BACHELOR OF MEDICAL LABORATORY TECHNOLOGY LAB MANUAL

2nd Semester

Prepared By

Paramedical & Allied Science Dept.

BMLT

MIDNAPORE CITY COLLEGE

MIDNAPORE CITY COLLEGE

Department of Paramedical and Allied Health Sciences

Bachelor of Medical Laboratory Technology (BMLT)

NEP

Semester: II

Paper Title: Basic Pathology (Practical)

Paper Code: BMLT MJ-2P

BMLT MJ-2P:

Syllabus:

1. Routine analysis of urine sample: Physical, Chemical (Protein, Sugar, ketone body, bile salt, bile pigment, blood) and Microscopical tests.

2. Sputum: Smear preparation, Staining (AFB).

1A: Physical examination of Urine

AIM: To examine the physical characteristics of normal and abnormal urine, wet preparation of urine and its microscopic examination and interpretation.

INTRODUCTION: Examination of urine is important for diagnosis and assistance in the diagnosis of various diseases. Routine (complete) examination of urine must start from physical and microscopic examination.

PRINCIPLE: The specimen should be properly collected in a clean container which should be properly labelled with name of the patient, age, sex, identity number with date and time of collection. It should not show signs of contamination. Physical examination of urine consists of volume, colour, odour, reaction/pH and specific gravity. After that observe by eye and light microscope.

SPECIMEN COLLECTION:

For routine examination a clean glass tube or capped jar is used; for bacteriologic examination a sterilized container is required.

For routine urinalysis: first morning sample is best since it is most concentrated.

For bacteriologic examination: mid-stream sample is preferable, i.e. first part of urine is discarded and mid-stream sample is collected.

For 24-hour sample: Collection of urine is started in the morning at 8 AM (first voided sample is discarded) and all subsequent samples are collected till next day 8 AM.

RESULTS & INTERPRETATION:

A. VOLUME:

Normal range: 700-2500 ml (average 1200 ml) of urine in 24 hours

Interpretation:

i) Nocturia	Excess of 500 ml during night with specific gravity of less than 1.018.	Sign of early renal failure.
ii) Polyuria	Excess of urine is passed in 24 hr (> 2000 ml) with low specific gravity.	Physiological: excess water intake, may be seasonal (e.g. in winter). Pathological: Diabetes insipidus, diabetes mellitus.
iii) Oliguria	Less than 500 ml of urine is passed in 24 hr.	Less intake of water, dehydration, renal ischaemia.
iv) Anuria	Almost complete suppression of urine (< 150 ml in 24 hours).	Renal stones, tumours, renal ischaemia

B. COLOUR:

Interpretation:

Normal: urine is clear, pale or straw-coloured due to pigment urochrome.

Colour	**Inference**
i) Colourless	diabetes mellitus, diabetes insipidus, excess intake of water.
ii) Deep amber	good muscular exercise, high grade fever.
iii) Orange	increased urobilinogen, concentrated urine
iv) Smoky urine	small amount of blood, administration of vitamin B12, aniline dye
v) Red	haematuria, haemoglobinuria
vi) Yellow-brown	bile and its derivatives
vii)Milky	pus, fat
viii) Green	putrefied sample, phenol poisoning

C. ODOUR:

Interpretation:

Normal: Faint aromatic odour.

Odour	Inference
i) Pungent	ammonia produced by bacterial contamination
ii) Putrid	UTI.
iii) Fruity	ketoacidosis
iv) Mousy	phenylketonuria

D. REACTION/pH:

Measured by pH indicator paper or by electronic pH meter.

Interpretation:

Normal urine: Slightly acidic and its pH ranges from 4.6-7.0 (average 6.0).

pH	Inference
Acidic urine	i. High protein intake, e.g. meats. ii. Ingestion of acidic fruits. iii. Respiratory and metabolic acidosis. iv. UTI by *E. coli*.
Alkaline urine	i. Citrus fruits. ii. Vegetables. iii. Respiratory and metabolic alkalosis. iv. UTI by *Proteus, Pseudomonas*.

E. SPECIFIC GRAVITY:

This is the ratio of weight of 1 ml volume of urine to that of weight of 1 ml of distilled water. It depends upon the concentration of various particles/solutes in the urine. Specific gravity is used to measure the concentrating and diluting power of the kidneys. Measured by urinometer, refractometer or reagent strips.

Assay by Urinometer:

Procedure:

1. Fill urinometer container 3/4th with urine.
2. Insert urinometer into it so that it floats in urine without touching the wall and bottom of container.

3. Read the graduation on the arm of urinometer at lower urinary meniscus.
4. Add or substract 0.001 from the final reading for each 3°C above or below the calibration temperature respectively marked on the urinometer.

Interpretation:

Normal specific gravity of urine: 1.003 to 1.030.

Specific Gravity	**Inference**
Low specific gravity	i. Excess water intake; ii. Diabetes insipidus
High specific gravity	i. Dehydration; ii. Albuminuria; iii. Glycosuria
Fixed specific gravity (1.010)	i. ADH deficiency; ii. Chronic nephritis

INFERENCE:

Record your observations in the following tabular format and write the inference from supplied sample.

Property	Results	Inference
Volume		
Colour		
Odour		
pH		
Specific gravity		

1B. MICROSCOPIC ANALYSIS OF URINE:

Microscopic analysis of urine sample has great clinical importance. Important structures like different casts, erythrocytes, leukocytes, and bacteria need to be observed.

PROCEDURE:

i. Agitate the urine sample to avoid any sediment that may settled to bottom.
ii. Fill the centrifuge tube with urine and centrifuge for 3 min at low rate of speed.
iii. Pour all the urine and there is sufficient urine on the slide to drain to the bottom and suspend the sediment.
iv. Pour a drop of sediment on a glass slide, and cover with a cover glass that has been wiped clean of oil and lint.
v. Examine under microscope with the lower power objective.
vi. Finding should be reported as few, many or abundant.
vii. If necessary, stain with new methylene blue.
viii. Organized sediments, epithelial cells.

OBSERVATION & INFERENCE:

Observe the followings and draw on your observation note book:

1. Cells - *RBCs, WBCs, epithelial cells*.

2. Casts -*Hyaline Cast, Red Cell Cast, Leucocyte Cast, Granular Casts, Waxy Casts, Fatty Cast, Epithelial Cast, Pigment Cast.*

3. Crystals - *Calcium oxalate, Uric acid, Amorphous urate, Tyrosine, Cystine, Cholesterol crystals, Sulphonamide, Amorphous phosphate, Triple phosphate, Calcium carbonate, Ammonium biurate*

4. Miscellaneous structures- *Spermatozoa, Parasites, Fungus, Tumour cells.*

1C: Bio-chemical analysis of urine.

Bio-chemical estimation of glucose in urine.

AIM: To study glucose present in supplied urine sample.

INTRODUCTION: Glucose is the most important of the sugars which may appear in urine. Normally, approximately 130 mg of glucose in urine is passed per 24 hours which is undetectable by qualitative tests. Above that level is called glucosuria that may be detected or estimated by qualitative or quantitative analysis.

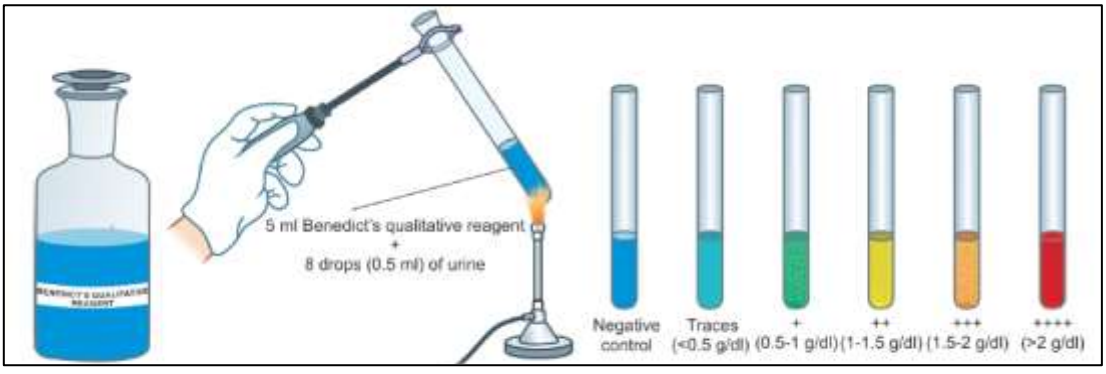
PRINCIPLE:

Cupric ion is reduced by glucose to cuprous oxide and a coloured precipitate is formed.

QUALITATIVE TESTS*: BENEDICT'S TEST:*

PROCEDURE: Benedict's test:

1. Take 5 ml of Benedict's qualitative reagent in a test tube.
2. Add 8 drops (or 0.5 ml) of urine.
3. Heat to boiling for 2 minutes (Fig. 42.5).
4. Cool in water bath or in running tap water and look for colour change and precipitation.



RESULTS & INTERPRETATION: Benedict's test:

No change of blue colour	= Negative
Greenish colour	= Traces (< 0.5 g/dl)
Green/cloudy green ppt	= + (0.5-1 g/dl)
Yellow ppt	= ++ (1-1.5 g/dl)
Orange ppt	= +++ (1.5-2 g/dl)
Brick red ppt	= ++++ (> 2 g/dl)

NOTE: Benedict's test is for reducing substances excreted in the urine, the test is positive for all reducing sugars (glucose, fructose, maltose, lactose but not for sucrose which is a nonreducing sugar) and other reducing substances (e.g. ascorbic acid, salicylates, antibiotics, L-dopa).

QUANTITATIVE TEST:

***PROCEDURE**:*

1. Take 25 ml of quantitative Benedict's reagent in a conical flask.
2. Add to it 15 gm of sodium carbonate (crystalline) and some pieces of porcelain.
3. Heat it to boil.
4. Add urine to it from a burette slowly till there is disappearance of blue colour of Benedict's reagent. This is same as common titration procedure.
5. Record the volume of urine used from burette.
6. Note: 0.05 gm of glucose reduces 25 ml of Benedict's reagent

CALCULATION: The amount of glucose present in urine as under: (0.05 × 100)/Amount of urine.

RESULTS & INFERENCE:

Record your results, calculate and interpret it.

Considering the other physical factor, causes of glucosuria may be due to diabetes mellitus, renal glucosuria, severe burns, administration of corticosteroids, severe sepsis, pregnancy.

Bio-chemical estimation of protein and ketone bodies in urine, bile salt, bile pigment, urobilinogen and blood in urine.

AIM: To learn the principle, perform the procedure and interpret the results of various routine tests for chemical

constituents of a urinary sample.

INTRODUCTION: Chemical constituents frequently tested in urine are: proteins, ketones, bile derivatives and blood. If urine is not clear, it should be filtered or centrifuged before testing.

TESTS FOR PROTEINURIA:

Urine may be tested for proteinuria by qualitative tests and quantitative methods.

Qualitative Tests:

1. Heat and Acetic Acid Test

PRINCIPLE: Heat causes coagulation of proteins.

PROCEDURE:

i. Take a 5 ml test tube.
ii. Fill 2/3rd with urine.
iii. Acidify by adding a few drops of 3% acetic acid if urine is alkaline.
iv. Boil upper portion for 2 minutes (lower part acts as control).
v. If precipitation or turbidity appears, add a few drops of 3% acetic acid.

INTERPRETATION:

Turbidity or precipitation disappears on addition of acetic acid, it is due to phosphates; if it persists after addition of acetic acid, then it is due to proteins.

