9. Although the colorimetric tests are useful primarily as screening tests for protein, these strip tests can be read semi-quantitatively as negative, trace, 1+, 2+, 3+, or 4+ to give a rough
estimate of the amount of protein present. To do this, the resulting color must be matched closely with the color chart provided with the test strips. The albustix and other multiple-reagent strips produced by ames co. are plastic strips with protein test areas impregnated with citrate buffer and tetra-bromphenol blue. The citrate buffer maintains the pH at
3. At pH 3 tetrabromphenol blue is yellow in the absence of protein and yellow - green, or blue in its presence. The shade of the color is dependent on the amount of protein present. Falsely positive reactions may occur when protein is absent, if the urine is exceptionally alkaline or highly buffered.

Procedure
Observe the precautions and follow the instructions supplied by the manufacturer.
1. Dip the reagent area of the strip briefly into the specimen.
2. Remove excess urine by tapping or drawing the edge of the strip along the rim of the urine container.
3. Compare the color that develops with the color chart supplied by the manufacturer and report as indicated on the chart.

Quantitative 24 hour Protein Determinations
Simple estimates of the protein content of urine are performed by quantitating the amount of precipitation formed following the addition of a specific chemical to the urine. The precipitate is measured either by comparison with known standards (sulphosalicylic acid turbidity test) or by recording the height of the column of precipitate in a speciallydesigned tube (Esbach's test).

A. Sulphosalicylic Acid Turbidity Test

Procedure
1. Pipette 2.5 ml of centrifuged urine into a test tube.
2. Add 7.5 ml of 3% sulphosalicylic acid.
3. Invert to mix
4. Let stand 30 minutes.

Compare the turbidity with known standards prepared from solutions containing 10, 20, 30, 40, 75 and 100mg albumin/dl, and estimate the concentration of the unknown. If the unknown urine contains more than 100mg/dl protein, dilute the urine and repeat the test.

B. Esbach's Test

1) If a 24 hour urine collection is used, first, measure the total volume; then filter some of the urine. The urine must be clear.
2) Do qualitative protein test, Robert's or strip test.
   - if the urine is +3, made 1:5 dilution.
   - if the urine is +4 make 1:10 dilution.
   - if the urine is trace, +1 or +2 non dilution is needed.
   - if the urine is negative, a quantitative test is not done.
3) Test measure the pH of the urine. It should be acidic. If not, add 10 % acetic acid.
4) Add pumice powder to the 0.5 mark of the Esbach's tube.

5) Add urine to the "U" mark.
6) Add Esbach's reagent to the "R" mark.
7) Mix slowly by inversion, 10 times.
8) Wait for 30 minutes. Read the highest of the column. Do not subtract the amount of the pumice.
9) The result is now in gram per liter of protein in the urine. If the urine has been diluted, multiply by the dilution factor, calculate, and record the g % and the g / 24 hrs

Final report should include total volume.
The following formula is used to calculate the amount of urinary protein excreted in 24 hrs.
g/24 hr = total volume x g/l
1000

Bence Jones Proteins

- This is a rare type of an abnormal protein with lower molecular weight than normal protein.
- They appear in patients suffering from multiple myeloma
- They have a peculiar properties of precipitating at a temperature between 40^oC – 60^oC but re-dissolves above and below this temperatures.
- Ordinary proteins precipitates irreversibly at temperatures above 60^oC
- Bence jones proteins are precipitated by 3% sulphosalicylic acid

Detection of Bence jones

1) Brandshaws test
   - Bence jones proteins are precipitated by conc. HCL forming white ring at the interface of the two fluids
2) Harrison's test (boiling method)
   - Bence jones protein precipitates between temperatures of between 40^oC – 60^oC and re-dissolves above and below these temperatures

Bile salts in urine

- ✓ Formed in the liver and stored in the gall bladder as bile
- ✓ Absorbed by the small intestines during digestion and passed back to liver through the portal circulation
- ✓ Bile salts lower the surface tension of aqueous liquid thereby facilitating digestion of fat
- ✓ Bile salts are sodium taurocholate and sodium deoxycholate
- ✓ Bile salts are not normally found in urine
- ✓ They are excreted in urine together with bilirubin in obstructive jaundice and small amount in hepatic jaundice

Hays test for bile salts

- Bile salts lower the surface tension of aqueous liquids and allow powdered sulphur to sink

NB: urine preserved with thymol are unsuitable for hays test because they give false positive results

Determination of Bilirubin

Introduction

Bilirubin is a waste product that must be eliminated from the body. It is formed by the breakdown of hemoglobin in the reticuloendothelial cells of the spleen and bone marrow, and

then transported to the liver. On its way to the liver it is not water-soluble, and is carried through the blood stream linked to plasma albumin. This water insoluble form of bilirubin is often referred to as free bilirubin or unconjugated bilirubin or indirect bilirubin. Since this albumin - bound form is insoluble in water; it does not appear in the urine. In the liver bilirubin is converted to a water soluble product by conjugation with glucuronic acid to form bilirubin glucuronide. The water-soluble form is called conjugated bilirubin. It is also called direct bilirubin. The liver cells that form the conjugated bilirubin excrete it into the bile and it is then excreted into the intestinal tract through the bile duct. In the small intestine this conjugated bilirubin is converted by intestinal bacteria to urobilinogen or stercobilinogen. Even though normally the level of conjugated bilirubin in the blood is not high enough to cause significant amounts to appear in the urine, this water soluble and conjugated bilirubin can be excreted by the kidneys.

Normal Value: approximately up to 0.02 mg/dl (This amount is not detected by routine qualitative or semi quantitative techniques).

Clinical Significance

Tests for urinary bilirubin and urobilingen were normally performed only indicated by abnormal color of the urine or when liver disease or a hemolytic condition was suspected from the patient's history. The presence of bilirubin and urobilinogen in the urine is an early sign of liver cell disease (hepatocellular disease) and obstruction of the bile flow from the liver (Obstructive or post - hepatic jaundice). Urine containing bilirubin will typically have been brown color and produce a yellow foam when shaken. Bilirubin is not stable in solution, but will be oxidized to biliverdin, which is a green pigment. Thus urinecontaining bilirubin will typically be red-brown when voided, and will turn
green on standing, especially if exposed to light. Tests for bilirubin will not be positive in the presence of biliverdin; so the urine must be examined when fresh.

Tests for Bilirubin

Tests for bilirubin are based on the oxidation of bilirubin to biliverdin.

Specimen: Freshly passed urine is required. Urine containing bilirubin should be analyzed immediately after collection (with in 2 hrs of voiding). If bilirubin exposed to sunlight, it will oxidize to biliverdin, which cannot be detected by the reagents used in any of the tests. The following tests are used to detect bilirubin in the urine.

A. Harrison's (Fouchet's) Test

Principle

This test depends on precipitation of bilirubin with barium chloride, which is then oxidized to biliverdin with Fouchet's reagent. The formation of biliverdin gives a green color, which constitutes a positive reaction.

Procedure

1. Add 5 ml of a 10% solution of barium chloride to 10 ml of urine. Mix, and let stand a Few minutes.
2. Filter through a small filter paper.
3. Spread the filter paper on a dry piece of filter paper and place one or two drops of Fouchet's reagent.
4. A blue to green color indicates a positive reaction.
5. Report as positive or negative.

B. Barium chloride Filter Paper Method

It is the modification of Harrison's test. The barium chloride is supplied on thick filter paper that has been soaked in a saturated solution of barium chloride.

Principle

The principle of the test is the same as Harrison's (Fouchet's) Test.

Procedure

1. Hold a strip of barium chloride paper perpendicularly in the urine for a few seconds
2. Place one or two drops of Fouchet's Reagent on the saturated area.
3. Look for the appearance of a green color, which constitutes a positive reaction.
4. Report as positive (formation of a green color) or negative (formation of any color except green, or no color formation).

Sensitivity

Harrison's Test is so sensitive that it detects as small as 0.1 - 0.2 mg of bilirubin in 100ml of urine.

