LECTURE NOTES

For Medical Laboratory Technology Students

Urinalysis

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Preface

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Acknowledgments

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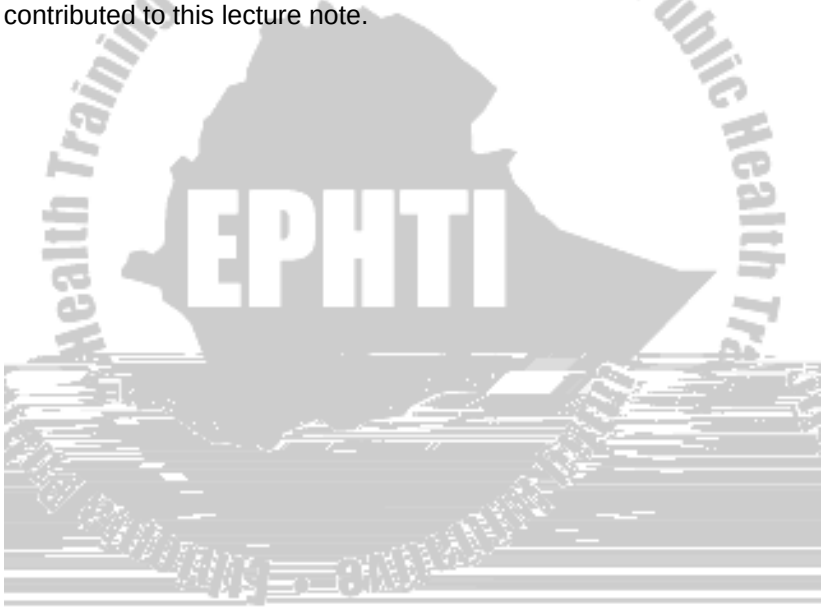
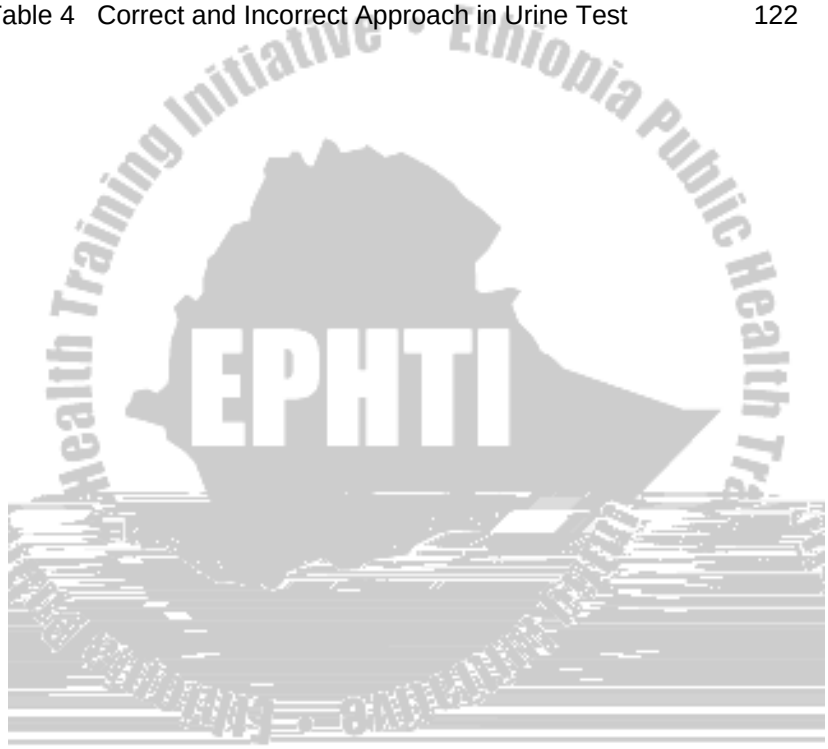


Contents

CHAPTER FOUR

CHAPTER FIVE



List of Figures

Abbreviations

ADH : Antidiuretic Hormone

RBC : Red Blood Cells

WBC : White Blood Cells

UT : Urinary Tract

UUTI : Upper Urinary Tract Infection

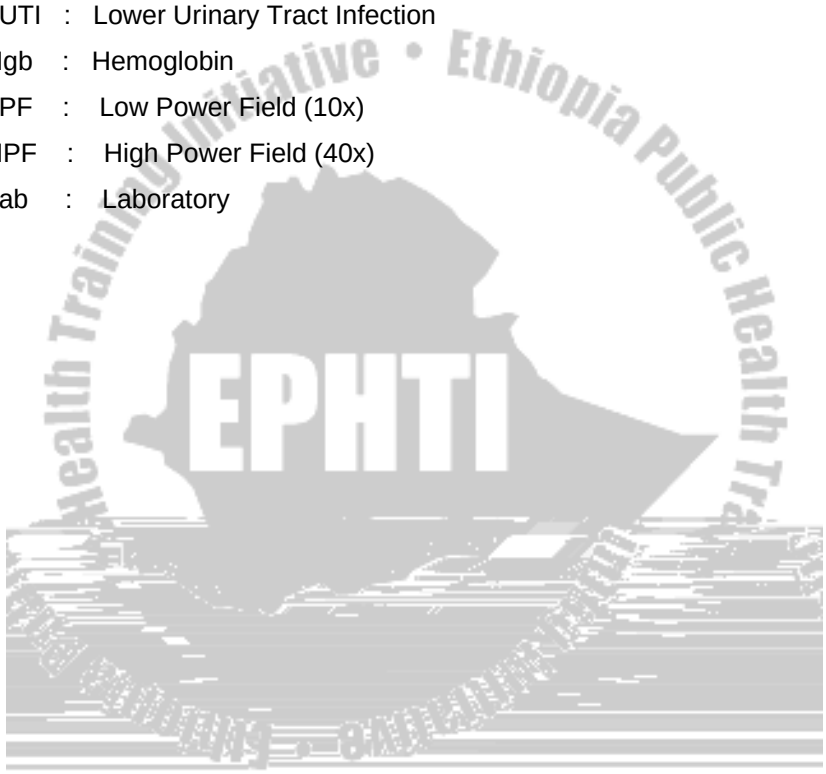
LUTI : Lower Urinary Tract Infection

Hgb : Hemoglobin

LPF : Low Power Field (10x)

HPF : High Power Field (40x)

Lab : Laboratory



Introduction

Examination of urine as an aid to diagnose a number of diseases is the oldest among tests in the history of Medical Laboratory Technology. It has been known for centuries that abnormalities in urine may indicate disease. Perhaps, one of the earliest known record of urine test was the technique of pouring urine on the ground and observing whether or not it attracted insects. The attraction of insects indicates " honey urine " which was known to be excreted by people with boils. Today checking sugar in urine is a test to detect diabetes (And untreated diabetes still suffer from boils). Around 1000 AD a Persian Physician named Ismail of Jordan described seven different observations made on urine such as Urine Consistency, Color, Odor, Transparency, Sediment and Froth. It was Walter Ames Compton who ushered in the modern era of Urinalysis in the early 1940's with the invention of 'CLINITEST'.

Urinalysis is a group of tests performed most frequently on random specimen. It is one of the most helpful indicators of health and disease, especially, it is useful as a screening test for the detection of various endocrine or metabolic abnormalities in which the kidneys function properly but they will excrete abnormal amounts of metabolic end-products specific of a particular disease. It is also used to detect intrinsic conditions that may adversely affect the kidneys or urinary tract. Diseased kidneys cannot function normally in regulating the volume and composition of body fluids, and in maintaining homeostasis. Consequently, substances normally retained by a kidney or excreted in small amounts may appear in the urine in large quantities, or substances normally excreted may be retained by kidney. Generally, urinalysis provides useful information concerning the presence or absence of renal and other diseases, and as a

This lecture note is prepared for Diploma Medical Laboratory Technology Students. It provides them with basic knowledge of Urine Examination. It also helps the students as well as other health

CHAPTER ONE

Anatomy and Physiology of The Kidney

Objective:

This chapter is intended

- To give a basic knowledge of the kidney structure and urine formation as an important aid in understanding urinalysis and test interpretation.

Introduction

The Renal System is a system which is composed of two kidneys, two ureters, one bladder and one urethra. As the components of the renal system the kidneys have the following functions:

- Regulation of water and electrolyte (such as chloride, potassium, calcium, hydrogen, magnessium, and phosphate ions) balances. - Regulation of acid – base balance of the blood.
- Regulation of body fluid osmolality and electrolyte concentratiions.
- Regulation of arterial pressure.
- Excretion of metabolic waste products and forein chemicals. The kidneys are the primary means for the eliminating waste products of body metabolism that are no longer needed by the body. These products include urea from the metabolism of amino acids, uric acid from the nucleic acids , creatinine from muscle creatine, bilirubin from the breakdown of hemoglobin.
- Secretion of hormones such as renin.
- Gluconeogenesis. The kidneys synthesize glucose from amino acids and other precursors, like lactate and glycerol, during prolonged fasting by the process called gluconeogenesis.