The test is semi-quantitative and can be graded from traces 0 to ++++ depending upon amount of protein as under:

No cloudiness	= Negative
Faint cloudiness	= Traces (less than 0.1 g/dl).
Cloudiness without granularity	= +(0.1 g/dl).
Granular cloudiness	= ++(0.1-0.2 g/dl)
Precipitation and flocculation	= +++(0.2-0.4 g/dl).
Thick solid precipitation	= ++++ (> 0.5 g/dl).

2. Sulphosalicylic Acid Test

PROCEDURE:

i. Make urine acidic by adding 3% acetic acid.
ii. With 2 ml of urine add a few drops (4-5) of 20% sulphosalicylic acid.

INTERPRETATION:

Appearance of turbidity which persists after heating indicates presence of proteins.

3. Heller's Test

PROCEDURE:

1. Take 2 ml of concentrated nitric acid in a test tube.
2. Add urine drop by drop by the side of test tube.

INTERPRETATION:

Appearance of white ring at the junction indicates presence of protein.

Quantitative Estimation:

1. Esbach's albumin meter method:

i. Fill the albuminometer with urine up to mark U.
ii. Add Esbach's reagent (picric acid + citric acid) up to mark R.
iii. Stopper the tube, mix it and let it stand for 24 hours.
iv. Take the reading from the level of precipitation in the albuminometer tube and divide it by 10 to get the percentage of proteins.

2. Turbidimetric method:

i. Take 1 ml of urine and 1 ml standard in two separate tubes.
ii. Add 4 ml of trichloroacetic acid to each tube.
iii. After 5 minutes take the reading with red filter (680 nm).

RESULTS & INFERENCE:

Write your results based on the different procedure and interpret as stated:

i. Normal: A very scanty amount of protein in urine (< 150 mg/day).
ii. *Heavy proteinuria* (> 3 gm/day) occurs due to nephrotic syndrome, renal vein thrombosis, diabetes mellitus and SLE.
iii. *Moderate proteinuria* (1-3 gm/day) due to Chronic glomerulonephritis, nephrosclerosis, multiple myeloma, pyelonephritis.
iv. *Mild proteinuria* (< 1.0 gm/day) occurs due to hypertension, polycystic kidney, chronic pyelonephritis, UTI, fever.
v. *Microalbuminuria* is excretion of albumin 30-300 mg/day or random urine albumin/ creatinine ratio of 30-300 mg/gm creatinine and is indicative of early and possibly reversible glomerular damage from hypertension and risk factor for cardiovascular disease. *Microalbuminuria* is estimated by radioimmunoassay.

TESTS FOR KETONURIA:

AIM: To detect the ketone bodies present in urine.

INTRODUCTION:

Ketone present in urine because of incomplete fat metabolism. The three ketone bodies excreted in urine are: acetoacetic acid (20%), acetone (2%), and β-hydroxybutyric acid (78%).

TEST PROCEDURE:

1. Rothera's Test:

Principle: Ketone bodies (acetone and acetoacetic acid) combine with alkaline solution of sodium nitroprusside forming purple complex.

Procedure:

i. Take 5 ml of urine in a test tube.
ii. Saturate it with solid ammonium sulphate salt; it will start settling to the bottom of the tube when saturated.
iii. Add a few crystals of sodium nitroprusside and shake.
iv. Add liquor ammonia from the side of test tube.

Interpretation:

Appearance of purple or permanganate coloured ring at the junction indicates presence of ketone bodies.

2. Gerhardt's Test:

Procedure:

i. Take 5 ml of urine in a test tube.
ii. Add 10% ferric chloride solution drop by drop.
iii. Filter it and add more ferric chloride.

Interpretation: Brownish-red colour indicates presence of ketone bodies.

RESULTS & INFERENCE:

Write your results/observation and interpret as stated below:

Ketone bodies found in urine sample may be due to:

i. Diabetic ketoacidosis

ii. Dehydration

iii. Hyperemesis gravidarum

iv. Fever

v. Cachexia

vi. After general anaesthesia.

TEST FOR BILE DERIVATIVES IN URINE:

AIM: To detect the bile derivatives (salts, pigments and urobilinogen) present in urine.

INTRODUCTION: Three bile derivatives excreted in urine are: urobilinogen, bile salts and bile pigments. While urobilinogen is normally excreted in urine in small amounts, bile salts and bile pigments appear in urine in liver diseases only.

A. TESTS FOR BILE SALTS:

Bile salts excreted in urine are cholic acid and chenodeoxycholic acid.

METHODS:

Hay's Test:

Principle. Bile salts if present in urine lower the surface tension of the urine.

Procedure:

i. Take a 50 ml beaker or 15 ml test tube.
ii. Fill the beaker or test tube upto 2/3rd with urine.
iii. Sprinkle finely powdered sulphur powder over it.

Interpretation: If bile salts are present in the urine then Sulphur powder sinks, otherwise it floats.

Cause for Bile Salts in Urine: Obstructive jaundice.

B. TESTS FOR UROBILINOGEN:

A small amount of urobilinogen is excreted in urine (4 mg/24 hr) normally. The sample should always be collected in a dark coloured bottle as urobilinogen gets oxidized on exposure to light. Urobilinogen in urine are detected by Ehrlich's test.

Ehrlich's Test:

Principle: Urobilinogen in urine combines with Ehrlich's aldehyde reagent to give a red purple coloured compound.

Procedure:

i. Take 10 ml of urine in a test tube.
ii. Add 1 ml of Ehrlich's aldehyde reagent.
iii. Wait for 3-5 minutes.
iv. If the test is positive, the test is repeated by preparing multiple dilutions, e.g. 1:10, 1:20, 1:40 and so on.

Interpretation:

i. Development of red purple colour indicates presence of urobilinogen.
ii. A positive test is subsequently done in dilutions; normally it is positive in up to 1:20 dilution.
iii. Increased urobilinogen in urine indicated haemolytic jaundice and haemolytic anaemia.
iv. Causes for absent urobilinogen in urine: Obstructive jaundice.

TESTS FOR BILIRUBIN (BILE PIGMENT):

Bilirubin is breakdown product of haemoglobin. Normally, no bilirubin is passed in urine.

1. Fouchet's Test:

Principle: Ferric chloride oxidizes bilirubin to green biliverdin.

Procedure:

i. Take 10 ml of urine in a test tube.
ii. Add 3-5 ml of 10% barium chloride.
iii. Filter through filter paper.
iv. To the precipitate on filter paper, add a few drops of Fouchet's reagent (ferric chloride + trichloroacetic acid).

Interpretation: Development of green colour indicates bilirubin.

2. Foam Test

Procedure:

i. Take 5/10 ml of urine in a test tube.
ii. Shake it vigorously.

Interpretation: Presence of yellow foam at the top indicates presence of bilirubin.

Causes of bilirubinuria: Obstructive jaundice, Hepatocellular jaundice.

TESTS FOR BLOOD IN URINE:

Presence of blood can be detected in urine by two important chemical test: Benzidine test and Orthotoluidine test

1. Benzidine Test

Procedure:

i. Take 2 ml of urine in a test tube.
ii. Add 2 ml of saturated solution of benzidine with glacial acetic acid.
iii. Add 1 ml of H_2O_2 to it.

Interpretation: Appearance of blue colour indicates presence of blood.

2. Orthotoluidine Test

Procedure:

i. Take 2 ml of urine in a test tube.
ii. Add a solution of 1 ml of orthotoluidine in glacial acetic acid.
iii. Add a few drops of H_2O_2.

Interpretation: Blue or green colour indicates presence of blood in urine.

Note: Causes of blood in urine:

i. Renal stones

ii. Renal tumours

iii. Polycystic kidney

iv. Bleeding disorders

v. Trauma.

2. ACID-FAST BACILLI STAINING

Introduction: The Ziehl–Neelsen stain, also known as the acid-fast stain, widely used differential staining procedure. The Ziehl – Neelsen stain was first described by two German doctors; Franz Ziehl (1859 to 1926), a bacteriologist and Friedrich Neelsen (1854 to 1894) a pathologist. In this type some bacteria resist decolourization by both acid and alcohol and hence they are referred as acidfast organisms. This staining technique divides bacteria into two groups namely acid-fast and non acid-fast. This procedure is extensively used in the diagnosis of tuberculosis and leprosy. Mycobacterium tuberculosis is the most important of this group, as it is responsible for the disease called tuberculosis (TB) along with some others of this genus Principle Mycobacterial cell walls contain a waxy substance composed of mycolic acids. These are β-hydroxy carboxylic acids with chain lengths of up to 90 carbon atoms. The property of acid fastness is related to the carbon chain length of the mycolic acid found in any particular species

Objectives: To differentiate between acid-fast bacilli and non-acid-fast bacilli. To stain *Mycobacterium* species.

Principle: The Ziehl-Neelsen stain uses basic fuchsin and phenol compounds to stain the cell wall of Mycobacterium species. Mycobacterium does not bind readily to simple stains and therefore the use of heat along with carbol-fuschin and phenol allows penetration through the bacterial cell wall for visualization. Mycobacterium cell wall contains high lipid content made up of mycolic acid on its cell wall making it waxy, hydrophobic, and impermeable. These are ß-hydroxycarboxylic acids made up of 90 carbon atoms that define the acid-fastness of the bacteria. Use of Carbol-fuschin which is basic strongly binds to the negative components of the bacteria which include the mycolic acid and the lipid cell wall. addition of acid alcohol along with the application of heat forms a strong complex that can not be easily washed off with solvents. The acid-fast bacilli take up the red color of the primary dye, carbol-fuschin. While non-acid-fast bacteria easily decolorize on the addition of the acid-alcohol and take up the counterstain dye of methylene blue and appear blue. This technique has been used in the identification of *Mycobacterium tuberculosis* and *Mycobacterium leprae*.