C. Diazotization Tests for Bilirubin

The tablet and reagent strip tests for bilirubin are based on the coupling of bilirubin with a diazonium salt in an acid medium to form azobilirubin, which gives a blue or purple color.

1. Icotest Tablet Test

The Ictotest tablet contains nitrobenzine diazonium, p-toluene sulfonate (bilazo), sulfosalicylic acid, and sodium bicarbonate. The mats are absorbent Asbestos cellulose.

Procedure

1. Place five drops of urine on either side of the special test mat supplied with the reagent tablets.
2. Place the tablet in the center of the moistened area.
3. Flow two drops of water on the tablet.
4. Observe the mat around the tablet for the appearance of a blue to purple color within 30 seconds.
5. Report the results as positive or negative according to the following criteria.

Negative: The mat shows no blue or purple within 30 seconds. Ignore any color that forms after 30 seconds or a slight pink or red that may appear.
Positive: The mat around the tablet turns blue or purple within 30 seconds. Ignore any color change on the tablet itself.
Sensitivity: Ictotest detects 0.1mg of bilirubin in 100ml of urine.

2. Reagent Strip Tests for Bilirubin (Ex. Multistix)

Principle

These tests for bilirubin are available only on multiple-reagent strips in conjugation with other tests. They are diazotization tests and are analogous to the Icotest tablet test. The test area for bilirubin on
Multistix and other Ames Co. reagent strip products is impregnated with 2,4-dichloro-aniline diazonium salt.
The reagent strip tests for bilirubin are difficult to read and the color formed after reaction with urine must be carefully compared with color chart supplied by the manufacturer.

Procedure

1. Dip the reagent strip briefly into the urine specimen.
2. Remove excess urine by tapping the edge of the strip against the rim of the urine Container. Hold the strip in a horizontal position to prevent mixing of chemicals from adjacent reagent areas.
3. Compare the test areas for bilirubin closely with the color chart supplied by the manufacturer. Multistix should be read seconds after dipping.

4. Report the results as positive or negative for bilirubin.

Sensitivity

Multistix detects 0.2-0.4 mg of bilirubin in 100ml of urine.

Determination of Urobilinogen

Introduction

In the intestine, most of the bilirubin is converted to urobilinogen or stercobilinogen by the action of certain bacteria that make up the intestinal flora. Approximately half of the urobilinogen formed in the intestine is absorbed into the portal blood circulation and returned to the liver. In the liver most of the urobilinogen is excreted into the bile once again and returned to the intestine. A very small amount of urobilinogen about 1 percent of the formed urobilinogen is excreted from the body in the urine as urobilinogen or can be also converted into urobilin, which gives the urine its characteristic color with the other color pigments (urochroms). Urobilinogen is also converted into urobilin when exposed to air.
Stercobilinogen in the intestine is either eliminated from the body unchanged or oxidized to the colored compound stercobilin, which gives the faces its characteristic color. Thus, urine normally contains only a very small amount of urobilinogen and no bilirubin. Both are abnormal urinary constituents. However, there are several serious conditions in which either one or both of these substances are found in the urine. When testing for urobilinogen the urine specimen must be fresh, since it is usually unstable and it is rapidly oxidized to urobilin. This oxidation takes place so readily that most urine specimens that contain urobilinogen will show an abnormal color caused by partial oxidation of urobilin. The presence of urobilinogen and that of urobilin have the same clinical significance, however, they take part in different chemical reactions, and urine is more frequently tested for urobilinogen.

Normal value: Normally 1-4 mg of urobilinogen is excreted in the urine each day.
Clinical Significance
Urine is often tested for increases in urobilinogen when investigating hemolytic jaundice or liver disorder in which liver function is impaired.

Test for Urobilinogen

A. Qualitative Ehrlich's Test for Urobilinogen

Principle
The test depends upon the reaction between urobilinogen and paradimethylaminobenzaldhyde to form a cherry (deep) red.
Procedure:

1. Place 10 ml urine in a test tube. Allow warming to room temperature.
2. Add 1 ml Ehrlich's reagent and mix.
3. Let stand 3 to 5 minutes
4. Normal amounts of urobilinogen present in the urine sample will change the solution to pink.

Abnormally high amounts of urobilinogen will change the solution to a Cherry red color. This must be reported
Positive for urobilinogen. Disregard any pink or light red coloration. This test is of no value in infections of the Urinary tract because some bacteria produce nitrites, which give false positive reaction. Formaline interferes with the test
and should not be used as a preservative.

B. Reagent Strip Tests for Urobilinogen- Urobilistix

The reagent strips have test areas for urobilinogen, which are based on the Ehrlich's reaction in which
P-dimethylaminobenzaldehyde reacts with urobilinogen in a strongly acidic medium to form a colored aldehyde.
The reddish brown color that is formed varies with the amount of urobilinogen present. After a timed interval, the color is compared with a graded color chart. However, the test is not specific for urobilinogen and reacts with substances know to react with Ehrlich's reagent.
Procedure:

1. Dip the reagent strip briefly into the specimen.
2. Remove excess urine by tapping the edge of the strip against the rim of the urine Container.

3. Compare the color of the test area after 60 seconds with the color chart supplied by the manufacturer.

Sensitivity

Urobilistix detects 0.1 mg in 100 ml of urine (0.1 Ehrlich units in 100 ml)

Test For Urobilin

Urobilin is an oxidation product of urobilinogen. Urobilin is colored and urobilinogen is colorless. Both compounds have the same clinical significance when present in urine; however, they undergo different chemical reactions.

Schilesinger's Test for Urobilin

Principle

When zinc acetate reacts with urobilin it produces a green fluorescence.

Procedure

1. Mix equal parts (10 ml each) of urine and alcoholic solution of zinc acetate in a test tube. Mix and filter the mixture.
2. Take 10 ml of the filtrate and add 2 drops of Lugol's solution. Mix by inversion.
3. Examine the solution for green fluorescence by viewing the tube from above as it is passed through the direct rays of a fairly strong light (sunlight or wood's light).
4. Report as positive or negative. Urobilin produce a green fluorescence while porphrins produce red fluorescence.

Determination of Hemoglobin

Introduction

Hemoglobin is a respiratory pigment in red blood cells composed of an iron- containing group (heme) and a complex protein (globin). In combination as hemoglobin it has the property of forming a reversible combination with oxygen. So, it serves as a transporter of oxygen in the blood from the lung to metabolically active tissues. It also transports carbon dioxide and hydrogen ions to the lung from metabolically active tissues. Hemoglobin appears in the urine when there is extensive or rapid destruction (hemolysis) of circulating erythrocytes that the reticuloendothelial system cannot metabolize or store the excessive amounts of free hemoglobin.

Normal Value: The renal threshold for hemoglobin is 1.0 - 1.4 g/1.

Clinical significance

The presence of free Hemoglobin in the urine is referred to as hemoglobinuria. Hemoglobinuria is usually related to hematuria- a condition when intact red blood cells are present in the urine. Hematuria is used to indicate bleeding somewhere in the urinary tract. Usually both red blood cells and hemoglobin mark this disorder. Therefore, hematuria can be distinguished from hemoglobinuria by a microscopic examination of the sediment from a fresh urine specimen. The presence of hemoglobin and the absence of red cells in the urine does not necessarily mean that the hemoglobin was originally free urinary hemoglobin. Red cells rapidly lysis in urine, especially when it has a specific gravity of 1.006 or less or is alkaline. For this reason urine should be absolutely fresh when examined for the presence of red cells. Hemoglobinuria may occur with severe intravascular hemolysis when the amount of hemoglobin being released into the plasma is more than can be taken up by haptoglobin (the plasma protein that binds free hemoglobin to prevent it being lost from the body). This results from a variety of conditions and disease states. It may be the result of hemolysis in the blood stream, in a particular organ, in the kidney of lower urinary tract or in the sample itself.

Causes of Intravascular Hemolysis

Intravascular Hemolysis is associated with:

- Hemolytic anemia.