Components of the renal system are shown in Figure 1.1

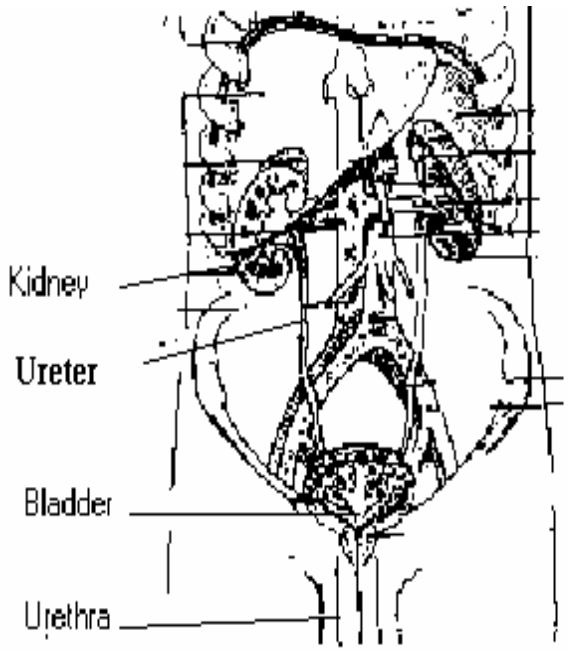


Fig 1.1 Renal System

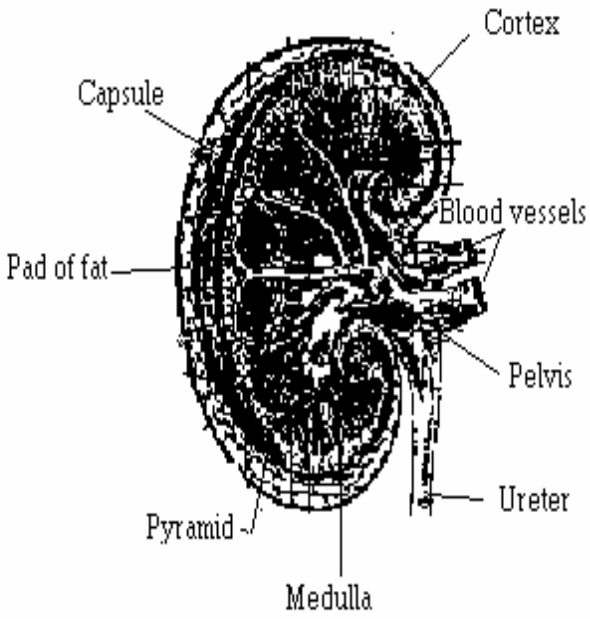


Fig 1.2 Anatomy of the Kidney

1.1 Anatomy of the Kidney

The kidneys are two bean shaped organs located under the lowermost part of the ribs in the posterior abdominal cavity. Each human kidney weighs 150 gms and measures 1x2x3 inches (thickness, width, and length). A coronal section of the kidney shows an outer reddish granular layer called renal medulla. In the renal medulla the triangular and wedge shaped structure is called renal pyramids. The tips of the pyramids found on the renal papillae at which urine is drained into cavities is called Renal Calyces. Renal Calyces drain urine into renal pelvis, then to ureter, which in turn drain to bladder and then through the urethra is voided out.

The gross anatomy of the kidney is shown in Figure 1.2.

The functional unit of the kidney is the nephron (Figure 1. 3). There are approximately one million nephrons in each kidney. Each nephron consists of a glomerulus, which is essentially filtering system, and a tubule through which the filtered liquid passes. Each glomerulus consists of a network of capillaries surrounded by a membrane called Bowman's (Glomerular) Capsule, which continues on to form Bowman's Space and the beginning of the renal tubule. The afferent arteriole, which carries blood from the renal artery into the glomerulus divides to form a capillary network. These capillaries re-unite to form the efferent arteriole, through which blood leaves the glomerulus. The blood vessels thus follow the course of the tubule, forming a surrounding capillary network. The tubular portion of each nephron has several distinct structural and functional segments. The uppermost portion, which continuous with the glomerulus, is the proximal convoluted tubule, followed by the thin walled segment and the distal convoluted tubule respectively. The descending limb of the proximal tubule (the thin-walled segment) and the distal tubule form a loop known as the Loop of Henle. The distal convoluted tubules from several nephrons drain into a

collecting tubule. A number of these collecting tubules form the collecting duct. The collecting ducts then join together to form the papillary ducts. The latter empty at the tips of the papillae into the calyces, which in turn drain into the renal pelvis.

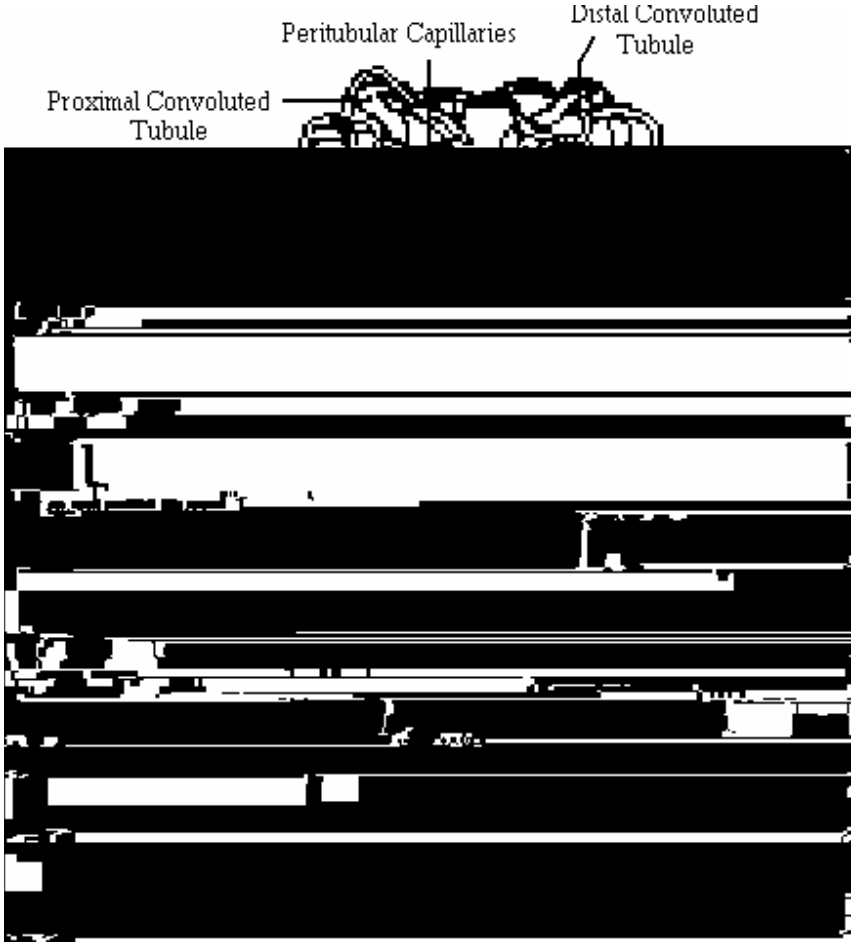


Figure 1.3 The Structural and Functional Segments of the Nephron

1.2 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 3(cid)5.3(sor blood)5(9701T-18.8683 -1.7

- Electrolytes
- Cast parasites and Bacterial microbes

1.4 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

1.5 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.