Reagents:

1. Carbol-Fuschin (Primary dye)
2. 20% sulphuric acid or acid-alcohol (Decolorizer)
3. Methylene Blue dye (counterstain) or malachite green

Preparation of reagents

Carbol fuschin

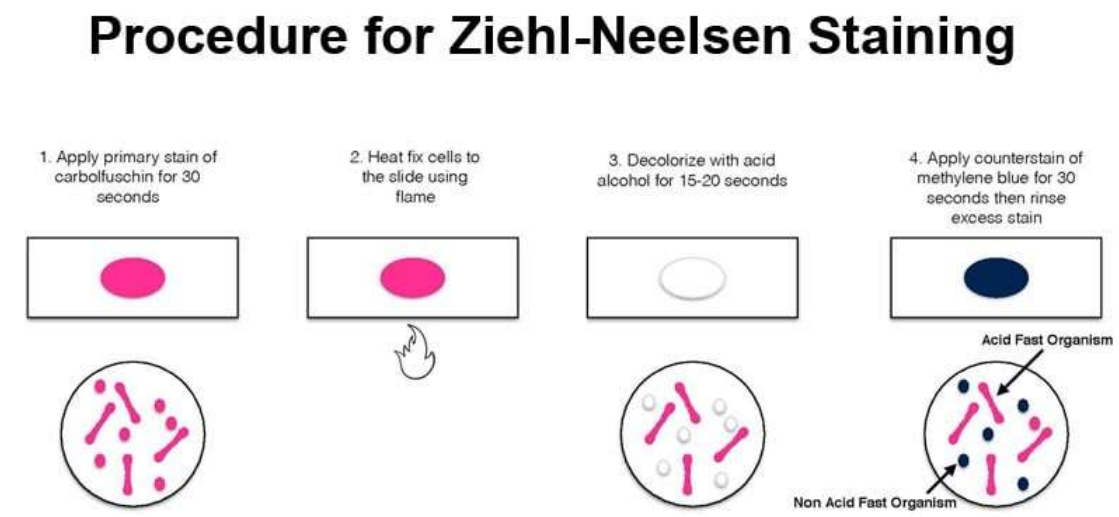
- Distilled water- 100ml
- Basic fuschin- 1g
- Ethyl alcohol (100% ethanol)- 10ml
- Phenol crystals- 5ml

Acid alcohol (3% hydrochloric acid in 95% ethyl alcohol)

- Ethyl alcohol- 95 ml
- Distilled water- 2 ml
- Concentrated hydrochloric acid- 3 ml

0.25% methylene blue in 1% acetic acid

- Methylene blue- 0.25g
- Distilled water- 99ml
- Acetic acid- 1ml



Procedure:

1. Make a smear. Air Dry. Heat Fix.
2. Flood smear with Carbol Fuchsin stain. Carbol Fuchsin is a lipid soluble, phenolic compound, which is able to penetrate the cell wall
3. Cover flooded smear with filter paper
4. Steam for 10 minutes. Add more Carbol Fuchsin stain as needed
5. Cool slide
6. Rinse with DI water
7. Flood slide with acid alcohol (leave 15 seconds). The acid alcohol contains 3% HCl and 95% ethanol, or you can declorase with 20% H_2SO_4
8. Tilt slide 45 degrees over the sink and add acid alcohol drop wise (drop by drop) until the red color stops streaming from the smear
9. Rinse with DI water.
10. Add Loeffler's Methylene Blue stain (counter stain). This stain adds blue color to non-acid fast cells. Leave Loeffler's Blue stain on smear for 1 minute

11. Rinse slide. Blot dry.
12. Use oil immersion objective to view.

Results: Acid-fast bacteria retain the primary dye, carbol-fuschin, and stain pink. Non-acid fat bacteria take up the methylene blue dye and appear blue.

Grading of slides in AFB Microscopy

Examination	Result	Grading	No. of fields to be examined
More than 10 AFB per oil immersion field	Positive	3 +	20
1–10 AFB per oil immersion field	Positive	2 +	50
10–99 AFB per 100 oil immersion fields	Positive	1 +	100
1–9 AFB per 100 oil immersion fields	Positive	Scanty (Record exact number seen)	100
No AFB per 100 oil immersion fields	Negative	—	100

3: Routine test and microscopical test for stool and Occult blood test.

Routine test and microscopical test for stool

AIM:

To analyze the stool (feces) by a series of tests done on it to help diagnose certain conditions affecting the digestive tract. These conditions can include infection (such as from parasites, viruses, or bacteria), poor nutrient absorption, or cancer and presence of hidden blood.

INTRODUCTION:

Feces analysis can be done by Physical Examination for color, volume, consistency, odour, mucus, pus, helminths; chemical examination for occult blood, fat, carbohydrate, protein etc.; and microscopic examination for cells, bacteria, protozoa, helminths etc.

SAMPLE:

Collect the stool in a dry, clean, leak proof container.

Make sure no urine, water, soil, toilet paper, soap gets in the container.

Morning Specimen is preferable.

PHYSICAL EXAMINATION:

PROCEDURE:

1. Note the color of the specimen.
2. Note the consistency of the specimen. Mushy or liquid stools suggest the possible presence of trophozoites or intestinal protozoa. Protozoan cysts are found most frequently in formed stools. Helminth eggs and larvae maybe found in either liquid or formed stools.
3. Examine the surface of the specimen for parasites (e.g., tape worm proglottids or, less commonly, adult pinworms).
4. Examine the stool for blood and/or mucous.
    a. Fresh blood (bright red) indicates acute lower intestinal tract bleeding.
    b. Bloody mucus suggests ulceration, and some of this material should be examined microscopically for trophozoites.
    c. Black, tarry stools are indicative of occult (hidden) blood from higher up in the intestinal tract.
5. Break up the stool with applicator sticks to check for the presence of adult helminths (e.g., *Ascaris*).
6. Feces should be sieved after drug treatment for tapeworm infections to assure recovery of the scolex.

RESULTS AND INFERENCE: Write your observation as above and note.

MACROSCOPIC EXAMINATION: Protozoa and Helminths:

PROCEDURE:

1. Direct saline wet mount

i. Place a drop of saline on the slide.

ii. Pick up a small amount of fecal material on the end of an applicator stick. NOTE: Take small amounts of material from several different areas, especially from bloody and/or mucoid areas.

iii. Emulsify in the saline and cover with a coverslip. Examine on low and high power. NOTE: A smear should be thin enough so that a printed page can be read through it.

iv. The entire preparation must be examined for the presence of eggs, larvae and protozoa. Low power is used to scan for large helminth eggs or larvae. High power is used to detect and identify smaller parasites and larger helminth eggs and larvae.

v. Any parasites detected are reported out by their scientific name and quantity observed. If no parasites are observed report out as "No parasites seen."

Density	Protozoa	Helminths
Rare	2 to 5 organisms per 22 mm square coverslip	2 to 5 organisms per 22mm square coverslip.
Few	1 organism per 5 to 10 high-power fields (40x)	1 egg/larva per 5 to 10 low-power fields (10x)
Moderate	1 to 2 organisms per high-power field, to as few as 1organism per 2 to 3 in high-power fields	1 to 2 eggs/larvae in low-power field
Many	Several organisms in every high-power field	Several eggs/larvae inevery low-power field

2. Iodine Wet mount

i. Place a drop of Lugol's iodine solution on a slide.
ii. Pick up a small amount of fecal material on an applicator stick using the same criteria in the saline procedure for selection of the proper areas.
iii. Emulsify in the iodine solution and cover with a coverslip.
iv. Examine on low and high power as described in the previous procedure.

RESULTS & INTERPRETATION:

Note your observation and interpret the results.

Detection of Occult Blood:

AIM: to detect the Occult blood (hidden) in supplied sample.

INTRODUCTION:

As blood passes through the intestinal tract it changes color and consistency due to the digestive processes. Stools with large amounts of occult blood are black and have a tarry consistency. Smaller amounts of blood may not be visibly detectable. Occult blood may indicate lesions in the intestinal tract due to parasitic infections, cancerous tumors, bleeding ulcers or other disease processes.

PRINCIPLE:

The occult blood test is primarily done to screen for colorectal cancer. It is an easy inexpensive procedure and the specimen is easily obtained by the patient. The chemical reaction involved is as follows:

Hemoglobin + Developer

$Hb + 2\ H_2O_2 = 2H_2O + O_2$

Oxidation of Guaiac

O_2 + Guaiac = Oxidized Guaiac

(Colorless) (Blue)

The presence of a blue color upon completion of the test is a positive test. A positive and negative control must be run concurrently with the patient sample. Most manufacturers now include this as part of the patient test card.

DIET recommendations/restrictions:

It is recommended that the patient be placed on a high residue diet starting 2 days before and continuing through the test period.

DIET MAY INCLUDE:

1. Meats: Only small amounts of chicken, turkey and tuna.
2. Vegetables: Generous amounts of both raw and cooked vegetables including lettuce, corn, spinach, carrots and celery. Avoid those with high peroxidase activity.
3. Fruits: Plenty of fruits, especially prunes and apples.
4. Cereals: Bran and bran-containing cereals.
5. Moderate amounts of peanuts and popcorn daily.

DIET TO BE AVOIDED:

1. Meat: Diet should not include any red or rare meat.
2. Fruits and vegetables containing high peroxidase activity:

Turnip Cauliflower, Broccoli Cantaloupe, Horseradish Parsnip

Alternately, the special diet may be omitted initially with dietary restrictions imposed upon the re-testing of all positive results.

Other factors which affect the test:

1. Medications: Do not ingest aspirin, tonics or vitamin preparations which contain Vitamin C (ascorbic acid) in excess of 250 mg per day.

2. Bleeding hemorrhoids
3. Collection of specimen during menstrual cycle.
4. Improper specimen collection
5. Other diseases of the gastrointestinal tract such as colitis, gastritis, diverticulitis and bleeding ulcers.

PROCEDURE:

1. Take a small test tube.
2. Pour a pinch of benzidine powder on it.
3. Add 2 -3 drops of glacial acetic acid and mix well.
4. Add 1.0 ml of H_2O_2 and mix well.
5. Take a small quantity of stool specimen on a clean glass slide.
6. One or two drop of benzidine-glacial acetic acid – H_2O_2 mixture on the stool specimen on the glass slide.
7. Observe the color change.

RESULT & INTERPRETATION:

Observe the color change and draw your inference as below:

i) Occult blood absent: No color change.
ii) Occult blood present: Color changes to green to blue.

MIDNAPORE CITY COLLEGE

Department of Paramedical and Allied Health Sciences

Bachelor of Medical Laboratory Technology (BMLT)

NEP

Semester: II

Paper Title: Basic Pathology

Paper Code: BMLT MJ-2

BMLT MJ-2:

Syllabus:

1. Introduction Pathology, Different domains. Clinical importance of pathological studies. 2. Pathological samples for clinical diagnosis: Collection procedure, Storing, Processing, Transportation-Precaution in above steps.

3. Composition of urine, collection & preservation of urine a) Physical examination- Colour, pH & specific gravity b) Chemical examination – Protein, Sugar, ketone body, bile salt, bile pigment, blood. c) Microscopic examination. - Cells, casts, crystals

4. Detection of micro albumin & 24 hrs urinary total protein estimation.

Introduction: Pathology

Pathology is the scientific study of the causes and effects of disease or injury. Pathology literally is the study (logos) of suffering (pathos) and it is the foundation of medical science & practice. Pathology is a bridging discipline devoted to the study of the structure & functional changes in cells, tissues & organs that underlie diseases. The word *pathology* also refers to the study of disease in general, incorporating a wide range of bioscience research fields and medical practices. 'Pathology' may also refer to the predicted or actual progression of particular diseases.

The scope of Pathology:

• Scientific knowledge about human diseases is derived from observations on patients or, by analogy, from experimental studies on animals & cell cultures.

• Clinical medicine is based on a longitudinal approach to a patient's illness.

• Clinical pathology is more concerned with a cross-sectional analysis at the level of the disease itself, studied in depth- the cause & mechanisms of the disease, & the effects of the disease upon the various organs & systems.

Goal of pathology course in MMLT is to foster understanding of the mechanisms of disease (pathogenesis) as a foundation for dealing with a vast amount of clinical information.

The Principal Objectives

• To use pathology to facilitate medical education

• Understanding mechanisms is more a function of logic.

• To leave students with a lasting knowledge of pathology

• To use pathology as the scientific basis of the" art" of medicine

Pathology is the study of disease.

Etiology is the study of the causes that helped in developing the disease.

Pathogenesis of a disease is the biological mechanism that leads to a diseased state. The term can also describe the origin and development of the disease, and whether it is acute, chronic, or recurrent.

Lesion is any damage or abnormal change in the organ or tissue through injury or disease or trauma such as a wound, ulcer, abscess, tumor, neurological disorders etc.

Aspects of a disease process that form the core of pathology

1. It's cause (etiology)

2. Mechanisms of its development (pathogenesis)

3. Structural alterations induced in cells & organs (morphological changes)

4. Functional consequences of the morphologic changes (Clinical significance)

Cause of Disease

Airborne: caused by pathogens and transmitted through the air.