- Severe infectious disease such as Falciparum Malaria, Yellow Fever, Small Pox and Typhoid Fever.
- Glucose - 6- phosphate dehydrogenase (G6PD) deficiency.
- The ingestion of certain drugs.
- Escherichia coli septicaemic.
- Incompatible blood transfusion.
- Snake bites that cause acute hemolysis.
- Sickle cell disease- crisis with sever hemolysis.
- Severe viral hemorrhagic fever accompanied by intravascular hemolysis.
- Poisonings with strong acids or mushrooms sever-burns and renal infarction.
- Significant amounts of free hemoglobin occur when ever excessive numbers of red cells are present as a result of various renal disorders.

Specimen

Urine containing hemoglobin appears brown or brown-gray on color and is usually cloudy. It should be tested as soon as possible after it has been passed.

Laboratory Tests for Hemoglobin

Numerous Tests are available for the detection of hemoglobin in urine.
The tests commonly used are:

- Guaiac
- Benzidine
- Occultest
- Reagent Strip Tests (Hemastix)

Benzidine is no longer used, as it is a carcinogen.

General Principle of the Tests

These tests are based on the same general principles and reaction. They all involve the presence of peroxidase activity of the hemoglobin and hydrogen peroxide (H2O2) or a suitable precursor which liberates oxygen. Peroxidase of the hemoglobin molecule liberates oxygen from hydrogen peroxide and the librated oxygen reacts with an organic reagent or chromogen (gum guaiac or 0-tolidine) to give a colored compound, which is usually blue or green. The intensity of the color depends on the amount of librated oxygen. The amount of liberated oxygen depends on the peroxidase ctivity of hemoglobin molecule, which intern depends on the amount of hemoglobin found in the urine.
These reactions are summarized below:

$$\text{Hemoglobin} + H_2O_2 \xrightarrow{\text{Perosidase}} \text{Oxygen}$$

$$\text{Oxygen} + \text{Chromogen} \rightarrow \text{Blue or green oxidation products}$$

Since all these tests are based on the peroxidase activity of hemoglobin, other substances with peroxidase activity also give positive reactions in the tests.

Factors that Affect Hemoglobin Determination

False negative

- High specific gravity such as heavy proteinuria (over 5 g/1). This prevent lysis of RBCs and may reduce the color reaction.
- Low to false negative results are obtained if the urine contains large amounts of ascorbic acid.
- Nitrite delays test reaction.
- Formaline used as preservative, fix the cell and prevent hemolysis.

False positive

- Low specific gravity < 1.010 enhances lysis and produces color reaction.
- Microbial peroxidase produces false the color reaction.
- False positive reactions can result from the presence of contaminating oxidizing detergents on the urine such as bleach.

A. Guaiac Test

Procedure:

1. Pour into a test tube 4 drops of urine and a few drops of concentrated acetic acid.
2. Pour the following into a second test tube
   - A knife point of Guaiac
   - 2 ml ethanol (95%)
   - 2 ml fresh 3% H_2O_2

   Mix the above slowly and pour the same into the side of the urine tube.
3. A green or blue color is a positive test. The chromogen in this test gum guaiac.

B. Occultest (tablet method)

Principle

When the tablet is moistened with water tartaric acid and calcium acetate react with strontium peroxide to form hydrogen peroxide. The hemoglobin in the urine catalytically decomposes hydrogen peroxide liberating oxygen, which oxidizes orthotolidine to a blue derivative.

Procedure:

1) Place a piece of filter paper, which comes with the reagent on a clean surface.
2) Place one drop of well-mixed urine in the middle of filter paper.
3) Place occultest tablet in middle of the moist area.
4) Flow two drops of water over the tablet.
5) Observe color of filter paper around tablet exactly two minutes later.
6) The test is positive, if a blue color appears on the filter paper around the tablet.
7) Report as positive or negative.

Content of the tablet

The tablet consists of orthotolidine, tartaric acid, strontium peroxide, sodium bicarbonate, calcium acetate, and red dye.

C. Reagent Strip Tests (Hemastix)

Principle:

When the strip is dipped in a urine containing hemoglobin, the action is similar to that of occultest tablets in that the hemoglobin (since it has a peroxidase activity) catalyzes the oxidation of orthotolidine by the peroxide. The oxidized orthotolidine is blue.

Procedure:

1) Follow the manufacturers directions and precautions.
2) Dip the test end of strip into a well-mixed specimen of urine and remove immediately.
3) After 30 seconds compare the test area with the color chart provided.
4) Report as indicated on the color chart.

Composition of the strip

The test area is impregnated with orthotolidine, cumene hydroperoxide and citrate buffer.

Determination of Urinary Calcium

Introduction

The bulk of calcium ions (Ca^{++}) discharged by body is excreted in the stool. However, there is small quantity of calcium that is normally excreted urine. But it may increase depending up on the quantity of dietary calcium ingested. The 24 hour test is most often ordered to determine the function of the parathyroid gland, which maintains a balance between calcium and

phosphorous by means of parathyroid hormone. Hyperparathyrpidism is a generalized disorder of calcium,
phosphate and bone that results from increased secretion of parathyroid hormones and an increased excretion of urinary calcium. In hypoparathyrodism the urinary calcium is decreased.

Interfering Factors

1. False positive
    - High sodium and magnesium intake.
    - High milk intake.
    - Some drugs.
    - If test done immediately after high calcium meal.
2. False negatives
    - Increased dietary phosphates.
    - Alkaline urine.
    - Some drugs.

Test for Calcium

The test for calcium in the urine is not included in the routine urinalysis. The qualitative test of Sulkowitch is used for calcium determination.

Sulkowitch Test

Principle of Test

In this test a solution of ammonium oxalate (about 4 %) is added to the urine. If calcium is present in excessive amounts, it drops out of solution as a heavy white precipitate of calcium oxalate.

Procedure

1) Pour 5 ml of urine into a test tube.
2) Add 5 ml of Sulkowitch reagent (see reagent No. 13).
3) Mix by inverting the tube several times.
4) Allow to stand 3 minutes.
5) If a fine white cloud appears, the calcium content is normal.
6) If no white cloud appears, the calcium content is decreased.
7) If a heavy white milky precipitate forms, the calcium content is increased.
8) Report the calcium content as normal, decreased or increased.

Determination of Nitrite

Introduction

Tests for nitrite was not a part of the routine urinalysis. It is included recently on commercial multiple-reagent strips such as Combur 9 and NMultistix. The detection of nitrite in the urine can be used to indicate the presence of bacteria such as Escherchi coli, proteius, klebsiella, enterobacter, citrobacter, and salmonella will reduce urinary nitrate to nitrite.The presence of urinary nitrite(in urine sample collected under sterile condition) indicates the existence of a urinary tract infection. Detection of such infections is particularly important in pregnant women and young girls to prevent permanent renal damage. Since the detection rate increases when the urine is held in the bladder for at least 4-6 hours, test for nitrite must be performed on the first morning urine specimen. For proper detection of nitrite the patient diet must also contain enough reducible nitrate on which the bacteria can act.

Types of Method Used for Detection of Nitrite

There are two methods that are used to detect bacteria in the urine during routine urinalysis.

1) Microscopic examination: urine sediment when examined microscopically can reveal bacteria when present. If the sample is collected under sterile condition.
2) Chemical dipstick method for nitrite can also give clue.