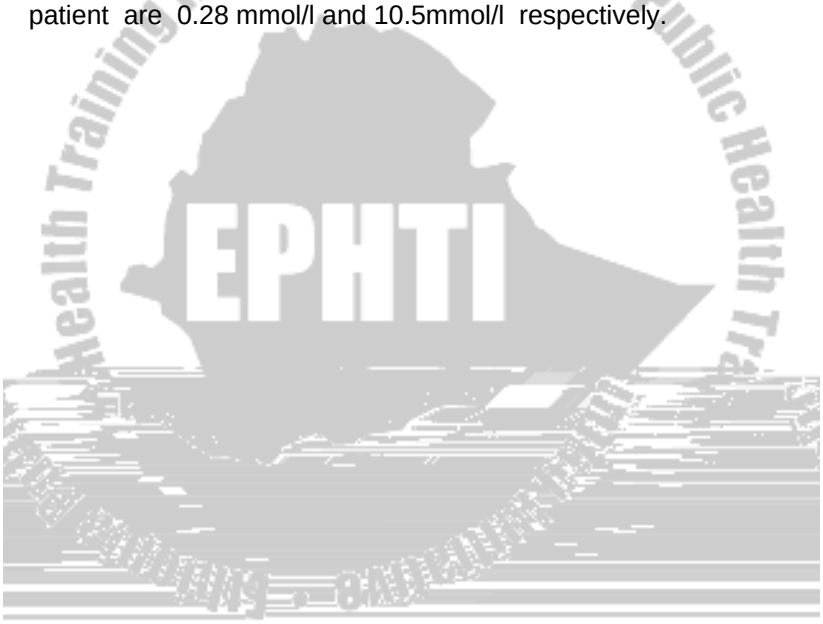
Renal Threshold

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.

Exercise 1.

Answer the Following Questions:

1. Describe the functions of the Urinay System.
2. Explain how Urine is formed by the Nephrons.
3. Mention the factors that determine the selective passage of molecules through the glomerular membrane.
4. Calculate the CcCr of a patient who voided 1500 ml of urine in 24 hrs. The serum and urine concentration of creatinine of the patient are 0.28 mmol/l and 10.5mmol/l respectively.



CHAPTER TWO

Collection And Preservation Of Urine Specimen

Objectives

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

- Identfy 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.

2.1 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 m(r.tic m12f)]TJT*0o 4 rs 10.02 85.08a(s of plperfo)5(rmee.l4.tic

plastic tube with a plastic snap-cap and self-adhesive identification label.Disposable tube holders are available for handling ten tubes at a

specimen. All specimens should be immediately covered and taken to the laboratory.

Types of Specimen

First Morning Specimen - a speciemen obtained during the first urination of the day.

- Most concentrated
- Bladder incubated

Best for:

- Nitrite
- Protein
- Microscopic examination

Random Specimen - a spciemen obtained at any time during examination.

- Most convenient
- Most common

Good for:

- Chemical Screen
- Microscopic examination

Second-voided Specimen - In this case first morning specimen is discardedand the second specimen is collected and tested. Such type of specimen is good for:

- Reflection of blood glucose.
- Keeping of formed elements intact.

Postprandial : a specimen obtained 2 hours after meal.

- Good for glucose.

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

1. 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.).
2. 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.
3. All the urine collected must be preserved.
4. The container should be labeled with :
   - The test order
   - The patient's name
   - Time of collection
   - The preservative added

Mid- stream Specimen - a specimen obtained from the middle part of the first urine.

- It is commonly used for routin e urinalysis.
- It is also important for bacteriological urine culture.

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 :

a. 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.

b. The patient should urinate a small amount and this is discarded.

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- Distruction of glucose by bacteria.
- Lysis of RBCs, WBCs and casts.

Method of Preservation of Urine Specimen

a. Physical Method

- Refrigeration
- Freezing

b. Chemical Method

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

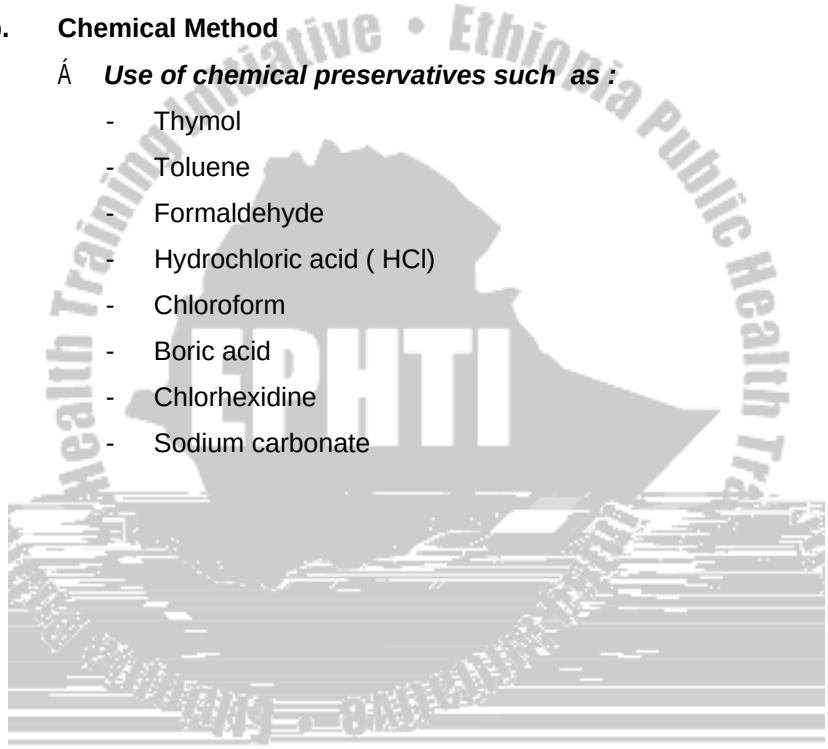

Table .1 Some Methods of Preservation, and their Advantages and Disadvantages

Methods	Advantages	Disadvantages
Refregeration (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
Thymole (small crystal 5 mm diametre/100ml urine)	Preserves most consitiuents	Can cause false positves for proteins
Chloroform (1 tablet/60 ml urine)	Preserves urine aldosterole 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

2.3 Type of Examination in Routine Urinalysis

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

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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

- 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.1(se)5m2492ssie400emli.

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 with out 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.
-

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.

3.4 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.
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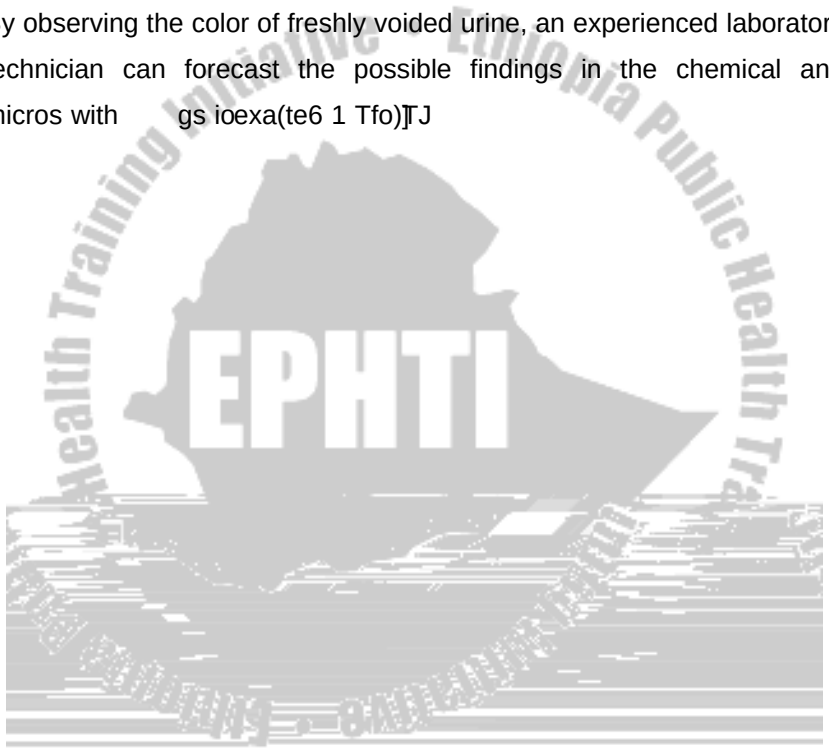
Procedure of the Test

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 micros with gs ioexa(te6 1 Tfo)]TJ



- 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.

Á 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.

Á 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 Hgb 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.

Á 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 B_{12} and riboflavin will give bright yellow color to urine.
- Rifampicilin 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.

3.5 Appearance (Transparency)

Fresh voided urine specimen is normally clear and transparent. On long

- 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

3.6 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.

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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 CO_2 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.

3.7 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 (Fig 3.1). 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.