Foodborne: Food poisoning is any illness resulting from the consumption of food contaminated with pathogenic bacteria, toxins, viruses, prions or parasites.

Infectious: Known as transmissible diseases or communicable diseases

Characteristic medical signs or symptoms of disease.

Resulting from the infection, presence and growth of pathogenic biological agents in an individual host organism.

Types:

contagious diseases—an infection, such as influenza or the common cold, that commonly spreads from one person to another.

communicable diseases—a disease that can spread from one person to another, but does not necessarily spread through everyday contact. Example: HIV, hepatitis A, B and C, measles, salmonella, measles, and blood-borne illnesses

Lifestyle

Any disease that appears to increase in frequency as countries become more industrialized and people live longer, especially if the risk factors include behavioral choices like a sedentary lifestyle or a diet high in unhealthful foods such as refined carbohydrates, trans fats, or alcoholic beverages.

Examples: obesity and type 2 diabetes

Non-communicable

It is a medical condition or disease that is non-transmissible. Non-communicable diseases cannot be spread directly from one person to another.

Examples: Heart disease and cancer.

Types of Clinical Specimens Required to Diagnose Diseases

Specimens collected from patients such as **blood, urine, feces, and cerebrospinal fluid (CSF)**, are known as clinical specimens.

Specimens commonly submitted to the hospital's Clinical Microbiology Laboratory (CML) include :blood, bone marrow, bronchial washings, sputum, CSF, cervical and vaginal swabs, feces, hair and nail clippings, pus, skin scrapings, sputum, synovial fluid, throat swabs, tissue specimens, urethral discharge material, urine, and urogenital secretions.

Blood: Blood samples can be collected from blood vessels (capillaries, veins, and sometimes arteries). The sample is obtained by needle puncture and withdrawn by suction through the needle into a special collection tube. Some specimens may be obtained by a finger puncture that produces a drop of blood, such as that used for glucose testing. The presence of bacteria in the bloodstream is known as bacteremia. Septicemia is the presence of bacteria or their toxins in the bloodstream.

Urine: Normally sterile in the bladder; becomes contaminated by indigenous microflora of the distal urethra during voiding. Contamination is reduced by collecting a clean-catch, midstream urine. Urine culture involves 2 parts: • Isolation and identification of the pathogen • Antimicrobial susceptibility testing

Cerebrospinal fluid (CSF): A sample of cerebrospinal fluid is obtained by lumbar puncture, often called a spinal tap. It is performed while the person is lying on their side in a curled up, fetal position or sometimes in a sitting position. The back is cleaned with an antiseptic and a local anesthetic is injected under the skin. A special needle is inserted through the skin, between two vertebrae, and into the spinal canal. The health practitioner collects a small amount of CSF in multiple sterile vials; the needle is withdrawn and a sterile dressing and pressure are applied to the puncture site. The patient will then be asked to lie quietly in a flat position, without lifting their head, for one or more hours to avoid a potential post-test spinal headache. The lumbar puncture procedure usually takes

less than half an hour. Meningitis is inflammation or infection of the membranes (meninges) that surround the brain and spinal column.

Sputum: Sputum is pus that accumulates deep within the lungs of a patient with pneumonia, tuberculosis, or other lower respiratory infection. If TB is suspected, extreme care should be taken. Better specimens can obtain by bronchial aspiration or trans tracheal aspiration.

Throat Swabs: Routine throat swabs are used to determine whether a patient has strep throat. Specific cultures may be necessary when Neisseria gonorrhoeae or Corynebacterium diphtheriae are suspected.

Wound: If a wound or sore is located in the outer layer of skin, the specimen is typically collected on a swab by brushing the swab over the area and gathering a sample of fluid or pus. Touching the open wound area may be temporarily painful since the wound is likely to be tender and sore. If a wound or infection is deep, however, a needle and syringe may be used to aspirate a sample of fluid or pus from the site.

Bacteriology Section: Bacterial pathogens are isolated from specimens, tests are performed to identify the bacterial pathogens, and antimicrobial susceptibility testing. Tests: Gram stain reaction, cell shape (cocci, bacilli, curved, spiral by simple stain), morphologic arrangement of cells ,growth or no growth on various types of media and colony morphology. Presence or absence of a capsule by capsule stain (Indian Ink). Motility and number and location of flagella. Also the ability of bacteria to sporulate by spore stain and Location of spores. Presence or absence of various enzymes (catalase, coagulase, etc), the ability to catabolize various carbohydrate and amino acids and type of hemolysis produced on blood agar. In Mycobacteriology Section: Mycobacterium spp. are identified by the acid-fast staining procedure and by using a combination of growth characteristics. GC Cultures (for Neisseria gonorrhoeae) N. gonorrhoeae is a fastidious bacterium. Cotton swabs used to collect GC specimens. Specimens (e.g., vaginal, cervical, urethral, throat, and rectal swabs) are cultured on special medium (e.g., Thayer-Martin medium) and incubated in a CO2 incubator

Fecal Specimens: Ideally, fecal (stool) specimens should be collected at the laboratory and processed immediately to prevent a decrease in temperature, which would allow the pH to drop and cause the death of some bacteria. A combination of direct microscopic examination, culture, biochemical tests, and immunologic tests may be performed to identify Gram-negative and Gram-positive bacteria, fungi, intestinal protozoa, and intestinal helminthes isolated from fecal specimens.

Mycology Section: Fungal infections (mycoses): The specimens processed here are the same as those that are processed in the Bacteriology Section, with the addition of hair and nail clippings and skin scrapings. A variety of procedures (macroscopic and microscopic) are used to identify fungal pathogens including special media , KOH preps. and biochemical tests (for yeasts).

Parasitology Section: Parasites are identified by observing and recognizing various parasite life cycle stages (e.g., trophozoites , cysts, microfilariae, eggs, larvae, adult worms) in specimens identified primarily by their physical appearance (e.g., size, shape, internal details)by general stool examination test (GSE) .

Virology Section: Techniques used in the identification of viruses include immuno-diagnostic tests, molecular techniques and cytopathic effect (CPE).

The Pathology Department: The pathology department is divided into **2 major divisions**:
Anatomical Pathology and Clinical Pathology

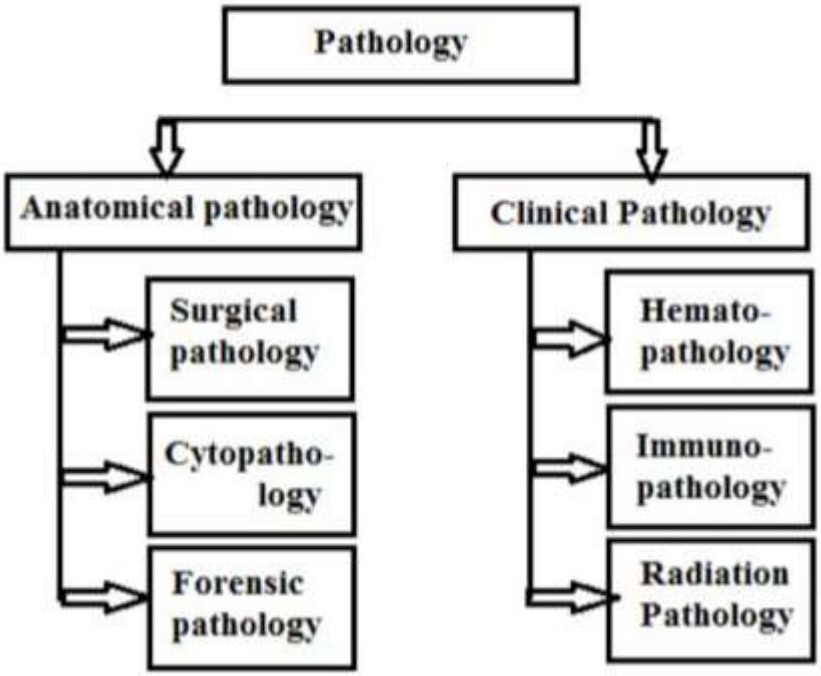
Anatomical Pathology: **Tissue biopsy** Samples of tissue may be obtained from a number of different body sites, such as breast, lung, lymph node, or skin. **Needle biopsy** a needle is inserted into the site and cells and/or fluid are withdrawn using a syringe. An excisional biopsy is a minor surgical procedure in which an incision is made and a portion or all of the tissue is cut from the site. A closed biopsy is a procedure in which a small incision is made and an instrument is inserted to help guide the surgeon to the appropriate site to obtain the sample. These biopsies are usually performed in a hospital operating room. A local or general anesthetic is used, depending on the procedure.

Bone marrow: The bone marrow aspiration and/or biopsy procedure is performed by a trained health care specialist. Both types of samples are most often collected from the hip bone (iliac crest). In some instances, marrow collection may be collected from the breast bone (sternum). Almost all patients are given a mild sedative before the procedure, then asked to lie down on their stomach or side for the collection. The site is cleaned with an antiseptic and injected with a local anesthetic, treating it as a typical surgical field. The patient is then instructed to lie quietly until their blood pressure, heart rate, and temperature are normal, and then to keep the collection site dry and covered for about 48 hour.

In addition, autopsies are performed in the morgue and some pathology departments have an Electron Microscopy Laboratory.

Clinical Pathology:

Clinical Pathology Consists of several laboratories in addition to the Clinical Microbiology Laboratory: Clinical Chemistry, Urinalysis, Hematology/Coagulation, Blood Bank, and Immunology.



Major Pathology Disciplines and Roles

Clinical biochemistry	**Study of the biochemical basis of disease**
Cytopathology	Study of disease in individual cells
Forensic pathology	Determination of cause and manner of death for legal purposes
Hematology	Study of blood disorders
Histopathology[a]	Study of disease in human tissue
Immunopathology	Study of the immunologic basis of disease
Medical microbiology	Study of infection
Molecular pathology and genetics	Study of the molecular and genetic basis of diseases and heritable conditions
Pediatric and perinatal pathology	Study of the diseases of pregnancy, childbirth, and children
Transfusion medicine	Study of the collection, preparation, storage, and clinical use of blood products

Urine:

Composition of urine:

Urinalysis is one of the most commonly performed laboratory tests in clinical practice. Composition of normal urine as:

Parameters Values

1. Volume: 600-2000 ml
2. Specific gravity 1.003-1.030
3. Osmolality 300-900 mOsm/kg
4. pH 4.6-8.0
5. Glucose < 3 mg
6. Proteins < 3 mg
7. Urobilinogen 0.5-4.0 mg
8. Porphobilinogen 0-2 mg
9. Creatinine 14-26 mg/kg (men), 11-20 mg/kg (women)
10. Urea nitrogen 12-20 gm
11. Uric acid 250-750 mg
12. Sodium 40-220 mEq
13. Potassium 25-125 mEq
14. Chloride 110-250 mEq
15. Calcium (low calcium diet) 50-150 mg
16. Formiminoglutamic acid (FIGlu) < 3 mg
17. Red cells, epithelial cells, and white blood cells

Urinalysis

Urine can be used to look for physical and biochemical abnormalities for the general information on health

• To *screen for and/or to aid in diagnosis of conditions:*

Urinary tract infections,
Kidney disorders,
Liver problems,
Diabetes
Other metabolic conditions,

Screening for drug abuse (eg. Sulfonamide or aminoglycosides)
Pregnancy

Ex-Persistent amounts of albumin and other proteins in the urine (proteinuria) indicate kidney damage.