The nitrite area in the multiple reagent strip is calibrated so that any shade of pink color that develops within 30 sec indicates an amount of nitrite produced by 105 or more organisms per milliliter in the urinespecimen. A positive result from the nitrite test is a reliable indication of significant bacteriuria (the presence of bacteria in the urine) and is an indication for urine culture unless the specimen has been improperly collected or stored after collection which allow bacterial growth. A negative result should never be interpreted as indicating absence of bacteriuria because:

- If an overnight sample were not used, there may have been insufficient time for the conversion of nitrate to nitrite to occur. Urine that has been left in the collection vessel for several hours may be falsely positive.
- Some amounts are caused by organisms that do not convert nitrate to nitrite (such as enterococci, acinetobacter spp and some pseudomonas species.).
- The patient was in a vegetable free diet, which is the important source for nitrate.
- Administration chemotherapeutic agents should be discontinued three days before the test, because antibiotic therapy may alter bacterial metabolism so as to render nitrite detection invalid.
- High doses of ascorbic acid.
- Presence of urobilinogen
- Low pH (< 6)

Even though the test depends upon the conversion of nitrate to nitrite by certain bacterial action in the urine, not all bacteria in the bladder convert nitrate to nitrite. Known nitrate reducing bacteria at significant levels produce false negative results by reducing nitrate to ammonia nitrite, and nitrous oxide, hydroxylamine, and nitrogen and will therefore give a negative nitrite test.

Principle

The Reagent Strip (Multistix with an acid pH) contains para-arsanilic acid which reacts with nitrite, to give a diazonium salt, which by looping with a benzoquinoline forms pink azo dye. The pink color is therefore related to the presence of bacteria in the urinary tract. However, the amounts of color produced cannot be related to the number of bacteria present and the result should be reported only as positive or negative. Falsely positive reactions may be caused by bacterial growth in "old" urine specimens or by medication such as phenazopyridine that colors the urine red or that turns red in an acidic medium.

Procedure

Follow the manufacturer's instruction and precautions:

1) Dip the test area of the strip briefly into the specimen.
2) Remove excess urine by tapping the edge of the strip along the rim of the container.
3) Compare the color that develops with the color chart supplied by the manufacturer.

Report as positive or negative within the time specified by the manufacturer.

Sensitivity

N-Multistix detect 0.075 mg of nitrite in 100 ml of urine and combur 9 test detects 0.05 mg per 100 ml of urine.

Leukocytes Test

Introduction

Tests for leukocytes has become part of the routine urinalysis since commercial multiple-reagent strips began to be marketed (N-Multistix or Combur 9). The presence of leukocytes indicates inflammation at some point along the urogenital tract.

Principle of Test

The reaction on the test strip reveals the presence of esterases that occur in granulocytes. These esterases cleave an indoxyl ester, and the indoxyl so liberated reacts with a diazonium salt to produce a violet dye.

Procedure

1) Insert the strip to the specimen. (no longer than 1 second).
2) Wipe off excess urine on the rim of the container.
3) After 60-120 seconds compare the test by matching with the color scale on the label supplied by the manufacturer.

Most tests are done on a random specimen of freshly voided urine by the patient. The specimen is collected in a clean dry container. If the determination is not done immediately, the specimen should be preserved at 5 0c in the refrigerator.

Determination of Indican /Indoscyle Sulphate

Introduction

Indican is driven from indole, which is the putrefaction product of protein, by protolytic bacteria. Patients with bacterial over growth in the small intestine excrete large amount of metabolites of amino acids such as tryptophan or tyrosine. Indole is produced by bacterial action on tryptophan in the intestine, which mostly eliminated with feces, some are absorbed and detoxified in the liver and excreted as indican in the urine.

 In normal urine the amount of indican excreted is small, it is increased in

1. High protein diet
2. Pathological conditions such as
   - Bacterial putrefactions.
   - Enteritis.
   - Pancreatic insufficiency.
   - Intestinal Infection.
   - Ulceration of intestinal mucosa.

To differentiate the pathological conditions from non-pathological, first restrict the patient from protein intake and then do the test.

Test for Indican

Obemyares Test

Principle

HCL liberates indoxyle from indican and ferric chloride ($FeCl_3$) oxidizes the indoxyle to indigo blue.

Procedure

1. Add 5 ml well mixed fresh urine and 5 ml obemyares reagent into a test tube.
2. Add 4 drops of chloroform and mix several times by inverting until all color dissolves out.
3. Add saturated ferric chloride drop by drop, mixing well after each addition and record the number of drops you add to decolorize.

When indican is present, the chloroform layer shows a deep violent to blue color. Normally when two drops are added, the color comes to light sky blue, if it needs more. It shows the presence of increased amount of indican.

Source of Errors

- Indican may be formed because of slow oxidation.
- Presence of iodide may case violent color removed by adding crystals of sodium thiosulfate.
- Bile pigments – removed by shaking $BaCl_2$ and filtering.

Determination of Melanin

Introduction

Melanin is pigment derived from tyrosine, which is normally present in hair, skin and in the choroid layer of the eye.

There are two recognized metabolic pathways for the conversion of tyrosine to melanin:

1) The eumelanin pathway, which polymerize to brown or black pigments.
2) The pheomelanin pathway – which polymerize to yellow or red pigments.

Melanomas with pigments are normally transferred from melanocytes to skin and mucus membrane cells.

In patients with tumors arising from the melanin producing cells, the melanomas, the melanin may be excreted in the urine in large amount, and its presence is indicative of metastasis of the tumor to the liver or other organ.20% of patients with disseminated malignant melanoma excrete a black urine due to melanin, or its precursor, the colorless melanogen in the urine. The urine becomes black upon standing (oxidation), where the chromogen /melanogen is changed into the

pigment called melanin which is a physical method to detect melanin.

Clinical Significance

Melanin occurs in metabolic tambours especially with metastasis of liver.

Chemical Tests for Melanin

There are two types of chemical test for melanin in urine depending up on either by

- Utilization of oxidizing agent (e.g. FeCl3)
- Utilization of reducing action of melanogen
- Oxidation by atmospheric air

A. Ferric Chloride Test

Principle: FeCl3 oxidizes the melanogen to melanin.

Procedure

- To 5 ml of freshly voided urine, add 1 ml 10% FeCl3 drop by drop to precipitate all the phosphates.
- Add drop by drop 10% HCl to dissolve all the precipitate and it forms different color.
- Centrifuge and examine for black or gray precipitate of melanin.

If melanin is present, decant the supernatant fluid and add Na2CO3 until alkaline to litmus, melanin precipitates will dissolve again, and then add 10% HCl until acid to litmus; melanin precipitates again as gray or black sediment when centrifuged.

B. Thormahlel Test

Principle

Sodium Nitroprusside is reduced to ferocyanide (prussian blue) by reducing action of melanogen.

Procedure

- Prepare fresh solution of sodium nitroprusside by shaking few crystals of sodium nitroprusside in 10 ml of distilled water.
- Add 0.2 ml aqueous sodium nitroprusside to 5ml urine
- Add 0.5 ml of 40% sodium hydroxide.

During this time the urine turns red due to the action of creatinine and other interfering substances. Add 3.5ml of 30% glacial acetic acid, and the color may change to blue or dark if melanin present which is indication for a positive reaction.

C. Oxidation by Atmospheric Air

Allow the urine sample `o stand exposed to the air undisturbed for 24 hours. If melanogen presents, it will slightly oxidize to melanin by the air, and turns dark brawn or black from the top downwards.
• Homogenstic acid gives the same effect, but the darkness of melanogen is not accelerated appreciably by alkali.
* Microscopic examination of sediment for melanin cast is also possible.

Exercises:

1. Discuss by comparison the Benedict's Qualitative and Glucose oxidase Tests.
2. List down the possible substances, which give false positive results in non-specific tests for glucose determination.
3. Mention the physiological effects of ketone accumulation in blood.
4. Write the principle of the test for determination of bilirubin and hemoglobin.
5. Write the general principles for the two types of determination of urinary protein.

Microscopic Examination of Urine

Objective:

It is expected that using the information presented in this chapter the students will be able to describe normal and abnormal urine sediments with their diagnostic features.

Introduction

Microscopic examination of urine is one of the routine tests of urinalysis. As mentioned in the introductory part of this lecture note, urine contains many substances in addition to water. The amounts of solid substances, which are found in the urine, may indicate an individual's health status, i.e. whether one is healthy or sick. Normally small amount of solid substances is found in the urine. But when their concentration become high, it may indicate the existence of abnormal physiological function of our body. Microscopic examination of urine to some extent can be considered as “renal biopsy” because it reveals more about the function of the kidneys. Repeated evaluation of urine sediment is frequently valuable in following the course and management of urinary tract disorders, because the appearance of cellular elements, and casts in the urine is a reflection of changes that take place in the kidney. Urine sediments can grossly be categorized into organized and non- organized sediments based on the substances they are composed of.