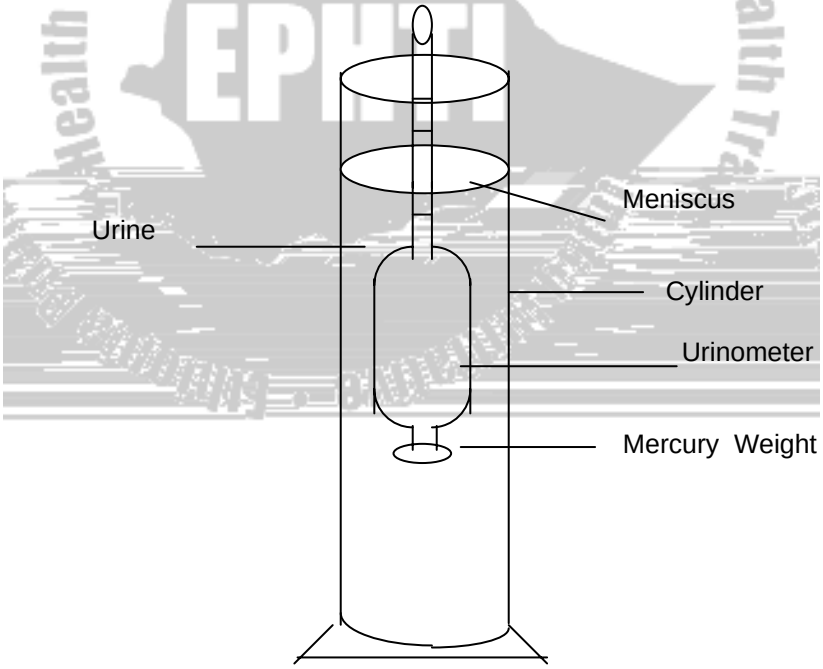


Fig 3.1 Urinometer and Urinometer Cylinder

Calibration

To obtain the correct specific gravit

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

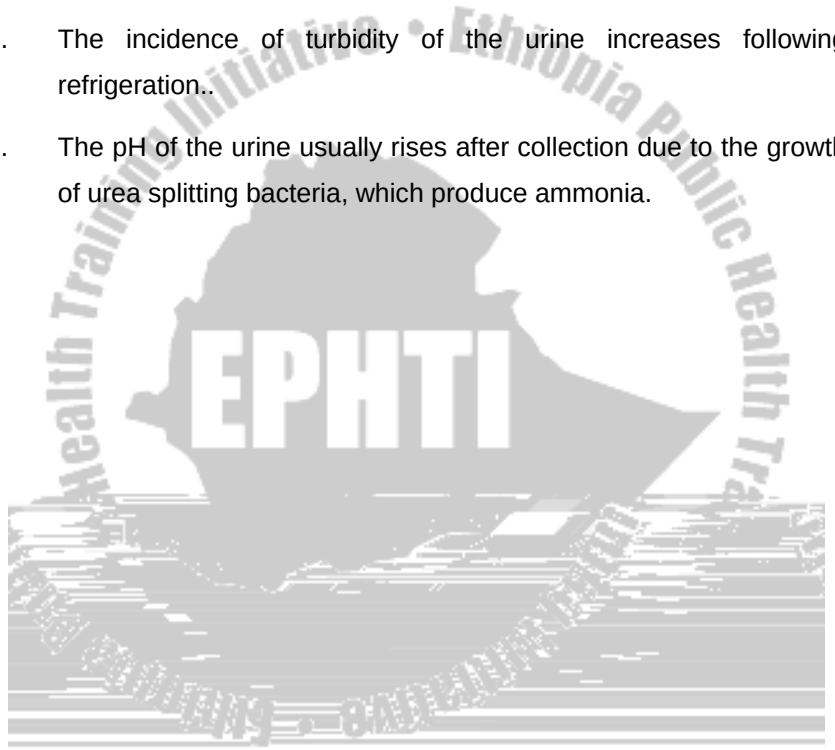
A test area to determine specific gravity in urine can be found in the multiple test strip of Ames called N-multistix. The reagent test area responds to the concentration of ions in the urine. It contains certain pretreated polyelectrolytes. The pKa of which changes depending up on the ionic concentration 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.

Exercises

Say True or False

1. Urine color and urine concentration commonly vary together.
2. The normal yellow color of the urine is due primarily to uroblin, uroerythrin and urochrome.
3. A turbid urine specimen always indicates a pathologic condition.
4. The incidence of turbidity of the urine increases following refrigeration..
5. The pH of the urine usually rises after collection due to the growth of urea splitting bacteria, which produce ammonia.



CHAPTER FOUR

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,

constituents of urine.

4.1 Determination of Urinary Sugar (Glucose)

Introduction

Glucose, a monosaccharide, is the pr

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 doesnot contain a

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Types of Urinary Sugar (Glucose)Tests

- Test for urine sugar is used to detect diabets mellitus and also used to monitor the effectiveness of diabetic control.
- 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.

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 $\xrightarrow{\text{alkali, heat}}$ Cuprous ions + Oxidized sugar

($CuSO_4$) (eg. glucose) (Cu_2O)(e.g. gluconic acid)

(Blue) (Orange-red)

The copper (II) ions are supplied in Benedict's qualitative reagent in the form of copper sulphate ($CuS0_4$). In the presence of a strong alkali this is converted to copper (I) oxide (Cu_2O). The heat is supplied by means of a boiling-water (100^{O}C) 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.

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)

Preparation of Benedict`s Reagent: Look at reagent numer 4

B. Clinitest Tablet Test

Principle:

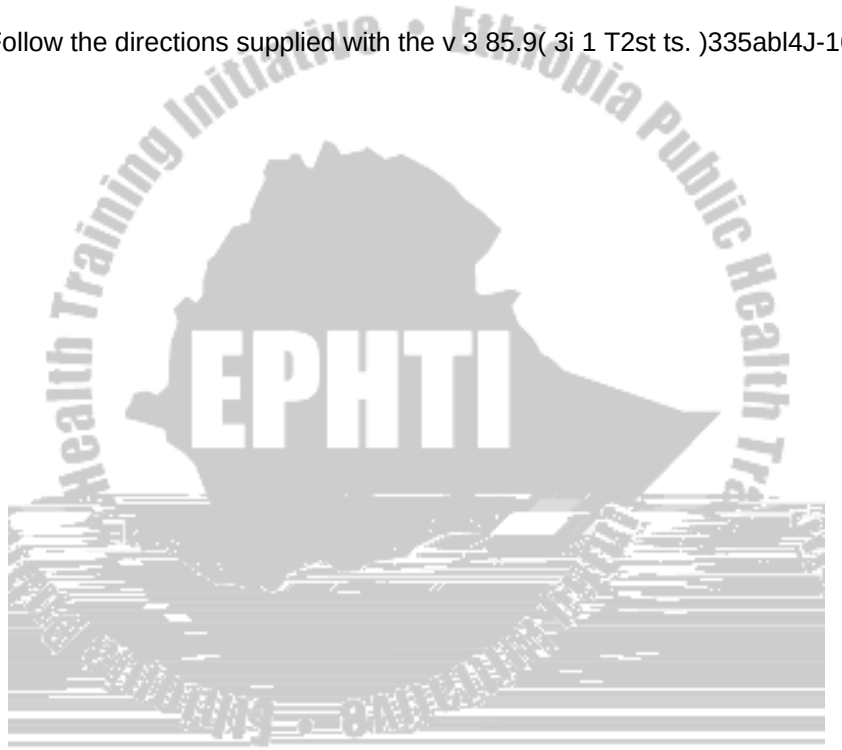
This is a qualitative, non-specific test for sugar. The principle of clinitest is essentially the same as that of Benedict's Qualitative Test. The clinitest tablet may be taught of as a solid form of Benedict's Qualitative reagent. In addition, the clinitest tablet contains anhydrous sodium hydroxide, 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 v 3 85.9(3i 1 T2st ts.)335abl4J-16.820 85.J-16.8.02i 61592.5483-0.ee335abTD-



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 :

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

$$H_2O_2 + \underset{(\text{red})}{\text{o-tolidine}} \xrightarrow{\text{peroxidase}} \underset{(\text{blue})}{\text{Oxidized o-tolidine}} + H_2O$$

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:

Á 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.

- 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

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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.