Collection of Urine:

Sample type	Sampling	Purpose
Random specimen	No specific time most common, taken anytime of day	Routine screening, chemical & FEME
Morning sample	First urine in the morning, most concentrated	Pregnancy test, microscopic test
Clean catch midstream	Discard first few ml, collect the rest	Culture
24 hours	All the urine passed during the day and night and next day Ist sample is collected.	used for quantitative and qualitative analysis of substances
Postprandial	2 hours after meal	Determine glucose in diabetic monitoring
Supra-pubic aspired	Needle aspiration	Obtaining sterile urine

Urine collection containers should be:

1. Sterile, wide mouthed, Clean and leak proof.
2. Break-resistant
3. Material of container should not interfere and
4. Container should not be re-used.
5. Capacity of at least 50 ml (routine) and at least 3 litre (24-hour sample)
6. Amber colored containers for light sensitive analytes

Collection Methods:

1. Midstream specimen: This is used for all types of examinations. After voiding initial half of urine into the toilet, a part of urine is collected in the bottle. First half of stream serves to flush out contaminating cells and microbes from urethra and perineum. Subsequent stream is collected which is from the urinary bladder.
2. Clean-catch specimen: This is recommended for bacteriologic culture. In men, glans penis is sufficiently exposed and cleaned with soap and water. In women urethral opening should be exposed, washed with soapy cotton balls, rinsed with water-saturated cotton, and holding the labia apart, the initial urine is allowed to pass into the toilet and the remaining is voided into the bottle (amount 20-100 ml). This method avoids contamination of urine with the vaginal fluids.
3. Catheter specimen: This is used for bacteriological study or culture in bedridden, ill patients or in patients with obstruction of urinary tract. It is usually avoided in ambulatory patients since it carries the risk of introduction of infection.

4. Infants: In infants, a clean plastic bag can be attached around the baby's genitalia and left in place for some time. For bacteriologic examination, urine is aspirated from bladder by passing a needle just above symphysis pubis.

Urine sample must be tested in the laboratory within 2 hours of collection to get the correct results.

If urine is left standing at room temperature for long after collection, following changes occur: • Increase in pH due to production of ammonia from urea by urease-producing bacteria. • Formation of crystals due to precipitation of phosphates and calcium (making the urine turbid) • Loss of ketone bodies, since they are volatile. • Decrease in glucose due to glycolysis and utilization of glucose by cells and bacteria. • Oxidation of bilirubin to biliverdin causing falsenegative test for bilirubin • Oxidation of urobilinogen to urobilin causing falsenegative test for urobilinogen • Bacterial proliferation • Disintegration of cellular elements, especially in alkaline and hypotonic urine.

Preservation/Storage of Urine Sample

• Not recommended for routine analysis as they interfere with reagent strip techniques and chemical test for protein.

Preservatives for 24-hour urine sample:

1. Hydrochloric acid: Used when detecting adrenaline, noradrenaline, vanillylmandelic acid (VMA) and steroids.

2. Toluene: It forms a thin layer and hence physical barrier against bacteria and air.

3. Boric acid: General preservative (sample can be kept for 24 hours without refrigeration)

4. Thymol: Inhibits bacteria and fungi.

5. Formalin: Excellent for preservation of formed elements.

Physical and Microscopic examination of Urine

Examination of urine is important for diagnosis and assistance in the diagnosis of various diseases. Routine (complete) examination of urine must start from physical and microscopic examination.

The specimen should be properly collected in a clean container which should be properly labelled with name of the patient, age, sex, identity number with date and time of collection. It should not show signs of contamination. Physical examination of urine consists of volume, colour, odour, reaction/pH and specific gravity. After that observe by eye and light microscope.

Specimen collection:

For routine examination a clean glass tube or capped jar is used; for bacteriologic examination a sterilized container is required.

For routine urinalysis: first morning sample is best since it is most concentrated.

For bacteriologic examination: mid-stream sample is preferable, i.e. first part of urine is discarded and mid-stream sample is collected.

For 24-hour sample: Collection of urine is started in the morning at 8 AM (first voided sample is discarded) and all subsequent samples are collected till next day 8 AM.

Results & interpretation:

F. Volume:

Normal range: 700-2500 ml (average 1200 ml) of urine in 24 hours

Interpretation:

i) Nocturia	Excess of 500 ml during night with specific gravity of less than 1.018.	Sign of early renal failure.
ii) Polyuria	Excess of urine is passed in 24 hr (> 2000 ml) with low specific gravity.	Physiological: excess water intake, may be seasonal (e.g. in winter). Pathological: Diabetes insipidus, diabetes mellitus.
iii) Oliguria	Less than 500 ml of urine is passed in 24 hr.	Less intake of water, dehydration, renal ischaemia.
iv) Anuria	Almost complete suppression of urine (< 150 ml in 24 hours).	Renal stones, tumours, renal ischaemia

G. Colour:

Interpretation:

Normal: urine is clear, pale or straw-coloured due to pigment urochrome.

Colour	Inference
i) Colourless	diabetes mellitus, diabetes insipidus, excess intake of water.
ii) Deep amber	good muscular exercise, high grade fever.
iii) Orange	increased urobilinogen, concentrated urine
iv) Smoky urine	small amount of blood, administration of vitamin B12, aniline dye
v) Red	haematuria, haemoglobinuria
vi) Yellow-brown	bile and its derivatives
vii)Milky	pus, fat
viii) Green	putrefied sample, phenol poisoning

H. Odour:

Interpretation:

Normal: Faint aromatic odour.

Odour	Inference
i) Pungent	ammonia produced by bacterial contamination
ii) Putrid	UTI.

iii) Fruity	ketoacidosis
iv) Mousy	phenylketonuria

I. Reaction/pH:

Measured by pH indicator paper or by electronic pH meter.

Interpretation:

Normal urine: Slightly acidic and its pH ranges from 4.6-7.0 (average 6.0).

pH	Inference
Acidic urine	i. High protein intake, e.g. meats. ii. Ingestion of acidic fruits. iii. Respiratory and metabolic acidosis. iv. UTI by *E. coli*.
Alkaline urine	i. Citrus fruits. ii. Vegetables. iii. Respiratory and metabolic alkalosis. iv. UTI by *Proteus, Pseudomonas*.

J. Specific gravity:

This is the ratio of weight of 1 ml volume of urine to that of weight of 1 ml of distilled water. It depends upon the concentration of various particles/solutes in the urine. Specific gravity is used to measure the concentrating and diluting power of the kidneys. Measured by urinometer, refractometer or reagent strips.

Assay by Urinometer:

Procedure:

5. Fill urinometer container 3/4th with urine.
6. Insert urinometer into it so that it floats in urine without touching the wall and bottom of container.
7. Read the graduation on the arm of urinometer at lower urinary meniscus.
8. Add or substract 0.001 from the final reading for each 3°C above or below the calibration temperature respectively marked on the urinometer.

Interpretation:

Normal specific gravity of urine: 1.003 to 1.030.

Specific Gravity	Inference
Low specific gravity	i. Excess water intake; ii. Diabetes insipidus
High specific gravity	i. Dehydration; ii. Albuminuria; iii. Glycosuria
Fixed specific gravity (1.010)	i. ADH deficiency; ii. Chronic nephritis

Microscopic analysis of urine:

Microscopic analysis of urine sample has great clinical importance. Important structures like different casts, erythrocytes, leukocytes, and bacteria need to be observed.

Procedure:

ix. Agitate the urine sample to avoid any sediment that may settled to bottom.
x. Fill the centrifuge tube with urine and centrifuge for 3 min at low rate of speed.
xi. Pour all the urine and there is sufficient urine on the slide to drain to the bottom and suspend the sediment.
xii. Pour a drop of sediment on a glass slide, and cover with a cover glass that has been wiped clean of oil and lint.
xiii. Examine under microscope with the lower power objective.
xiv. Finding should be reported as few, many or abundant.
xv. If necessary, stain with new methylene blue.
xvi. Organized sediments, epithelial cells.

Observation & inference:

Observe the followings and draw on your observation note book:

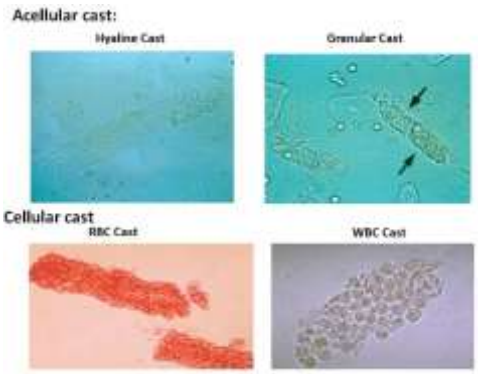
1. Cells - *RBCs, WBCs, epithelial cells*.

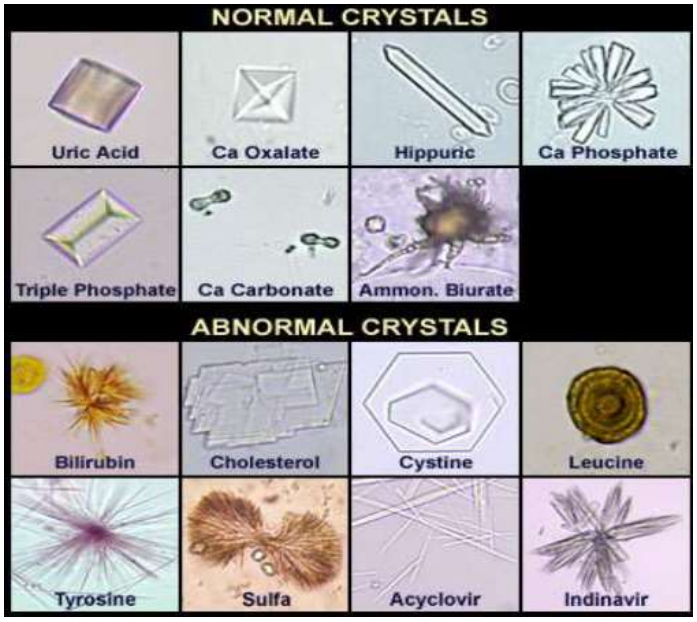
2. Casts -*Hyaline Cast, Red Cell Cast, Leucocyte Cast, Granular Casts, Waxy Casts, Fatty Cast, Epithelial Cast, Pigment Cast.*

3. Crystals - *Calcium oxalate, Uric acid, Amorphous urate, Tyrosine, Cystine, Cholesterol crystals, Sulphonamide, Amorphous phosphate, Triple phosphate, Calcium carbonate, Ammonium biurate*

4. Miscellaneous structures- *Spermatozoa, Parasites, Fungus, Tumour cells.*

A variety of normal and abnormal crystals may be found in the urine sediment:





Urinary casts are cylindrical aggregations of particles that form in the distal nephron, dislodge, and pass into the urine.

In urinalysis they indicate kidney disease.

Cast form via precipitation of Tamm-Horsfall mucoprotein which is secreted by renal tubule cells.

Types of cast seen :

Acellular cast: Hyaline casts, Granular casts, Waxy casts, Fatty casts, Pigment casts, Crystal casts.

Cellular cast: Red cell casts, White cell casts, Epithelial cell cast.

Hematuria is the presence of abnormal numbers of red cells in urine due to any of several possible causes.

glomerular damage,

tumors which erode the urinary tract anywhere along its length,

kidney trauma,

urinary tract stones,

acute tubular necrosis,

upper and lower urinary tract infections,

nephrotoxins

WBC in high numbers indicate inflammation or infection somewhere along the urinary or genital tract.