Procedure for Microscopic Examination of Urine

1. Assemble all necessary materials used for the collection,
   Centrifugation and examination. This include:
   - ƒ Clean dry plastic or Glass containers, which enable to collect at least up to 15 ml of urine for routine urinalysis.
   - ƒ Hand (manual), or electrical centrifuge.
   - ƒ Conical centrifuge tubes, or regular test tubes.
   - ƒ Pasture pipette with rubber fit or automatic pipettes if possible.
   - ƒ Slides and cover slides 20 x 20 mm.
   - ƒ Electrical or solar microscope, which has 10x and 40 x objectives.
2. Preparation of patient

- ƒ Explain the purpose of the test by using simple language. Do not use medical terms or try to explain details of the procedure.
- ƒ Advise the patient how to collect the specimen. The first morning urine or mid-stream urine specimen is more preferable, because it is more concentrated.
- ƒ If the patient is female, advise her to wash her genital organ before giving the specimen. This is because bacteria that are normally found on the genital tract may contaminate the sample and affect the result.
- ƒ Advise the patient to collect at least 15 ml of urine in to the clean, sterilize and dry urine cup that is supplied from the laboratory.

3. The collected urine sample should arrive at a diagnostic laboratory as soon as possible.
   - ƒ If the urine sample is delayed by more than 2 hours, without preservation, urine sediment appearance and constituent may be changed and false results may be obtained and reported.
   - ƒ If it is difficult to deliver within 2 hrs, it is better to preserve specimen in the refrigerator at the temperature between 2-6 0C or use chemical preservatives.
4. Centrifugation of the urine specimen
   a) Mix the urine specimen
   b) Transfer about 10 ml of urine in the centrifuge tube. Balance tubes in the centrifuge.
   c) Centrifuge the specimen at a medium speed (from 1500 – 2000 rpm) for 3-5 minutes
   d) Discard the supernatant by quick inversion of the tube
   e) Re suspend the sediment that is at the bottom of the tube, by tapping the tube by your fingers
   f) Take the sediment by Pasteur pipette from the tube and transfer a drop into the clean, sterilized and dry slide.
   g) If Pasteur pipette is not available, gently incline the tube and place drop of sediment into the clean, sterilized and dry slide.
   h) Apply cover slide on the urine sediment that is on the slide. This will make specimen to be spread on the slide on one cell thickness.
   i) Put the slide on the stage of microscope and tie it by clips on the stage.
   j) Lower the condenser, close the diaphragm and look under 10x objective of the microscope. Casts tend to concentrate near the edge of cover slide.
   k) Then after looking through at least 20 fields of the low power objective, change the objective in to 40x objective. Do not forget to raise the condenser and opening of the diaphragm when you change the objective in to the high power (40x). Under high power objective also you should have to look for a minimum of 10-15 fields).
   l) Then report what you get under10 x (low power) and 40 x (high power) on the laboratory request form of the patient. For determination of cellular elements, casts, etc, the number of elements seen under at least 10 fields should be counted and the average of this number is used for report value. Other elements such as parasites are usually reported as well.

Source of Errors in the Microscopic Examination of Urine

Possible errors that may encounter during microscopical examination of urine include:

- ƒ Drying of the specimen on the slide.
- ƒ During trial of observing 2 specimens in a single slide by putting at each side of slide, (mix up of the specimens).

ƒ If the supernatant fluid after centrifugation is not poured off properly, that is if some drop is left in the tube, it may decrease concentration of urine sediments and false result may be reported

ƒ If the whole sediment with supernatant is discarded during inverting down the tube for long period, the whole sediments will be discarded and so again false negative result will be reported.

Urinary Sediments

Classification of Urinary Sediments

MICROSCOPICAL EXAMINATION OF URINE SEDIMENTS

Organized Elements (Formed for Non-living Material)

- RBCs/HPF
- WBCs/HPF
- Epithelial cells / LPF
- Casts / LPF
- Parasites/LPF
- Bacteria / HPF

- Yeast Cells /`LPF
- Mucus trade/LPF
- Spermatozoa
- Miscellaneous substances (common contaminants)

Non-organized Elements (Formed from Living Materials)

(Crystals)

I. Acidic urine crystals

-Amorphous Urates,
-Uric acid crystals,
-Cystine crystals
-Calcium Phosphate
-Cholesterol
-Ammonium Biurates
-Tyrosine, Leucien, Bilirubin,
-Calcium sulfates (urates)
- Calcium carbonate

II. Acidic, Neutral, or slightly alkaline Urine crystals
- Calcium Oxalate crystals

III. Alkaline, Neutral, or Slightly acidic urine
- Triple phosphates

IV. Alkaline Urine Crystals
- Amorphous phosphate
- Calcium carbonate
- Calcium phosphate

Organized Urinary Sediments

RED BLOOD CELLS

Appearance: Normally RBCs appear in the fresh sample as intact, small and faint yellowish discs, darker at the edges

- Measure 7-8 µm
- In concentrated urine may be crenated, and their size became small (5-6 µm)
- In diluted urine, RBCs may be turgid and increase in size (9-10 µm)

- In alkaline urine, they may be small or entirely destroyed forming massive of brownish granules

Clinical Implications: When the number of RBCs is found more than their normal range, usually greater than 5 RBCs/HPF it may indicate:

ƒ Presence of disease conditions in the urinary tract, such as:

- Acute and chronic glomerulonephritis
- Renal stone
- Cystitis
- Prostates
- Trauma of the kidney
- Presence of parasites, such as: schistosoma.
- Presence of bacterial infection, such as: renal tuberculosis
- Other disease conditions, such as hemophilia, malignant hypertension.

Temporarily (transient) increased RBC may be seen

- After strenuous exercise
- Exposure to cold temperature

Other substances confusing with RBCs Yeast cells, and fat droplets may confuse with RBCs morphologically. They may be differentiated by their morphology. Red blood cells are somewhat round or disc shaped, and uniform in size: while yeast cells are oval in shape, and have budding at the surface. On the other hand fat droplets are irregular in size and they are shiny. Another means of differentiating RBCs from yeast and fat droplets is that, when 5% of acetic acid is added under the cover slide, RBCs will hemolize, while yeast cell and fat droplets will not show any change.

How to report result:

• After looking RBCs under the 40x objective, they can be reported by mentioning the average number of RBCs/HPF.

Interfering factors: Factors that may result falsely in high number of RBCs, i.e. without the presence of actual renal or other normal physiological disturbances included:

- Menstrual bleeding
- Vaginal bleeding
- Trauma to peranal area in female patients
- Following traumatic cateterization
- Due to some drugs, such as,

- Aspirin ingestion or over dose
- Anticoagulant therapy over dose

LEUKOCYTES (WBCs)

Normal range: 0-4 WBC/HPF.

Appearance: normally, clear granular disc shaped,

- Measure 10-15 µm, the nuclei may be visible.
- In alkaline urine, they may increase their size and become irregular.
- Predominantly, polymorph nuclear neutrophils are seen.