4.2 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 -odhIme. butyratdhIme.icdhIm 10acdhIm 1id)dhIme.. TJ-15.4533 -1.7245 TD0.0003

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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

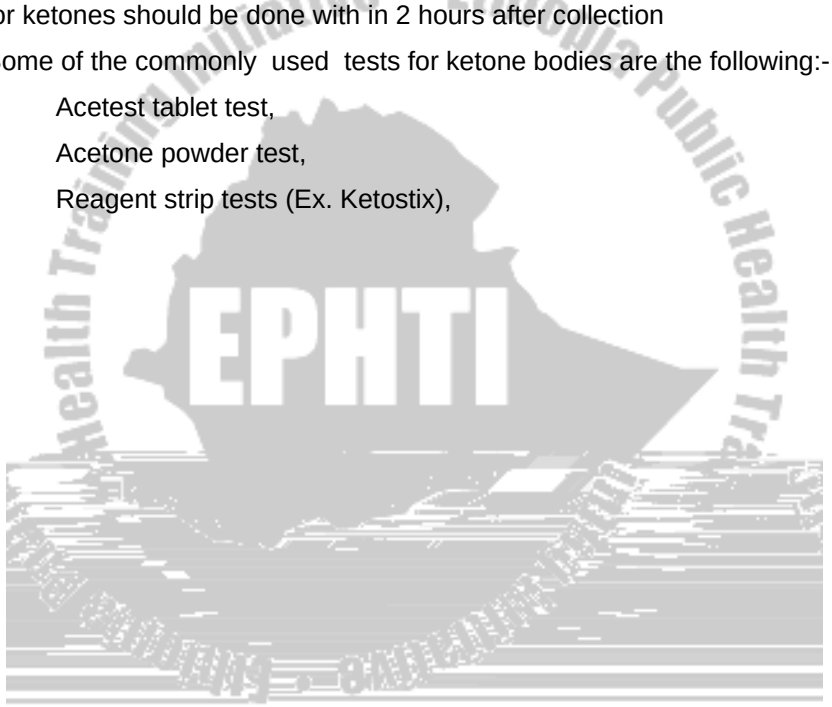
concentration and acetone in the smallest concentrations. However most of the tests for ketonuria are most sensitive to the presence of acetoacetate. There are no simple laboratory tests for β-hydroxybutyric acid. Most tests react with acetone and acetoacetate or both.

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),



4. If acetone or acetoacetate is present, a red to purple color will

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, Gerhar

of the functions are acting as enzyme(e.g trypsin), transport protein (

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
- Sulphosalicylic Acid Test
- Heat and Acetic Acid Test

Turbidimetric test based on acid reagents are non-specific since any urine components, which is insoluble in acid, will give a positive result.

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due to urates, uric acid urea and bile, acids. These are not to be reported positive for protein.

6. Report the result according to the chart given on the above for ring tests.
7. 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.

Preparation of Robert's Reagent (See Reagent Number 11)

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

Preparation of Acetic Acid Reagent (See Reagent Number 1)

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 semiquantitatively 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 tetrabromphenol 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 specially-designed 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.

$$g/24\ hr = \frac{\text{total volume}}{1000} \times g/l$$

4.4 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 cunjugated 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

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4. A blue to green color indicates a positive reaction.
5. Report as positive or negative.

Prparation of Fouchet's Reagent (See Reagent Number 9)

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.

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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 20 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.

4.5 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 para-dimethylaminobenzaldhyde 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.

Preparation of Ehrlich's Reagent- see reagent number 7

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)

4.6 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.

Preparation of Alcoholic Solution of Zinc Acetate (See Reagent Number 13)

4.7 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 dehyphoCoghyen(r)6(s17.7pE(G6P6(e6Falci)6.4(pa/D)ghyfJ0 -1.3(a)-r. (r. r7( )]TJ-17.

- 53v9r witro.6(a)6cip7ga

Specimen

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

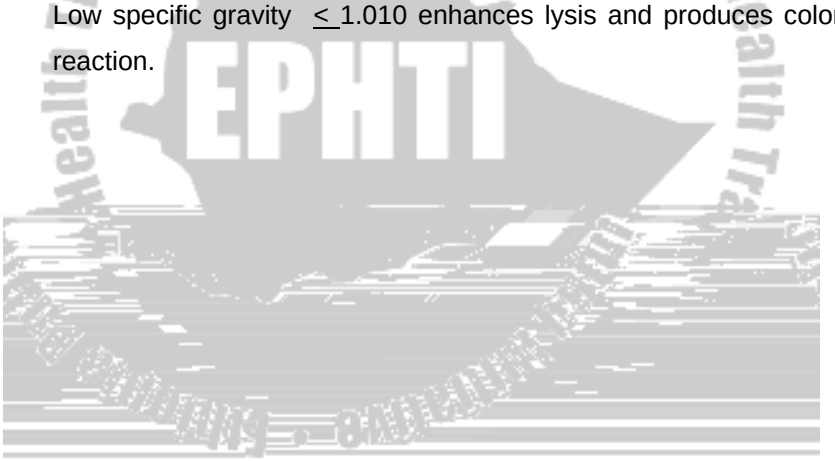
Factors that Affect Hemoglobin Determination

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 $\leq$ 1.010 enhances lysis and produces color reaction.



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.

4.8 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

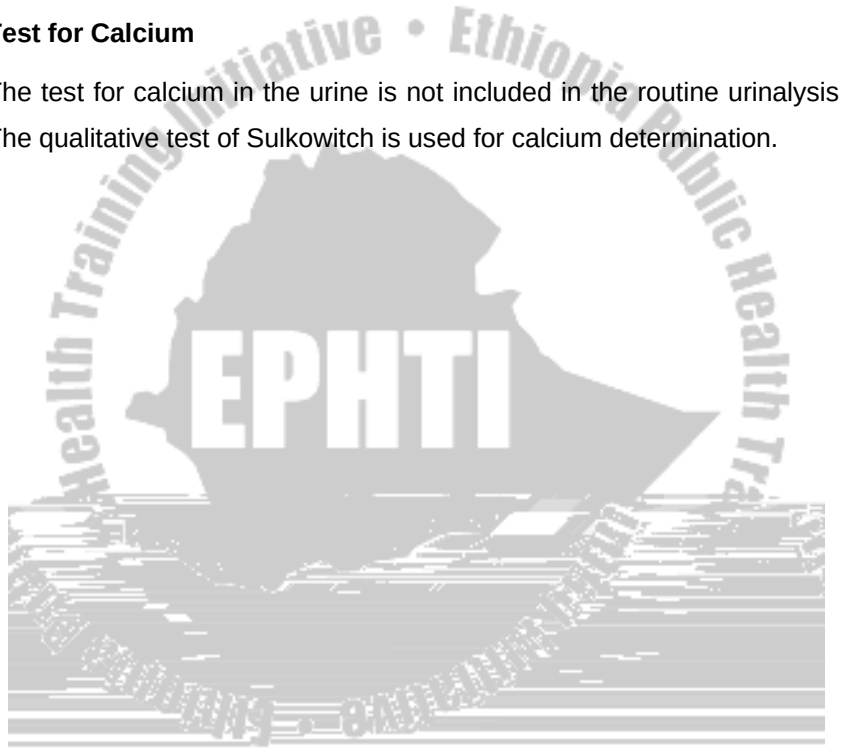
1. False positive
   - High sodium and magnesium intake.

- 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.



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:

a. 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.
b. Some amounts are caused by organisms that do not convert nitrate to nitrite (such as enterococci, acinetobacter spp and some pseudomonas species.).
c. The patient was in a vegetable free diet, which is the important source for nitrate.
d. 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.
e. High doses of ascorbic acid.
f. Presence of urobilinogen.

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.

4.10 Leukocytes Test

Introduction

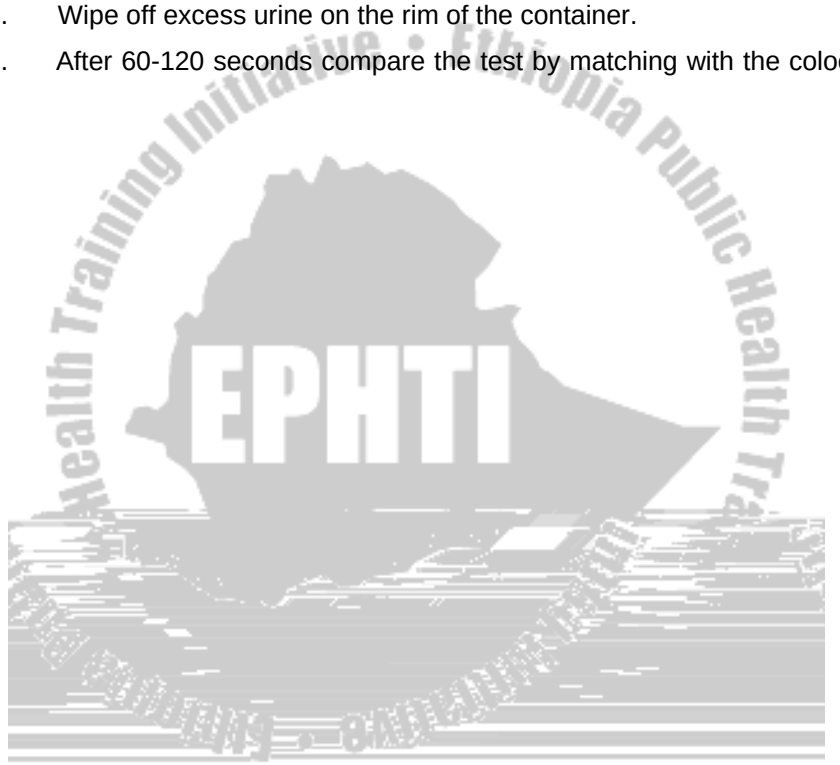
Tests for leukocytes has become part of the routine urinalysis since commercial multiple-reagent strips began to be marketed (N-Multistix or Combur 9). The presence of leukocytes indicates inflammation at some point along the urogenital tract.