Reagent strips are the primary method used for the chemical examination of urine.

• They represent multiple complex, state-of-the-art chemical reactions.

Advantages of Multistix Strip

• Quick screening of urine chemistry.

• Reliable, specific and sensitive.

• Avoids use of various corrosive reagents, different type of glass wares and other laboratory material required for wet chemical testing of urine.

• It can be performed in uncentrifuged urine and doesn't require acidification.

• Less labour intensive and can be automated for large laboratories.

• Less chance of human error.

Glucose in urine:

Glucose is the most important of the sugars which may appear in urine. Normally, approximately 130 mg of glucose in urine is passed per 24 hours which is undetectable by qualitative tests. Above that level is called glucosuria that may be detected or estimated by qualitative or quantitative analysis.

Cupric ion is reduced by glucose to cuprous oxide and a coloured precipitate is formed.

The presence of significant amounts of glucose in the urine is called glycosuria (or glucosuria).

The quantity of glucose in the urine is dependent upon

the blood glucose level,

the rate of glomerular filtration, and

the degree of tubular reabsorption.

Usually, glucose will not be present in the urine until the blood level exceeds 160–180 mg/dL, which is the normal renal threshold for glucose.

The fasting level in an adult is only about 2–20 mg of glucose/dL of urine.

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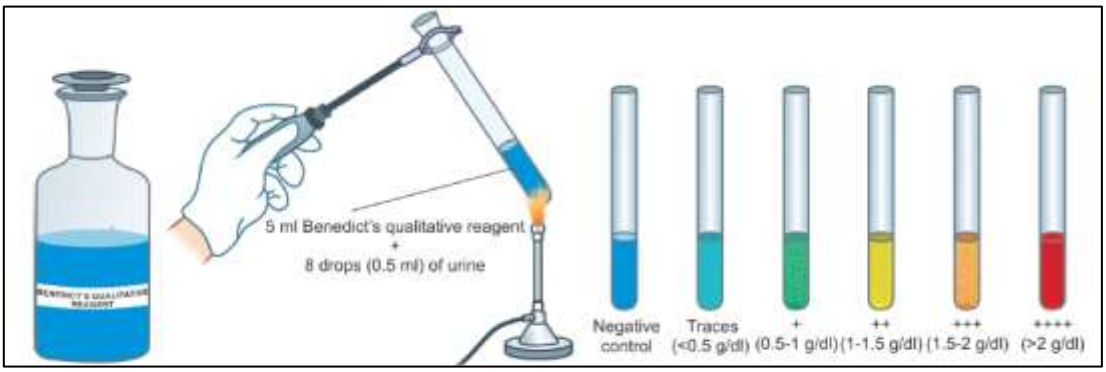
QUALITATIVE TESTS*: BENEDICT'S TEST:*

PROCEDURE: Benedict's test:

5. Take 5 ml of Benedict's qualitative reagent in a test tube.
6. Add 8 drops (or 0.5 ml) of urine.
7. Heat to boiling for 2 minutes,
8. Cool in water bath or in running tap water and look for colour change and precipitation.



RESULTS & INTERPRETATION: Benedict's test:

No change of blue colour	= Negative
Greenish colour	= Traces (< 0.5 g/dl)
Green/cloudy green ppt	= + (0.5-1 g/dl)
Yellow ppt	= ++ (1-1.5 g/dl)
Orange ppt	= +++ (1.5-2 g/dl)
Brick red ppt	= ++++ (> 2 g/dl)

Benedict's test is for reducing substances excreted in the urine, the test is positive for all reducing sugars (glucose, fructose, maltose, lactose but not for sucrose which is a nonreducing sugar) and other reducing substances (e.g. ascorbic acid, salicylates, antibiotics, L-dopa).

QUANTITATIVE TEST:

PROCEDURE:

7. Take 25 ml of quantitative Benedict's reagent in a conical flask.
8. Add to it 15 gm of sodium carbonate (crystalline) and some pieces of porcelain.
9. Heat it to boil.
10. Add urine to it from a burette slowly till there is disappearance of blue colour of Benedict's reagent. This is same as common titration procedure.
11. Record the volume of urine used from burette.
12. Note: 0.05 gm of glucose reduces 25 ml of Benedict's reagent

The amount of glucose present in urine as under: (0.05 × 100)/Amount of urine.

Considering the other physical factor, causes of glucosuria may be due to diabetes mellitus, renal glucosuria, severe burns, administration of corticosteroids, severe sepsis, pregnancy.

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TESTS FOR KETONURIA*:*

Ketone present in urine because of incomplete fat metabolism. The three ketone bodies excreted in urine are: acetoacetic acid (20%), acetone (2%), and β-hydroxybutyric acid (78%).

1. Rothera's Test:

Principle: Ketone bodies (acetone and acetoacetic acid) combine with alkaline solution of sodium nitroprusside forming purple complex.

Procedure:

v. Take 5 ml of urine in a test tube.
vi. Saturate it with solid ammonium sulphate salt; it will start settling to the bottom of the tube when saturated.
vii. Add a few crystals of sodium nitroprusside and shake.
viii. Add liquor ammonia from the side of test tube.

Interpretation:Appearance of purple or permanganate coloured ring at the junction indicates presence of ketone bodies.

2. Gerhardt's Test:

Procedure:

iv. Take 5 ml of urine in a test tube.
v. Add 10% ferric chloride solution drop by drop.
vi. Filter it and add more ferric chloride.

Interpretation: Brownish-red colour indicates presence of ketone bodies.

Ketone bodies found in urine sample may be due to:

i. Diabetic ketoacidosis

ii. Dehydration

iii. Hyperemesis gravidarum

iv. Fever

v. Cachexia

vi. After general anaesthesia.

Bile derivatives in urine:

Three bile derivatives excreted in urine are: urobilinogen, bile salts and bile pigments. While urobilinogen is normally excreted in urine in small amounts, bile salts and bile pigments appear in urine in liver diseases only.

C. Tests for bile salts: Bile salts excreted in urine are cholic acid and chenodeoxycholic acid.

Hay's Test:

Principle. Bile salts if present in urine lower the surface tension of the urine.

Procedure:

iv. Take a 50 ml beaker or 15 ml test tube.
v. Fill the beaker or test tube upto 2/3rd with urine.
vi. Sprinkle finely powdered sulphur powder over it.

Interpretation: If bile salts are present in the urine then Sulphur powder sinks, otherwise it floats.

Cause for Bile Salts in Urine: Obstructive jaundice.

D. Tests for urobilinogen:

A small amount of urobilinogen is excreted in urine (4 mg/24 hr) normally. The sample should always be collected in a dark coloured bottle as urobilinogen gets oxidized on exposure to light. Urobilinogen in urine are detected by Ehrlich's test.

Ehrlich's Test:

Urobilinogen in urine combines with Ehrlich's aldehyde reagent to give a red purple coloured compound.

Procedure:

v. Take 10 ml of urine in a test tube.

vi. Add 1 ml of Ehrlich's aldehyde reagent.
vii. Wait for 3-5 minutes.
viii. If the test is positive, the test is repeated by preparing multiple dilutions, e.g. 1:10, 1:20, 1:40 and so on.

Interpretation:

v. Development of red purple colour indicates presence of urobilinogen.
vi. A positive test is subsequently done in dilutions; normally it is positive in up to 1:20 dilution.
vii. Increased urobilinogen in urine indicated haemolytic jaundice and haemolytic anaemia.
viii. Causes for absent urobilinogen in urine: Obstructive jaundice.

E. Tests for bilirubin (bile pigment):

Bilirubin is breakdown product of haemoglobin. Normally, no bilirubin is passed in urine.

1. Fouchet's Test:

Principle: Ferric chloride oxidizes bilirubin to green biliverdin.

Procedure:

v. Take 10 ml of urine in a test tube.
vi. Add 3-5 ml of 10% barium chloride.
vii. Filter through filter paper.
viii. To the precipitate on filter paper, add a few drops of Fouchet's reagent (ferric chloride + trichloroacetic acid).

Interpretation: Development of green colour indicates bilirubin.

2. Foam Test

Procedure:

iii. Take 5/10 ml of urine in a test tube.
iv. Shake it vigorously.

Interpretation: Presence of yellow foam at the top indicates presence of bilirubin.

Causes of bilirubinuria: Obstructive jaundice, Hepatocellular jaundice.

Tests for blood in urine:

Presence of blood can be detected in urine by two important chemical test: Benzidine test and Orthotoluidine test

3. Benzidine Test

Procedure:

iv. Take 2 ml of urine in a test tube.
v. Add 2 ml of saturated solution of benzidine with glacial acetic acid.
vi. Add 1 ml of H_2O_2 to it.

Interpretation: Appearance of blue colour indicates presence of blood.

4. Orthotoluidine Test

Procedure:

iv. Take 2 ml of urine in a test tube.
v. Add a solution of 1 ml of orthotoluidine in glacial acetic acid.
vi. Add a few drops of H2O2.

Interpretation: Blue or green colour indicates presence of blood in urine.

Note: Causes of blood in urine:

i. Renal stones

ii. Renal tumours

iii. Polycystic kidney

iv. Bleeding disorders

v. Trauma.

9. Detection of micro albumin & 24 hrs urinary total protein estimation

Protein in Urine:

Chemical constituents frequently tested in urine are: proteins, ketones, bile derivatives and blood. If urine is not clear, it should be filtered or centrifuged before testing.

Tests for proteinuria:

Urine may be tested for proteinuria by qualitative tests and quantitative methods.

Qualitative Tests:

1. Heat and Acetic Acid Test

Heat causes coagulation of proteins.

Procedure:

vi. Take a 5 ml test tube.
vii. Fill 2/3rd with urine.
viii. Acidify by adding a few drops of 3% acetic acid if urine is alkaline.
ix. Boil upper portion for 2 minutes (lower part acts as control).
x. If precipitation or turbidity appears, add a few drops of 3% acetic acid.

Interpretation:

Turbidity or precipitation disappears on addition of acetic acid, it is due to phosphates; if it persists after addition of acetic acid, then it is due to proteins.

The test is semi-quantitative and can be graded from traces 0 to ++++ depending upon amount of protein as under:

No cloudiness	= Negative
Faint cloudiness	= Traces (less than 0.1 g/dl).
Cloudiness without granularity	= +(0.1 g/dl).
Granular cloudiness	= ++(0.1-0.2 g/dl)
Precipitation and flocculation	= +++(0.2-0.4 g/dl).
Thick solid precipitation	= ++++ (> 0.5 g/dl).

2. Sulphosalicylic Acid Test

Make urine acidic by adding 3% acetic acid.

With 2 ml of urine add a few drops (4-5) of 20% sulphosalicylic acid.

Interpretation:

Appearance of turbidity which persists after heating indicates presence of proteins.

4. Heller's Test

Procedure:

3. Take 2 ml of concentrated nitric acid in a test tube.
4. Add urine drop by drop by the side of test tube.

Interpretation: Appearance of white ring at the junction indicates presence of protein.

Quantitative Estimation:

1. Esbach's albumin meter method:

v. Fill the albuminometer with urine up to mark U.
vi. Add Esbach's reagent (picric acid + citric acid) up to mark R.
vii. Stopper the tube, mix it and let it stand for 24 hours.
viii. Take the reading from the level of precipitation in the albuminometer tube and divide it by 10 to get the percentage of proteins.