- Sometimes because of predominance of neutrophils and the occurrence of bacterial cell together with polymorphonuclear cells, WBCs are called pus cells.
- WBCs (pus cells) may be seen in clumps.
- It is also possible to see single irregular nuclei and small round lobed nuclei in the WBCs, that are seen in the urine sediment

Clinical implication: increased number of leukocyte urine are seen in case of:

- Urinary tract infection
- All renal disease
- Bladder tumor
- Cystitis
- Prostates
- Acute or chronic bacterial infection such as renal tuberculosis, temporarily increased number of leukocytes are also seen during:
  - Fever,
  - After strenuous exercise

How to report the result: - After observing the distribution of leukocytes under 40x objective, at least 10 fields of microscope, it is possible to report as : 0-5 leukocytes / HPF, 20-39 leukocytes / HPF etc, that is by counting the total leukocytes in 10 HPF and divide by 10.
Or,

When 0-5 leukocytes / HPF are seen..... normal
5-10 leukocytes / HPF are seen..... few leukocytes / HPF
10-20 leukocytes/HPF are seen.....moderate leukocytes/ HPF
20-30 leukocytes /HPF are seen ..... many leukocytes / HPF
Above 30 leukocytes / HPF / are seen ..... full/field

EPITHELIAL CELLS • Normally few epithelial cells (0-2 / HPF) can be found

• Appearance ƒ Their size differs depending on the site from which they originated.

a. Those coming from renal cells - Size is small as compared to other epithelial cells - It measures 10µ to 18 µm in length, i.e., slightly larger than leukocytes - Very granular - Have refractive and clearly visible nucleus - Usually seen in association with proteins or casts (in renal disease).

b. Cells from pelvis and urethra of the kidney - Size is larger than renal epithelia's - Those from pelvis area are granular with sort of tail, while those from urethra are oval in shape - Most of the time urethral epithelia is seen with together of leukocytes and filaments (mucus trades and large in number) - Pelvic epithelia's seen usually with no leukocyte and mucus trade, and are few in number

c. Bladder cells - Are squamious epithelial cells? - Very large in size. - Shape seems rectangular and often with irregular border. - Have single nucleus. * Here it is important to keep in mind that it is not expected from an experienced Lab. technician after simply observing epithelial cells, to say that these are urethral cells, and of pelvic origin and reporting such a false result in the laboratory request form.

* Knowing the origin of the epithelial cells and reporting it, may have more meaning when requested by the physician for special purpose, especially by the urologists.

Clinical implication

Presence of epithelial cells in large number, mostly renal types may indicate:

- Acute tubular damage
- Acute glomerulonephritis
- Silicate over dose

* The presence of large number of epithelial cells with large number of Leukocytes and mucus trades (filaments) may indicate Urinary Tract Infections (UTI).

Reporting of the result:

• Epithelial cells distribution reported after looking under 10x (low power objective) of the microscope. • Usually they are reported semi quantitatively by saying

- Occasional epithelial cells /LPF ……1-3 epithelial cells seen in the whole LPF
- Few epithelial cells / LPF…….. 2-4 epithelial / LPF
- Moderate epithelial cells / LPF…….. 6-14 epithelial / LPF
- Many epithelial cells / LPF…….. 15-25 epithelial/ LPF
- Full of epithelial cells / LPF……..when the whole field of 10 x objective covered by epithelial cells.

Interfering factors

• Squameous epithelial cells from female patients that shade from vaginal area (together with vaginal discharge) may give false result of high epithelial cells

CASTS

• Formed by precipitation of proteins, and aggregation of cells within the renal tubules. Most of them dissociate in alkaline urine, and diluted urine (specific gravity ≤ 1.010) even in the presence of proteinurea. Most of them are transparent. Thus to look them clearly, it is important to lower the condenser and close (partially) the diaphragm. Look them under 10 x (low power objective) of the microscope. There are different kinds of casts based on their shape and content (morphologically) may be grouped in to the following.

a. **Hyaline Casts**

• Normal range: 0-2/HPF

• Appearance

- Transparent (clear), cylindrical shape
- Have parallels side with slightly round ends
- Their appearance in urine depends on rate of urine flow, i.e. many hyaline casts are seen when the flow rate is slow, and are not seen in alkaline urine mostly; and as the degree of proteinurea is high, there concentration also increase.

Clinical Implication

Presence of large number of hyaline casts may show possible damage of glomerular capillary membrane. This damage permits leakage of protein through glomerulus and result in precipitate and gel formation (i.e. hyaline casts) in the tubule. Thus this may indicate:

- Nephritis
- Meningitis
- Chronic renal disease
- Congenital heart failure

- Diabetic nephropathy

Hyaline casts may also be seen in moderate number temporarily in the case of:

- Fever
- Postural orthostatic strain
- Emotional stress
- Strenuous exercise
- After anesthesia

b. **Granular Casts**

- More similar in appearance with hyaline casts and in which homogenous, course granules are seen. More dense (opaque) than hyaline cast, thus can be more easily seen than hyaline casts. They are also shorter and broader than hyaline casts. May represent the first stage of epithelial cell cast degeneration. Some other studies also suggest that, they are formed independently from cellular cast degeneration, and stated that they result from aggregation of serum proteins into cast matrix of mucoproteins

- Based on the amount and type of granules, they can be further divided into fine, and course granular casts.

Clinical implication

Granular casts may be seen in

- Acute tubular necrosis
- Advanced granulonephritis
- Pyelonephrites
- Malignant nephrosicosis
- Chronic lead poisoning
- In healthy individuals these casts may be seen after strenuous exercise

c. **Waxy Casts (Renal Failure Casts)**

Normal value

- Not seen in normal individuals.

Appearance

- Shorter and broader than hyaline casts.
- Composed of homogeneous, yellowish materials.
- Broad waxy casts are from two to six times the width of ordinary
- casts and appear waxy and granular.
- Have high retractile index.
- May occur from cells (WBC, RBC, or Epithelial) casts, hyaline casts.

Clinical significance

Waxy casts are found in

- Chronic renal disease.
- Tubular inflammation and degeneration.
- Localized nephron obstruction.

* The presence of waxy casts indicates severity of renal disease.

d. **Fatty Casts**

Normal range: normally not seen in health individuals.
Appearance:

- These are casts, which contain fat droplets inside them.
- Fat droplets are formed after accumulation of fat in the tubular vessels, especially tubular epithelial and finally disintegrated.

Clinical Implication:

• The occurrence of fat droplets, oval, fat bodies, or fat casts is very important sign of nephritic syndrome.
• Chronic renal disease.
• Inflammation and degeneration of renal tubules.

e. Cellular Casts

Cellular casts are casts, which contain • Epithelial cells • White blood cells • Red blood cells

Normal range: normally not seen in normal individual

Appearance • These are casts in which cellular elements are seen. • Formed usually after accumulation of cellular element in the renal tubules

Clinical Significance

• Epithelial / renal / casts mostly seen in tubular degeneration.
• Red cell cast usually seen in acute glomerulonephritis cases.
• White blood cell casts seen mostly during pyelonephrites conditions.

NOTE: Casts are very significant findings of urine microscopic examination. This is because their presence indicates the existence of renal disease. Sometimes it is possible to get a single cast having course granules, fine granules and fat droplets, i.e. different substances in a single cast, at the same time. At this time decision is made after looking and evaluation of other fields and based on the majorities.

Reporting of Laboratory Result

• Casts are examined under 10x objective of the microscope.
• Always the condenser should be lowered and at the same time in order to have good contrast, the diaphragm should be partially closed.
• Casts are reported quantitatively by saying:
  - o Occasional casts / LPF
  - o Few casts / LPF
  - o Moderate casts / LPF a
  - o Many casts / LPF

During the report the type of cast that is seen should also be mentioned
Example: few hyaline casts / LPF are seen

PARASITES

Parasites that can be seen in urine microscopy are:

- ƒ Trichomonas vaginalis
- ƒ Schistosoma haematobium
- ƒ Wuchereria bancroftie

* Other parasites also may occur due to contamination of the urine with stool.

a. **Trichomonas Vaginalis**

It is a protozoal parasite that infect the genitourinary tract.

Appearance - Size is about 15 µm. - Shape is round, globular. - Has vibratory, whirls and turns type of movement. - Has also undulating membrane that is like the fin of a fish, on one side very motile. - Have 4 flagella.

b. **Schistosoma Haematobium**

It is fluke that infect venules of the bladder.

Appearance of the egg

- It is found in the urine sediment.
- Has pale yellow brown color.
- Large and oval in shape.
- Has characteristic small spine at one end (terminal spine).
- Measure about 145 x 55 µm.
- The egg contains a full-developed miracedium.