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 coloe



- 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.

4. 12 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

a. Utilization of oxidizing agent (e.g. $FeCl_3$)
b Utilization of reducing action of melanogen
c. Oxidation by atmospheric air

A. Ferric Chloride Test

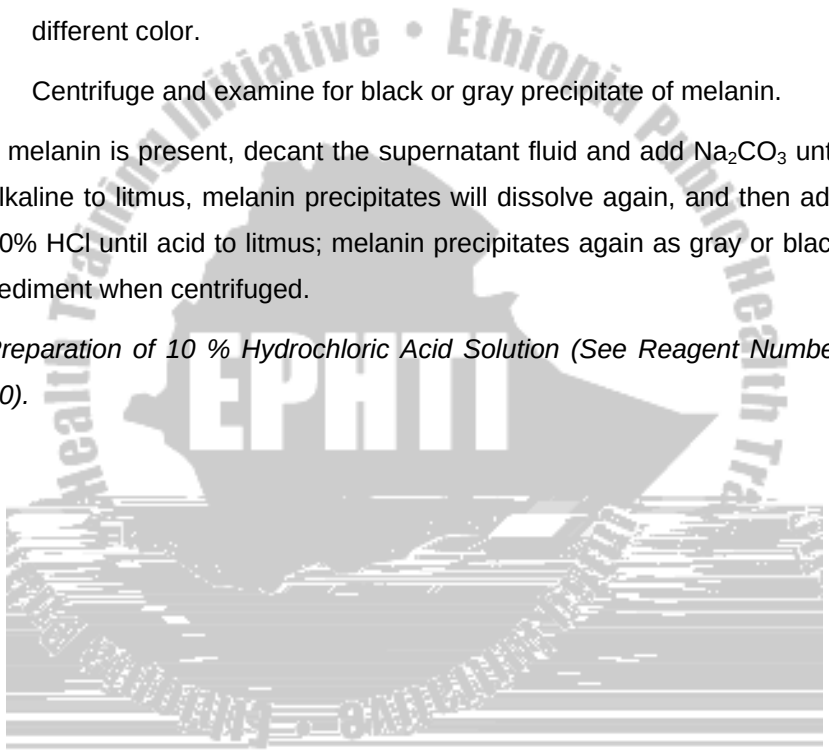
Principle: $FeCl_3$ oxidizes the melanogen to melanin.

Procedure

- To 5 ml of freshly voided urine, add 1 ml 10% $FeCl_3$ 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 Na_2CO_3 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.

Preparation of 10 % Hydrochloric Acid Solution (See Reagent Number 10).



Procedure

1. Prepare fresh solution of sodium nitroprusside by shaking few crystals of sodium nitroprusside in 10 ml of distilled water.

CHAPTER FIVE

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.

5.1. Procedure for Microscopic Examination of Urine

1. Assemble all necessary materials used for the collection,

centrifugation and examination. This include:

- Clean dry plastic or Glass containers, which enable to collect at least up to 15 ml of urine for routine urinalysis.
- Hand (manual), or electrical centrifuge.
- Conical centrifuge tubes, or regular test tubes.
- Pasture pipette with rubber fit or automatic pipettes if possible.
- Slides and cover slides 20 x 20 mm.
- Electrical or solar microscope, which has 10x and 40 x objectives.

2. Preparation of patient
   - Explain the purpose of the test by using simple language. Do not use medical terms or try to explain details of the procedure.
   - 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.
   - If the patient is female, advice 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.
   - 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.
   - 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.

- If it is difficult to deliver within 2 hrs, it is better to preserve specimen in the refrigerator at the temperature between 2-6 ^{0}C 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. If

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).

5.3 Urinary Sediments

Classification of Urinary Sediments

MICROSCOPICAL EXAMINATION OF
URINE SEDIMENTS

Organized Elements (Formed from Living Materials)

Non-organized Elements (Formed for Non-living Material)

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- 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, and
  - 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

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 memb round e)9(ca)5.9(st0 Tw( )Tj/Tof ge41.0017 Ts.ea9(e4osne u)5.5(i.b00.5(.5(be)5.1

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 ex7amined under 10x objective of the microscope.

- Always the condenser should be lowered and at the same time in

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

- 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

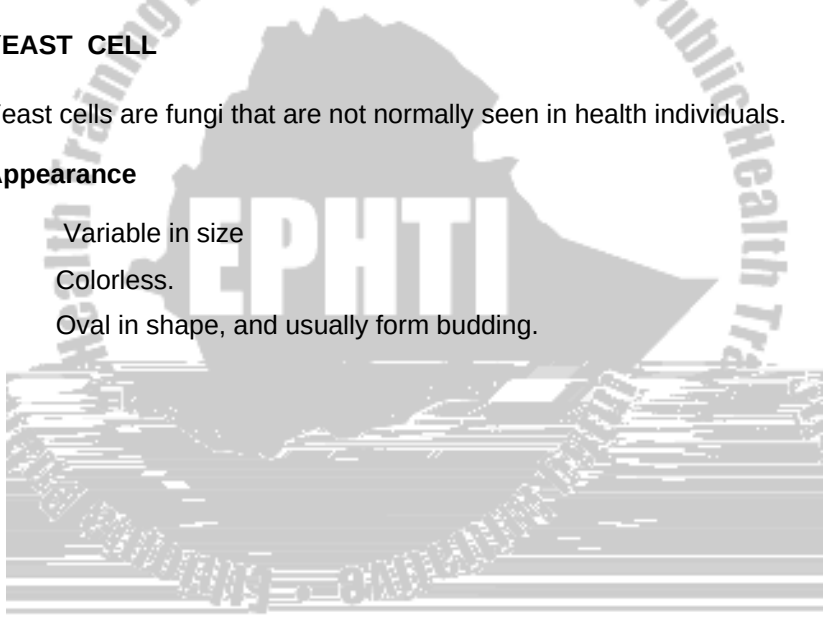
- The parasite usually found in high concentration during night from 10:00 p.m. – 4:00 a.m. and i.e. it has nocturnal periodicity.
- Differentiate from B. malai and L. loa by its tail feature.
- Differentiate from Mansonella species by its large size and sheath.

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.



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.

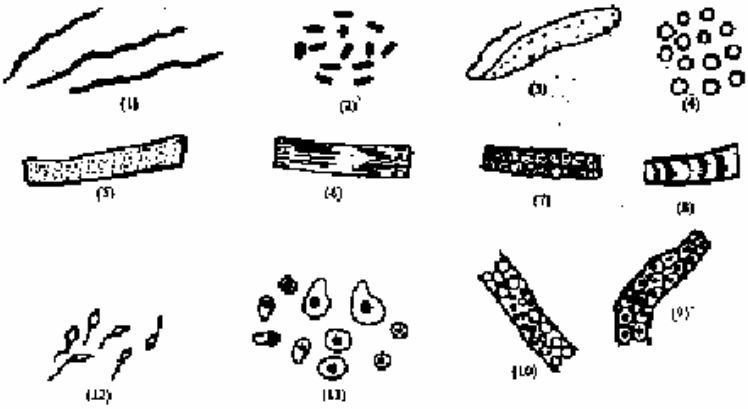
Clinical Significance

- 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.