2. Turbidimetric method:

iv. Take 1 ml of urine and 1 ml standard in two separate tubes.
v. Add 4 ml of trichloroacetic acid to each tube.
vi. After 5 minutes take the reading with red filter (680 nm).

Results & inference:

Write your results based on the different procedure and interpret as stated:

vi. Normal: A very scanty amount of protein in urine (< 150 mg/day).
vii. *Heavy proteinuria* (> 3 gm/day) occurs due to nephrotic syndrome, renal vein thrombosis, diabetes mellitus and SLE.
viii. *Moderate proteinuria* (1-3 gm/day) due to Chronic glomerulonephritis, nephrosclerosis, multiple myeloma, pyelonephritis.
ix. *Mild proteinuria* (< 1.0 gm/day) occurs due to hypertension, polycystic kidney, chronic pyelonephritis, UTI, fever.
x. *Microalbuminuria* is excretion of albumin 30-300 mg/day or random urine albumin/ creatinine ratio of 30-300 mg/gm creatinine and is indicative of early and possibly reversible glomerular damage from hypertension and risk factor for cardiovascular disease. *Microalbuminuria* is estimated by radioimmunoassay.

Microalbuminuria: This is defined as urinary excretion of 30 to 300 mg/24 hours (or 2-20 mg/dl) of albumin in urine. Significance of microalbuminuria 1. Microalbuminuria is considered as the earliest sign of renal damage in diabetes mellitus (diabetic nephropathy). It indicates increase in capillary permeability to albumin and denotes microvascular disease. Microalbuminuria precedes the development of diabetic nephropathy by a few years. If blood glucose level and hypertension are tightly controlled at this stage by aggressive treatment then progression to irreversible renal disease and subsequent renal failure can be delayed or prevented. 2. Microalbuminuria is an independent risk factor for cardiovascular disease in diabetes mellitus. Detection of microalbuminuria: Microalbuminuria cannot be detected by routine tests for proteinuria. Methods for detection include: • Measurement of albumin-creatinine ratio in a random urine sample • Measurement of albumin in an early morning or random urine sample • Measurement of albumin in a 24 hr sample Test strips that screen for microalbuminuria are available commercially. Exact quantitation can be done by immunologic assays like radioimmunoassay or enzyme linked immunosorbent assay.

Bence Jones Proteinuria: Bence Jones proteins are monoclonal immunoglobulin light chains (either κ or λ) that are synthesized by neoplastic plasma cells. Excess production of these light chains occurs in plasma cell dyscrasias like multiple myeloma and primary amyloidosis. Because of their low molecular weight and high concentration they are excreted in urine (overflow proteinuria). Bence Jones proteins have a characteristic thermal behaviour. When heated, Bence Jones proteins precipitate at temperatures between 40°C to 60°C (other proteins precipitate between 60-70°C), and precipitate disappears on further heating at 85-100°C (while precipitate of other proteins does not). When cooled (60-85°C), there is reappearance of precipitate of Bence Jones proteins. This test, however, is not specific for Bence Jones proteins and both false-positive and -negative results can occur. This test has been replaced by protein electrophoresis of concentrated urine sample

Stool/Feces

Collection of Stool Specimen:
Collection of sufficient quantity: Morning specimen (at least about 5 to 6 ml capacity) is collected in a 50 ml clean & dry container • It should be uncontaminated with urine or other body secretions, such as menstrual blood • Deliver immediately after collection

Containers: Disposable ,wide mouthed plastic bottle or glass bottle Waxed Cardboard box Glass Jar with fitting Lid

Precaution after Collection of Stool Specimen:
• Labelling • Should not be left uncovered • Examined within 1 hour of collection • Disposed properly after examination

Type of Stool Examination:
• Gross & Physical Examination of stool • Chemical Examination • Microscopic Examination

Routine test and microscopical test for stool and Occult blood test.

Stool analysis is done to:

- Identify diseases of the digestive tract, liver, and pancreas.
- Enzymes (such as trypsin or elastase) may be evaluated in the stool to help determine how well the pancreas is functioning.
- Find the cause of symptoms affecting the digestive tract, including prolonged diarrhea, bloody diarrhea, an increased amount of gas, nausea, vomiting, loss of appetite, bloating, abdominal pain and cramping, and fever.
- Screen for colon cancer by checking for hidden (occult) blood.
- Look for parasites, such as pinworms or *Giardia*.
- Look for the cause of an infection, such as bacteria, a fungus, or a virus.
- Check for poor absorption of nutrients by the digestive tract (malabsorption syndrome). For this test, all stool is collected over a 72-hour period and then checked for fat (and sometimes for meat fibers). This test is called a 72-hour stool collection or quantitative fecal fat test.

Risks:

- Any stool sample may contain germs that can spread disease.
- It is important to carefully wash your hands and use careful handling techniques and precaution to avoid spreading infection.

REFERENCE RANGES	
Bulk:	100-200 grams/day
Color:	Brown
Water:	Up to 75%
pH:	7.0-7.5
RBC:	Absent
WBC	Few
Epithelial cells	Present
Crystals:	Calcium oxalate, triple phosphate
Sugars	<0.25 g/dL
Fat (Adults):	<7 gm/day (gravimetric method), <6 gm/day (titrimetric method)
Fat droplets:	2.5/ high power field in random sample
Urobilinogen:	50-300 mg/24 h
Parasites	Absent
Ova, cysts, trophozoites:	Absent

Normal:	Abnormal:
The stool appears brown, soft, and well-formed in consistency.	The stool is black, red, white, yellow, or green. The stool is liquid or very hard.
The stool does not contain blood, mucus, pus, undigested meat fibers, harmful bacteria, viruses, fungi, or parasites.	The stool contains blood, mucus, pus, undigested meat fibers, harmful bacteria, viruses, fungi, or parasites.
The stool is shaped like a tube.	The stool contains low levels of enzymes, such as trypsin or elastase.
The pH of the stool is 7.0–7.5.	The pH of the stool is less than 7.0 or greater than 7.5.
The stool contains less than 0.25 g/dL of sugars called reducing factors.	The stool contains 0.25 g/dL or more of sugars called reducing factors.
The stool contains 2–7 g/24h of fat.	The stool contains more than 7 g/24h of fat (if your fat intake is about 100 g a day).

Abnormal Indications

- High levels of fat in the stool may be caused by diseases such as pancreatitis, sprue (celiac disease), cystic fibrosis, or other disorders that affect the absorption of fats.
- Presence of undigested meat fibers in the stool may be caused by pancreatitis.
- Low pH (<5.6) may be caused by poor absorption of carbohydrate or fat.
- High pH may mean inflammation in the intestine (colitis), cancer, or antibiotic use.
- Blood in the stool may be caused by bleeding in the digestive tract.
- WBCs in the stool may be caused by inflammation of the intestines, such as ulcerative colitis, or a bacterial infection.
- Diarrhea is present, testing may be done to look for rotaviruses in the stool.
- High levels of reducing factors in the stool may mean a problem digesting some sugars.
- Low levels of reducing factors may be caused by sprue (celiac disease), cystic fibrosis, or malnutrition. Medicine such as colchicine (for gout) or birth control pills may also cause low levels.
- Occult blood in the stool may indicate colon cancer or polyps in the colon or rectum — though not all cancers or polyps bleed.

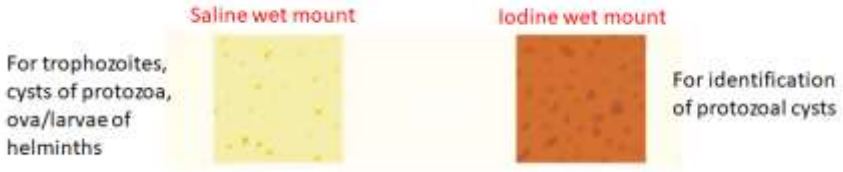
Color/Appearance of Fecal Specimens:	
Brown	Normal
Black	Bleeding in upper GI tract (proximal to cecum), Drugs (iron salts, bismuth salts, charcoal)
Red	Bleeding in large intestine, Undigested tomatoes or beets
Clay-colored (gray-white)	Biliary obstruction
Silvery	Carcinoma of ampulla of Vater
Watery	Strains of *Escherichia coli*, Rotavirus enteritis, Cryptosporidiosis
Rice water	Cholera
Unformed with blood and mucus	Amebiasis, inflammatory bowel disease
Unformed with blood, mucus, and pus	Bacillary Dysentery
Unformed, frothy, foul smelling, which float on water	Steatorrhea

Preparation of Slides:

Saline and iodine wet mounts:

I. A drop of normal saline is placed near one end of a glass slide and a drop of Lugol iodine solution is placed near the other end.

II. A small amount of feces is mixed with a drop each of saline and iodine using a wire loop.
III. A cover slip is placed over each preparation separately.



If the specimen contains blood or mucus, that portion should be included for examination (trophozoites are more readily found in mucus).

If the stools are liquid, select the portion from the surface for examination.

Saline wet mount:

- ✓ Used for demonstration of eggs and larvae of helminths, and trophozoites and cysts of protozoa.
- ✓ Trophozoites become non-motile in iodine mounts.
- ✓ Also detect red cells and white cells.

Iodine wet mount:

- ✓ Useful for identification of protozoal cysts.
- ✓ Iodine stains glycogen and nuclei of the cysts
- ✓ A liquid, diarrheal stool can be examined directly without adding saline.

Concentration of fecal specimen is required if very small numbers of parasites are present.

It is used for detection of ova, cysts, and larvae of parasites.

Limitation: amebic trophozoites can no longer be detected as destroyed.

Two types:

1. *Sedimentation techniques*: Ova and cysts settle at the bottom.

Excessive fecal debris may make the detection of parasites difficult.

Example: Formol-ethyl acetate sedimentation procedure.

2. Floatation techniques: Ova and cysts float on surface.

Examples: Saturated salt floatation technique and zinc sulphate concentration technique.

Formol-ethyl acetate concentration technique

i) Detect eggs and larvae of all helminths, and cysts of protozoa,

(ii) Preserves their morphology well,

(iii) Rapid, and

(iv) Risk of infection to the laboratory worker is minimal because pathogens are killed by formalin.

Fecal suspension is prepared in 10% formalin (10 ml formalin + 1 gm feces).

Passed through a gauze filter till 7 ml of filtered material is obtained.

Ethyl acetate (3 ml) is added.

Mixture is centrifuged for 1 minute.

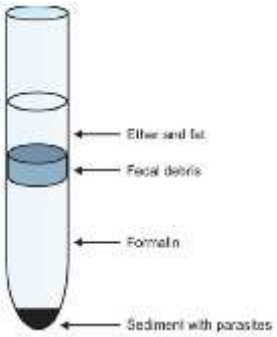
Eggs, larvae, and cysts sediment at the bottom of the centrifuge tube.

Fecal debris is loosened with an applicator stick and the supernatant is poured off.

One drop of sediment is placed on one end of a glass slide and one drop is placed at the other end.

One of the drops is stained with iodine, cover slips are placed.

Examined under the microscope



Density	Protozoa	Helminths
Rare	2 to 5 organisms per 22 mm square coverslip	2 to 5 organisms per 22mm square coverslip.
Few	1 organism per 5 to 10 high-power fields (40x)	1 egg/larva per 5 to 10 low-power fields (10x)
Moderate	1 to 2 organisms per high-power field, to as few as 1organism per 2 to 3 in high-power fields	1 to 2 eggs/larvae in low-power field
Many	Several organisms in every high-power field	Several eggs/larvae inevery low-power field

Detection of Occult Blood:

Occult blood (hidden) in supplied stool or urine sample. As blood passes through the intestinal tract it changes color and consistency due to the digestive processes. Stools with large amounts of occult blood are black and have a tarry consistency. Smaller amounts of blood may not be visibly detectable.