Sometimes the miracedium hatch from the egg and can be seen swimming in the urine. The miracedium swim in the urine by the help of ciliates that are surrounding it.

High excretion of S. haematobium egg can be seen usual between 10.00 a.m. and 2 p.m. It is also important to remember that even when persons are highly infected, eggs may not be present in the urine. Therefore that is important to examine several specimens collected on different days and examine carefully, that is due to the irregular pattern of egg excretion.

c. **Wuchereria Bancroftie**

- It is tissue nematode that invades lymph vessels. It is usually attack lower limb.
- In chronic bancroftie filariasis, a condition called chyluria can occur i.e. passing of chyle in the urine. It occurs when the urogenital lymphatic vessels, which are linked to those, that transport chyle from the intestine became blocked and rupture.
- Chile consists of lymph and particles of digested fat (soluble in ether).
- Urine containing chyle appears creamy white. When blood is also present, the urine appears pinkish-white.
- Large, measuring 275-399 x 8-10 µm.
- Body curves are few, nuclei are distinct.
- Sheath stains pink with Giemsa and palely with haematoxylin.
- There is no nuclei in the tip of at the tail.

Other points that should be considered also

- The parasite usually found in high concentration during night from 10:00 p.m. – 4:00 a.m. and i.e. it has nocturnal periodicity.
- Differentiate from B. malai and L. loa by its tail feature.
- Differentiate from Mansonella species by its large size and sheath.

YEAST CELL

Yeast cells are fungi that are not normally seen in health individuals.

Appearance

- Variable in size

- Colorless.
- Oval in shape, and usually form budding.
- Have high refractive index.
- Usually confused with Red Blood Cells.

The way in which one can differentiate yeast cells from RBC is discussed in detail under Red Blood Cells.

Clinical Significance

• They are usually of candida species (candida albicans) and are common in patients with
- Urinary tract infection
- Vaginitis
- Diabetic mellitus
- Intensive antibiotic or immunosuppressive therapy.

BACTERIA

Bacteria are the most common cause of UTI and aerobic gram-negative bacilli, particularly, members of the enterobacteriacea, are the most dominant agents. The Gram-positives account for proportionately large number of infections in hospital inpatients. Normally, bacteria are not seen in the healthy individual's urine.

To check the presence or absence of bacteria a technician can either check for Nitrate that was formed in the urine after breakdown of nitrite into nitrate by the metabolic action of bacteria. Hence, dipstick test can give indirect clue. Or one can use urine microscopy test to check the presence of pus cells within the drop of urine or its sediment. Further the observed bacterial cell can be identified by bacteriological culture.

Appearance

- Bacteria that are seen in the microscopic examination of the drop of urine sample. Their shape varies with the type of bacteria observed..
- Depending on the type of bacteria they can be either motile or non motile organisms.
- They can be observed when examined under less than 40 x (high power) objective of the microscope.

Clinical Significance

- Presence of bacteria may indicate the presence of UTI or contamination by genital or intestinal microflora. - To confirm what type of bacteria they are and whether or not they are the causes of the disease, it is important to culture them in appropriate media and perform biochemical tests for identification.

Report of the Result

The bacteria concentration before or without performing culture and identification of the bacteria, can be reported as: • Occasional bacteria HPF • Few bacteria / HPF • Moderate bacteria / HPF • Many bacteria / HPF • Full of bacteria / HPF.

Non-organized Elements (Urine Crystals)

Appear usually after the specimen (urine) collected and left without examination. Mostly occur during metabolic abnormalities and excessive consumption of certain foodstuffs. May be classified into acidic, basic, and both acidic and basic based on:

- pH of urine in which they are usually seen.
- Solubility characters.

Identification of particular urine crystals from patient urine-sediment mainly serves as

- Guide to diagnose most likely type of calculus present.
- Mode of therapy of calculus by adjusting of urine, and by avoiding the intake of certain calculus precursors.
- Occurrence of certain abnormal urine crystals, such as cystine. Leucine, and Tyrosine, indicate the patient is in certain metabolic disorders and Some drug crystals in the urine include, sulfonamides, aspirin, caffeine, used to follow the treatment condition.

I. Acidic Urine Crystals

a. **Amorphous Urates (Anhydrous uric acid)**

- Normally present in urine in different quantity.
- Have pink to "brick red" color.
- From very small granules and seen in cluster.
- Dissolve in urine when the sample is gently heated.
- When urine is left in the refrigerator, it shows heavy precipitation of urates.

b. **Uric Acid Crystals**

- Polymorphs (different in shape) i.e. square, prism, hexagonal, rostelles etc.
- Yellow to yellow brown in color.
- Size is 30-150 µm
- Small quantity found in normal urine, but increases in association with:
  - Increased Purine metabolism in case of gout.
  - Increased Nucleic Acid turn over, such as leukemia.

c. **Cystine Crystals**

- Rarely found.
- Flat, hexagonal plates with well defined edges.
- Colorless, and highly retractile.
- Size is 30-60 µm.
- Found only in fresh urine, because if there is delay, they are soluble and not seen.
- Appeared during cystinosis, which is a hereditary disease (Wilson disease), or during transient acute phase of pyelonephritis. Its appearance in the urine is called cystinuria.

d. **Cholesterol**

- Rarely found.
- Colorless and retractile.
- Have "broken window" shape, with notches on one side.
- 50-100 µm in size.
- Soluble in ether.
- Seen in case of elevated cholesterol, chyluria.

e. **Tyrosine**

- Rarely found.
- Colorless or yellowish.

- Have fine silky needle in sheaves or rosettes shape.
- Indicate protein break down problem, or severe liver disease.

f. **Leucine**

- Rarely found.
- Yellow to yellow brown in color.
- Spheroid in shape with striation.
- Seen in case of protein breakdown problem, or severe liver disease.
- Lucien and Tyrosine crystals may occur together. Both are amino acids usually; in case of severe liver disease, they will not be metabolized, and excreted in urine.

g. **Bilirubin**

- Very rarely seen.
- Have reddish brown color.
- Seen in case of elevated Bilirubin.
- Have various tiny squarish, beads or amorphous needle shape.
- Size is 5 µm (half RBC).
- Chemical test for bile pigments positive.

h. **Calcium Sulfate Crystals**

- Have large prism or flat bladder shaped.
- Seen separately or in bundles.
- Size 50-100 µm.
- Can be distinguished from calcium phosphate crystals by measuring pH of urine.

II. Acidic, Neutral, or Basic Urine Crystals

Calcium Oxalate Crystal - Are colorless and refractive. - Have octahedral, envelope, shape. - Size 10-12 µm. - Normally seen in small amount. - After consumption of high calcium, or oxalate rich foods, such as milk, tomatoes, asparagus, and orange, normally the crystals may be seen. - In dehydration condition, such as, in hot weather where there is high perspiration and only small amount of water is consumed per day Calcium oxalate crystals may be seen. - Pathologically in large quantity may be seen in (severe chronic renal disease, and urinary calculus).

III. Alkaline, Neutral, or Slight Acidic Urine Crystals

Triple Phosphates

- Colorless and refractive.
- Have “coffin lids” 3 to 4 to 6 – sided prism.
- Shape, or fern leaf or star shape.
- Size 13 0- 150µm.
- Seen in urine stasis (obstructive uropathy), or in urinary tract infections.
- Their presence is frequently indicative of bacterial infection by proteus marbles.

IV. Alkaline Urine Crystals

a. Amorphous Phosphates

- Normally seen in alkaline urine.
- Small, whitish granules usually seen scattered.
- Soluble in 100g/1 acetic acid.

b. Calcium Carbonate

- Less commonly seen.
- Colorless.