Formed urinar Sediment: (1) Mucous threads, (2) Bacteria, (3) Cylindroid, (4) RBCs (5-10, are casts), (5) Granular, (6) Hyaline, (7) pus cells, (8) Waxy, (9) Epithelial Cell, (10) Fatty, (11)Epithelial Cells, & (12) Spermatozoa

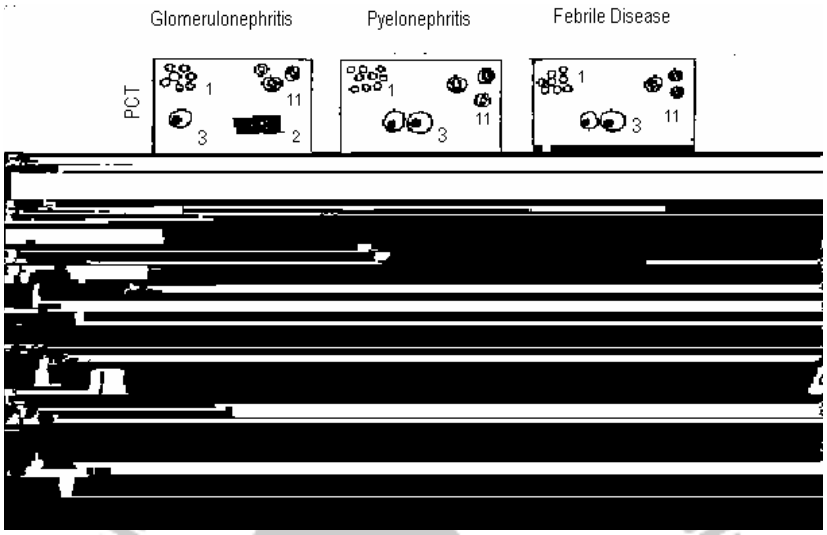


Fig 5. Formed Urine Elements

5.5 Non-organized Elements (Urine Crystals)

Appear usually after the specimen (urine) collected and left with out 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.

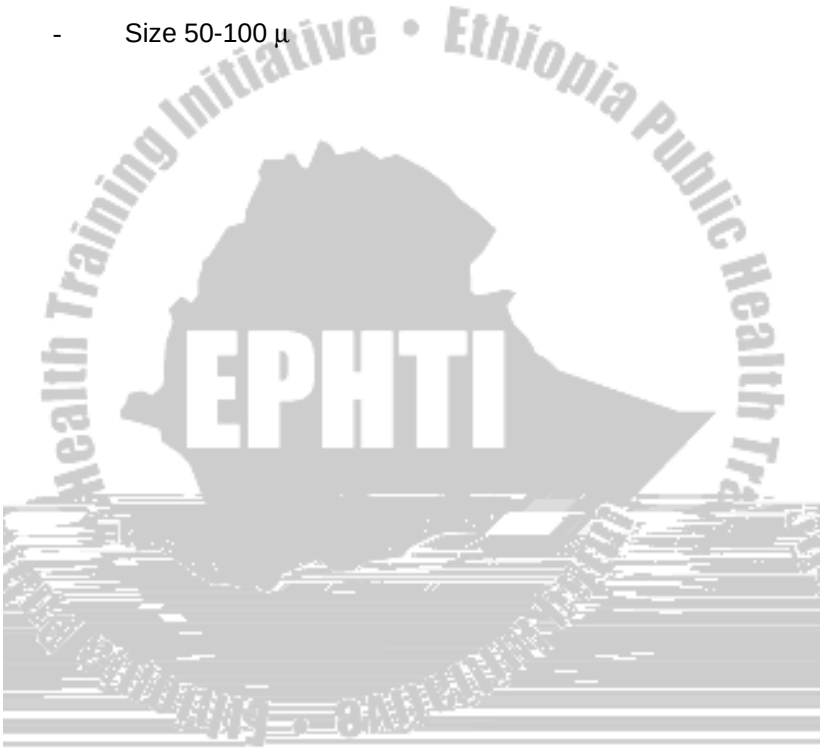
g. Bilirubin

- 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 μ



- 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.

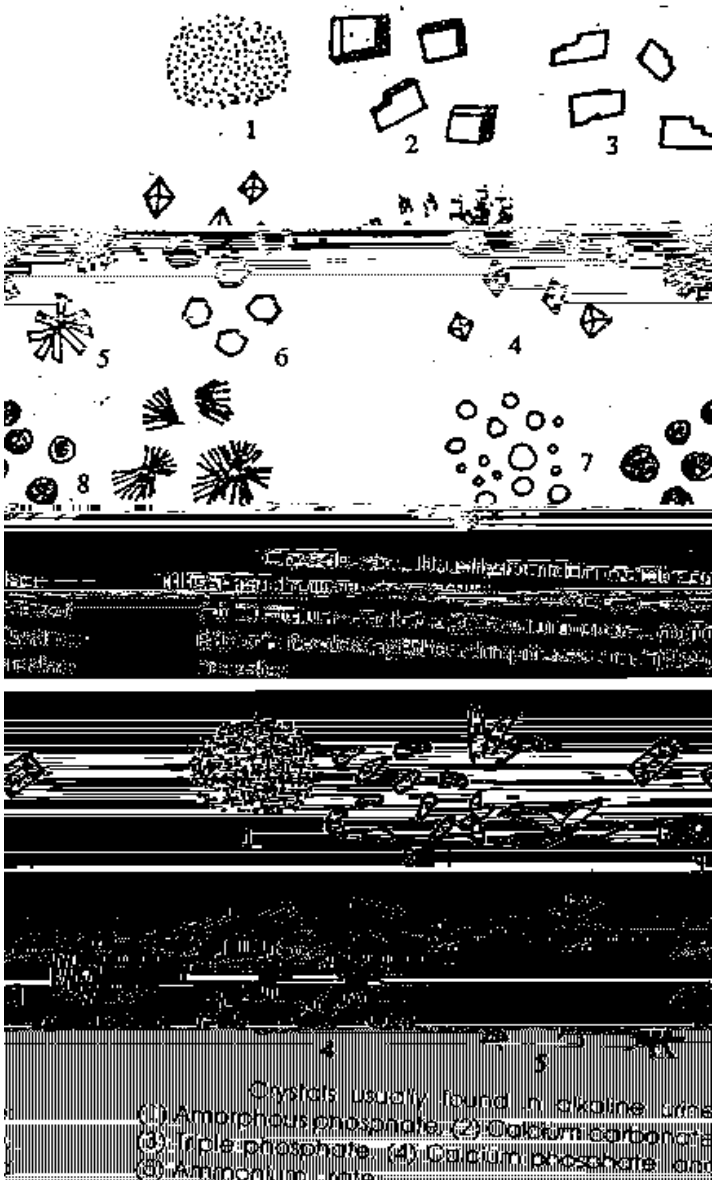


Fig 6. Urine Crystals

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-uniformin size.

Fat droplets (other bubbles)

- Oil droplets

— not evenly distributed.

- Pollen greens — are seasonal.
- Starch granules — incomplete digestion of starch

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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:

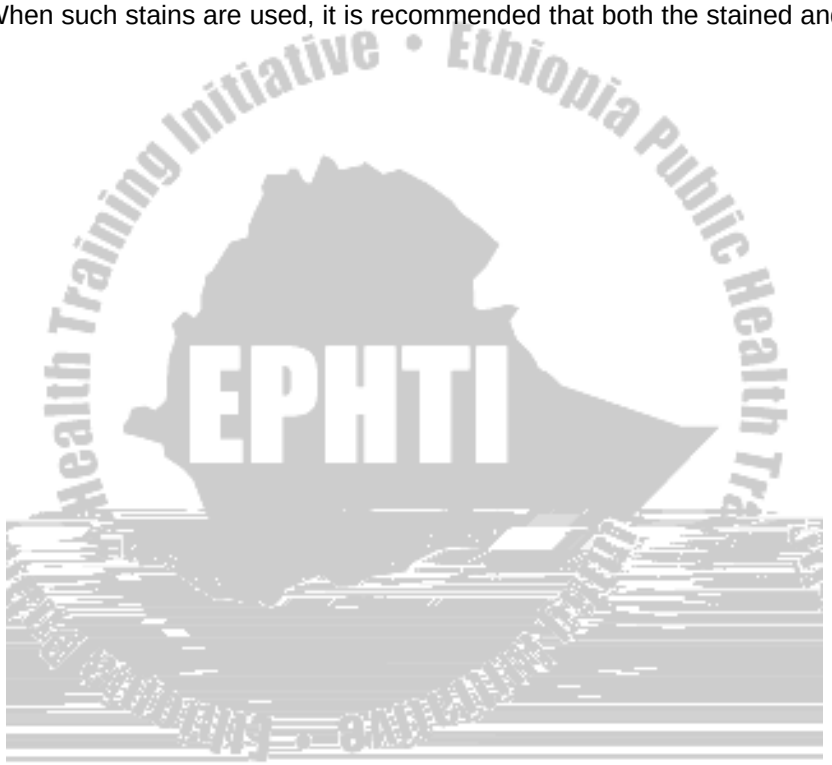
RBC – Purple to dark purple.
WBC – Cytoplasm -violet to blue.
Nucleus – reddish purple.
Glitter cells – blue.