- Have needle, spherical or dumbbells shape.
- Have very small crystals.
- If 100g/1, i.e. 10% acetic acid is added, they dissolve, give off bubbles of gas.

c. Calcium Phosphates
- Seen in small amount in normal individual urine, and when they are in large amount, may indicate chronic cystitis, or prosthetic hypertrophy.
- Have star or needle shape.
- Colorless.

d. Ammonium Bruits (Urates)
- Normally seen in alkaline urine.
- Have bundle of needles or “thorn apple” sphere shape.
- Size is about 20 µm.
- Often found together with phosphates.
- Yellowish or brown refractive color.

MISCELLANEOUS

I. Spermatozoa
- Are small structures consisting of a head and tail, connected by a short middle piece (neck).
- Easily recognized especially if they are motile.
- Frequently seen in the urine of males.
- They may see in the urine of females, when the urine collected after coitus usually not reported, unless the physician has special interest in it.

II Mucus Trades
- Formed by the precipitation of mucoprotein in cooled urine.
- Normally little mucus trades seen in normal individuals.
- Have fine, fiber like appearance.
- Wavy in shape and tapered at ends.
- If not examined carefully may confuse with hyaline casts.
- Their presence in large amount with WBCs, may indicate UTI.

III. Other Contaminates and Artifact Structure
- Muscle fibers
- Vegetable cells

- Cotton fibers (wool fibers) — All are fairly seen and easily recognizable.

- Structure from slide or cover slide — high retractile and non-uniformed size
- Fat droplets (other bubbles)
- Oil droplets — not evenly distributed.
- Pollen greens — are seasonal.
- Starch granules — incomplete digestion of starch. They can be confirmed by lugos iodine.

* To minimize the above mentioned contaminants and artifacts
- Don't use dirty containers, slides and cover slides.
- Don't let urine specimen to open-air.
- Avoid contamination of urine with fats and oils.

- Avoid the drying of sediments.

Methods for Examining Urine Sediments

A. Unstained Urine Sediment

1. **Bright field microscopy of the unstained urine sediment**

Traditionally, the urinary sediment has been examined microscopically by placing a drop of urine sediment on a microscopic slide, cover with cover slide and observing the preparation with the lower and high power, objective of the bright field microscope. When the sediment is examined under the bright field microscope, correct light adjustment is essential, and the light must be sufficiently reduced, by the correct positioning of the condenser and the iris diaphragm to give contrast between the unstained structures and the back ground liquid.

2. **Phase Contrasts (PC)**

P.C. illumination is useful in the examination of unstained urinary sediment, particularly for translucent elements such as hyaline casts and mucus threads, which have a refractive index similar to that of urine in which they are suspended. Phase contrast has the advantage of hardening the outlines even the most ephemeral formed elements.

B. Stained Preparation Cellular detail is best seen with stained preparation.

The following stains are commonly used:

1. A crystal violet safranin stain (sternheimer and malbin) is useful in the identification of cellular elements. It is commercially available as sedi- stain.

Preparation of Reagents

Solution (1) Crystal violet ---------- 3g
Ethanol (95%)---------- 20 ml
Ammonium Oxalate---------- 0.8 g
Distilled water ---------- 80 ml

Solution (2) Safranin ----------1 g
Ethanol (95%)----------40 ml
Distilled water---------- 400 ml

The mixture should be filtered every 2 weeks!

- Discard after 3 months
- Separately, solution (1) and solution (2) keep indefinitely at room temperature.

In highly alkaline urine, the stains will precipitate.

Procedure Add 1 or 2 drops of crystal violet safranin stain to approximately 1 ml of concentrated urine sediment. Mix and place a drop of this suspension on a slide and cover with cover slide.

Staining reaction to crystal – violet safranin stain:

RBC – Purple to dark purple.
WBC – Cytoplasm -violet to blue.
Nucleus – reddish purple.
Glitter cells – blue.

	Nucleus	Cytoplasm
Squameous epithelial	purple	pink to violet
Uroepithelial	dark blue	blue
Renal tubular cells	dark purple	range purple

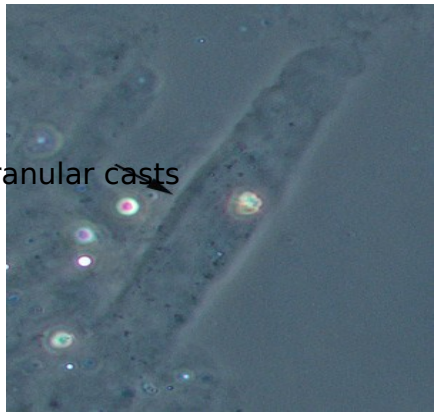
2. Methyl blue (Loeffler's stain)
3. Cyto-Diachrome stains

When such stains are used, it is recommended that both the stained and unstained sediment be mounted and observed, as the stain may cause precipitation of some constituents. This is especially the problem with alkaline urine specimens, because the precipitated materials may obscure important pathological constituents.

Casts



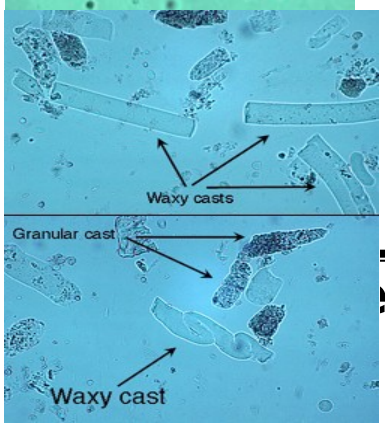
Granular casts



Hyaline cast

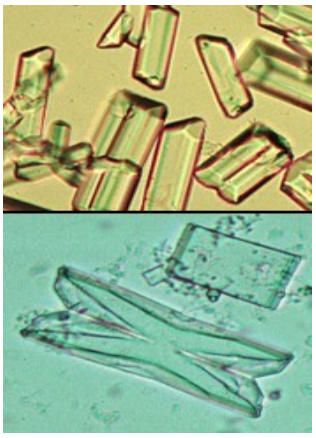


Fatty Cast

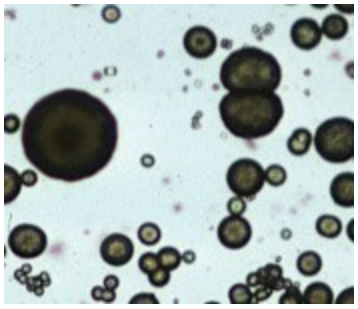


Waxy cast

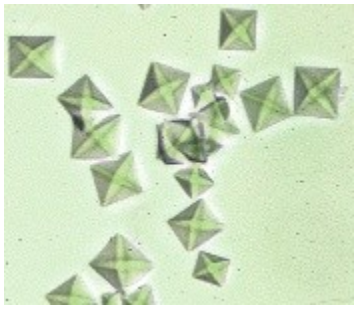
Crystals



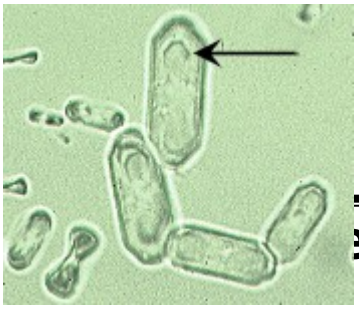
Struvite crystals (magnesium ammonium phosphate, triple phosphate)



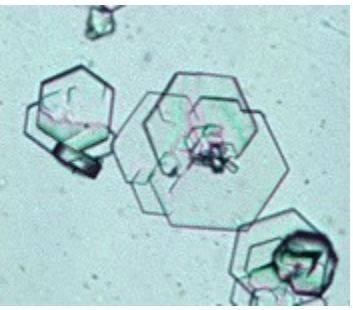
Calcium carbonate



Calcium oxalate dihydrate crystals



Calcium oxalate monohydrate



Cystine

Exercise:

Say True or False

1. The number of casts preserved decrease as the pH of the urine decreases.
2. Presence of RBCs in the urine is always indicative of a renal disease.
3. Waxy casts are the end stage in the degeneration of cellular casts.
4. Pyuria refers to elevated numbers of leucocytes in the urine.
5. The presence of Bacteria in the Urine is determined using only Microscope.