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

2. *Methyl blue (Loeffler's stain)*

3. *Cyto Diachrome stains*

When such stains are used, it is recommended that both the stained and



APPENDIX

I. Reagent Preparation

Reagent No.1 Acetic Acid Reagent (10%)

Pipette 10 ml of glacial acetic acid into a 100ml volumetric flask that is 1/2 filled with distilled water. Dilute to 100-ml volume with distilled water and mix.

Reagent No.2 Acetone Powder Reagent

Weigh each of the following with the rough balance. Grind each separately with a mortar and pestle and put into a reagent bottle.

Ammonium Sulphate 20g
Sodium Carbonate 20g
Sodium Nitroprusside 1g

After all have been added to the reagent bottle, mix well.

Reagent No.3 Barium Chloride Filter Paper for Bilirubin

Soak thick filter paper in saturated barium chloride
Dry & cut in to small strips (4 x 1/2 inch strips).

Reagent No.4 Benedict Reagent for Glucose

To make 1 lit

A - 173 gm.----------Trisodium citra
100 gm---------- Sodium carbonate anhydrates
800 ml---------- Distilled H_2O
Dissolve to make to 850 ml
B- 17,3 gm ---------- $CuSO_4$
100 ml ---------- Distilled H_2O
Stir slowly in to the first solution bring up to 1 lit. with distilled H_2O.

Reagent No.5 Bleach for Preservation of S.Hematobium egg in urine

S. hematobium. egg preserved by adding 1ml (1-% v/v).

Domestic bleach in every 10 ml urine.

Reagent No.6 Boric Acid Preservative (1-%w/v) 10 gm/l

Boric acid ----------10gm.

Dissolve in 1000ml distilled water.

Reagent No.7 Ehrlich's Reagent for Uroblinogen

To make 200ml

para-dimethylaminobenzaldehyde ---------- 4 gm

HCl concentrated ---------- 40 ml

Distilled H_2O ---------- 160ml

a. Weigh the para-dimethylaminobenzaldeyde and transfer it to clean, leak proof bottle.
b. Measure the water and add to chemical and mix
c. Add conc. HCl and mix well.
d. Label the bottle and mark, as it is corrosive. Store at room temperature the reagent is stable for several weeks.

Reagent No. 8 10 % Ferric Chloride Reagent

Weigh 10 g of ferric chloride and transfer to a 100-ml volumetric flask. Dissolve and dilute to 100ml volume with water and mix.

Reagent No. 9 Fouchet's Reagent

Trichloroacetic acid ---------- 25gm

Distilled H_2O ----------100ml

10% Ferric chloride ($FeCl_3$) ----------10ml

Mix well.

Reagent No. 10 10% Hydrochloric Acid (HCl) Reagent

Measure 10 ml HCl and dissolve in 90ml-distilled water.

Reagent No. 11 Robert's Reagent for Protein

To 1 lit of distilled water add magnesium sulphate ($MgSO_4$) with stirring until no more to dissolve.

Add 200-ml concentrated $HN0_3$ & mix.

Reagent No.12 Sulkowitch Reagent for Calcium

Weigh 4 gm Ammonium Oxalate and dissolved in 100 ml distilled water.

Reagent No.13 Alcoholic Solution of Zinc Acetate for Urobilin

Place 100 ml of ethyl alcohol in a beaker.

Add Zinc Acetate with string until no more goes into solution.

Weigh 5 gm of Iodine and 10 gm of KI.

Transfer all reagents to a brown bottle.

Reagent No.14 Sulphosalicylic Acid Reagent (20% W/V)

Sulphosalcylic acid ---------- 200 gm

Dilute to 1 lit. Volume with distilled H_2O.

Reagent No.15 Sodiumnitroprusside Rreagent

Weigh 10 gm of Sodium Nitroprusside (Nitroferricyanide).

Transfer to a flask containing 95ml of water and 2ml of concentrated H_2SO_4.

Mix and store in a brown bottle.

Reagent No.16 Saturated Sodium Nitroprusside

Add 40 gm of Sodium Nitroprusside to 100 ml of water is a brown bottle. Shake to dissolve as much as possible. Allow any undissolved salt to remain in the bottom of the bottle.

III. Table 4: Correct and Incorrect Approach in Urine Testing

Correct Approach	**Incorrect Approach**
Use fresh urine	Delay in the testing of urine without preservation
Make quality control of reagents	Using expired reagents
Be aware of normal as well as abnormal results which are significant	Believing urine results have little significance in the overall diagnostic picture of the patient
Follow the directions carefully	Being careless
Accept only clear and proper collection bottles	Using any container.
Be familiar with interfering substances	Not giving due attention to cross reaction and artifacts
Mix Urine properly	Not mixing well
Record results accurately	Not checking the results recorded during the training of new personnel
Give proper training to professionals	New personnel always jumping into urinalysis because it is the easiest to do and least significant

Glossary

***Alkaptouria**:* Genetically determined defect of metabolism in which homogenestic acid is excreted in the urine, which turns dark on standing.

***Alkaline**:* Containing alkali, strictly a fluid with pH greater than 7.

***Anuria**:* Cessation of the production of urine.

Bile Pigments: Breakdown products of hemoglobin.

***Cystine**:* Sulfur containing amino acid.

Cystinosis: A rare inborn error of metabolism of cystine and other amino acids.

Cystinuria: Presence of abnormal amount of cystine in the urine.

***Cystitis**:* Inflammation of the urinary bladder.

***Diabetes Insipidus**:* A syndrome caused by deficient secretion of anti-diuretic hormone (ADH) by the pituitary gland, and characterized by polyuria.

***Diabetes Mellitus**:* A syndrome caused by a relative deficiency of insulin.

***Diuresis**:* An increased secretion of urine.

Diuretics: Drugs, which increase the volume of urine excreted.

***Diurnal**:* Daily

***Endocarditis**:* Inflammation of the endocardial lining of the heart.

***Glomerulus's**:* The filtration unit of a nephrone, consists of a coil of fine capillaries opposed to an expansion of urinary epithelium.

***Glomeurlar Filtrate**:* Ultra filtered blood through the glomerular membrane.

***Glomeurlar Filtration** Rate:* The rate by which the glomerular filtrate is formed.

***Glomerulonephritis**:* one cause of acute nephritic syndrome. The exact pathogenesis is unknown but mostly associated with streptococcal infection of throat or elsewhere.

***Glycaemia**:* Presence of sugar in blood .

***Glycosuria**:* Presence of sugar in urine.

***Hemoglobinuria**:* Hemoglobin, freed by lysis of red blood cells, in the urine.

***Haematuria**:* Presence of intact red blood cells in the urine.

***Jaundice**:* A syndrome characterized by an increased level of bile pigments in the blood and tissue fluids.

***Ketonaemia**:* Presence of Kenton bodies in the blood.

***Ketone**:* Chemical compound containing carbonyl radical (C=O).

***Ketonuria**:* the presence of ketone bodies in the urine.

***Ketosis**:* Acidosis due to increased level of Ketone bodies in the blood.

Oliguria: A diminution in the volume of urine produced by the kidney.

***Orthostatic Proteinuria**:* Protein if urine due to standing upright anatomic position for long period.

***Polyuria**:* Excessive production of urine.

***Polymorph Nuclear**:* having nuclei of various shape.

***Postprandial**:* After meal.

Postural Proteinuria: Protein in urine pertaining to posture.

***Pylonephritis**:* Inflammation of the kidney its pelvic.

***Renal**:* Related to kidney.

***Renal Calculus**:* A stone in the kidney.

***Renal Failure**:* Acute renal failure presents as sudden inability of the kidney to produce urine.

Renal Threshold: Concentration of the substance on the blood at which it appears in the urine.

***Renal Tubular Acidosis**:* Defective renal tubular function in which there is a failure to secrete hydrogen ion in to the urine with consequent inability to reduce the acidity of the blood in the normal way.

***Specific Gravity**:* The ratio between the weight of a substance and the weight of an equal volume of water.

***Tyrosinaemia**:* Presence of tyrosine in the urine.

***Uremia**:* An elevation of the urea concentration in the blood above the normal value of about 5 mm/liter.

***Urea**:* Principal excretory product of protein metabolism.

***Uresis**:* Urination.

***Ureter**:* The canal between the kidney and the bladder, down which the urine passes.

***Ureteral**:* Pertaining to the ureter.

***Ureteritis**:* Inflammation of the ureter.

***Urinalysis**:* Analysis of the urine.

***Urinary**:* Pertaining to the urine.

***Urination**:* Micturition (the act of discharging urine).

***Urine**:* Excretory product of the kidney.

***Urinometer**:* A small glass instrument with a graduated stem used for

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