Occult blood may indicate lesions in the intestinal tract due to parasitic infections, cancerous tumors, bleeding ulcers or other disease processes.

PRINCIPLE:

The occult blood test is primarily done to screen for colorectal cancer. It is an easy inexpensive procedure and the specimen is easily obtained by the patient. The chemical reaction involved is as follows:

Hemoglobin + Developer

$Hb + 2\ H_2O_2 = 2H_2O + O_2$

Oxidation of Guaiac

O_2 + Guaiac = Oxidized Guaiac

(Colorless) (Blue)

The presence of a blue color upon completion of the test is a positive test. A positive and negative control must be run concurrently with the patient sample. Most manufacturers now include this as part of the patient test card.

DIET recommendations/restrictions:

It is recommended that the patient be placed on a high residue diet starting 2 days before and continuing through the test period.

DIET MAY INCLUDE:

1. Meats: Only small amounts of chicken, turkey and tuna.
2. Vegetables: Generous amounts of both raw and cooked vegetables including lettuce, corn, spinach, carrots and celery. Avoid those with high peroxidase activity.
3. Fruits: Plenty of fruits, especially prunes and apples.
4. Cereals: Bran and bran-containing cereals.
5. Moderate amounts of peanuts and popcorn daily.

DIET TO BE AVOIDED:

1. Meat: Diet should not include any red or rare meat.
2. Fruits and vegetables containing high peroxidase activity:

Turnip Cauliflower, Broccoli Cantaloupe, Horseradish Parsnip

Alternately, the special diet may be omitted initially with dietary restrictions imposed upon the re-testing of all positive results.

Other factors which affect the test:

1. Medications: Do not ingest aspirin, tonics or vitamin preparations which contain Vitamin C (ascorbic acid) in excess of 250 mg per day.
2. Bleeding hemorrhoids
3. Collection of specimen during menstrual cycle.
4. Improper specimen collection
5. Other diseases of the gastrointestinal tract such as colitis, gastritis, diverticulitis and bleeding ulcers.

PROCEDURE:

8. Take a small test tube.
9. Pour a pinch of benzidine powder on it.
10. Add 2 -3 drops of glacial acetic acid and mix well.
11. Add 1.0 ml of H_2O_2 and mix well.
12. Take a small quantity of stool specimen on a clean glass slide.
13. One or two drop of benzidine-glacial acetic acid – H_2O_2 mixture on the stool specimen on the glass slide.
14. Observe the color change.

Observe the color change and draw your inference as below:

iii) Occult blood absent: No color change.
Occult blood present: Color changes to green to blue

Sputum collection, Smear preparation- Routine diagnostic tests:

Tuberculosis (TB) is a potentially fatal contagious disease that can affect almost any part of the body but is mainly an infection of the lungs. Sputum is mucus that is coughed up from the lower airways. This process is known sputilization. It is a secretion that is produced in the lungs and the bronchi. This mucus-like secretion may become infected, bloodstained, or contain abnormal cells that may lead to a diagnosis.

Purulent sputum contains pus, composed of white blood cells, cellular debris, dead tissue, serous fluid, and viscous liquid (mucus). It is typically yellow or green. It is seen in cases of bronchitis or acute upper respiratory tract infection (common cold, laryngitis).

Microbiological sputum samples are usually used to look for infections by *Moraxella catarrhalis*, *Mycobacterium tuberculosis*, *Streptococcus pneumoniae*, and *Haemophilus influenza.*

When Sputum analysis?

Symptoms may include: Cough, Fever, Muscle aches, Fatigue, Trouble breathing, Chest Pain

1. Smear and culture: Identification of causative organism in a suspected infection - like pneumonia, TB, fungal infection, *P. carinii* in HIV, bronchiectasis

2. Cytological examination: for malignant cells, looking for viral inclusions, asbestosis

Revised National TB Control Programme (RNTCP): The National TB Programme (NTP) was launched by the Government of India in 1962 in the form of District TB Centre model involved with BCG vaccination and TB treatment.

Collection of sputum:

1. Early morning deep cough sample is preferred

2. If unable to cough, induction of sputum can be done by:

    a. 15% NaCl aerosol spray & propylene glycol for 20 min or

    b. Nebulized hypertonic saline and distilled water

Collected in:

1. dry wide mouthed container with 25 ml capacity

2. leak proof – to prevent aerosols

3. break resistant – to prevent desiccation

Sample transport

1. Samples should be immediately transported to laboratory as such if nearby

2. If distant laboratory, transport in 25 ml of the following solution:

    N-acetyl pyridinium chloride 5g

    Sodium chloride 10g

    Distilled water 1 lt

If sputum is allowed to stand without medium–

a. rapid proliferation of contaminating bacterial flora from oral cavity and throat

b. *H. influenzae* donot survive for long

Do not refrigerate in any case

Microbiological examination:

GRAM STAIN:

Prerequisites

1. There should not be squamous cells covered with masses of bacteria – indicates sample is mostly from mouth or throat

2. If PMNs are <10 per epithelial cell – no need for culture

3. Knowledge of flora of mouth and pharynx necessary before analyzing

Culture:

Ideal sample for culture

1. should contain <25 squamous cells per low power filed or <10 squamous cells per high power field

2. sample should contain alveolar macrophages

3. neutrophils should be >10 per epithelial cell or >5 per high power field

4. bronchial epithelial cells present

5. sample should be washed with normal saline to wash the saliva

Method:

1. Inoculate the sample on blood agar and chocolate agar
2. Incubate in an atmosphere of extra CO_2
3. Inspect plates after 18 hours.
4. If growth is significant, antibiotic sensitivity testing is carried out

Blood in sputum [hemoptysis]: TB, Pneumonia, Bronchiectasis, Lung abscess, Mitral Stenosis, Bronchogenic carcinoma.

EXAMINATION FOR ACID FAST BACILLI

1. ZEIHL NEELSON STAINING (AFB stain)

Sample:

1. According to RNTCP guidelines [Revised National TB Control Programme (RNTCP)], two samples are collected, one stat and one early next morning sample. It should be deep cough sputum sample.
2. for children, gastric aspirate can be used as they often swallow sputum.

Preparation:

- smear is prepared with blood tinged/opaque/grayish/yellowish portion of the sputum
- stained with ZN stain

Examined under microscope:

Reporting guidelines (RNTCP):

1. Mycobacteria appear as bright red, slightly curved or red beaded rods, 2-4 µm in length and 0.2 to 0.5 µm wide, against a blue green background.

2. At least 100 fields should be examined before declaring negative.

Grading of slides in AFB Microscopy

Examination	Result	Grading	No. of fields to be examined
More than 10 AFB per oil immersion field	Positive	3 +	20
1–10 AFB per oil immersion field	Positive	2 +	50
10–99 AFB per 100 oil immersion fields	Positive	1 +	100
1–9 AFB per 100 oil immersion fields	Positive	Scanty (Record exact number seen)	100
No AFB per 100 oil immersion fields	Negative	—	100

Drawbacks:

1. sensitivity 60-80%

2. minimum 5000-10000 bacilli / ml should be present for smear to be positive

Bleaching technique:

1. A solution of sodium hypochlorite is added to sputum sample – it leads to liquefaction of mucous and killing of microbes

2. smears are prepared from sediment and stained with ZN stain

2. FLUORESCENCE MICROSCOPY

1. Slides are stained with fluorescent auramine-rhodamine or auramine O.

2. Observed under fluorescent microscope – Mycobacteria appear bright yellow against green background

3. CULTURE ON CONVENTIONAL MEDIA

Indications:

1. drug susceptibility testing

2. species identification if other than M. tuberculosis suspected

3. sputum smear negative and strong clinical suspicion

Prerequisites:

1. 4% NaOH should be added before inoculation

2. this is because sputum samples are contaminated with normal flora, which grow and digest the media before MTB can grow.

Media used:

1. Solid media – LJ media (egg based) or Middle brook (agar based)

2. Liquid media – middle brook, TH9, TH 12

Advantage:

1. Sensitivity 80-85%

2. Can detect as low as 10-100 bacteria/ml

Drawbacks: expensive, requires 6 weeks for results

4. COMMERCIAL AUTOMATED CULTURE METHODS (BACTEC)

1. Can give results in 2 weeks

2. Mycobacteria are inoculated in a broth containing 14C palmitate

3. M.tb. metabolize 14C palmitate and release 14CO2 which is detected by the Instrument

MOLECULAR METHODS (PCR)

1. DNA sequences identified in MTB genome by PCR

2. can detect bacteria as low as 10-100 organisms / ml of sputum

3. direct sputum sample or culture samples can be used

4. laboratory cross contamination is an important issue here

PROCEDURE USING FLUORESCENT METHOD

AURAMINE STAINY OF SLIDE

1. Arrange slide in serial order on stainy bridge with the smear up

2. Apply filtered 0.1% Auramine on the slide and keep for at least 25minutes

3. Rinse with water and drain.

4. Apply 0.5% HCL decolorizing solution for 3 minutes

5. Rinse with water and drain

6. Apply 0.5% potassium permanganate for 1 minute

7. Rinse with water and drain

8. Air dry on slide rack.

Ziehl-Neelsen Staining- Principle and Procedure with Results

The Ziehl-Neelsen staining technique is a differential staining technique that was initially developed by Ziehl and modified later by Neelsen, hence the name Ziehl-Neelsen stain.

- Neelsen used carbol-fuschin from Ziehl's experiment, with heat and added a decolorizing agent using acid-alcohol and a counterstain using methylene blue dye, thus developing the Ziehl-Neelsen Technique of staining. The use of acid-alcohol in the technique earned it the name **Acid-Fast Stain** and the application of heat in the technique gives it the name the **hot method of Acid-Fast staining** which is a synonymous name for the **Ziehl-Neelsen Staining technique.** This technique is used on microorganisms that are not easily stained by basic stains such as Negative staining or Gram staining. One of the most complex micro-organisms that require harsh treatment of the Ziehl-Neelsen compounds is the *Mycobacterium* spp. *Mycobacterium, Actinomycetes, Norcadia, Isospora, Cryptosporidium,* and some fungi contain a thick cell wall made up of lipoidal complexes known as mycolic acid. Mycolic acid is difficult to stain and therefore simple stains like gram staining cannot penetrate the thick cell wall of these organisms. They require harsher treatments to allow stain penetration for identification and examination and hence the use of the Ziehl-Neelsen or the hot method of Acid-fast stain

Objectives: To differentiate between acid-fast bacilli and non-acid-fast bacilli. To stain *Mycobacterium* species.

Principle: The Ziehl-Neelsen stain uses basic fuchsin and phenol compounds to stain the cell wall of Mycobacterium species. Mycobacterium does not bind readily to simple stains and therefore the use of heat along with carbol-fuschin and phenol allows penetration through the bacterial cell wall for visualization. Mycobacterium cell wall contains high lipid content made up of mycolic acid on its cell wall making it waxy, hydrophobic, and impermeable. These are ß-hydroxycarboxylic acids made up of 90 carbon atoms that define the acid-fastness of the bacteria. Use of Carbol-fuschin which is basic strongly binds to the negative components of the bacteria which include the mycolic acid and the lipid cell wall. addition of acid alcohol along with the application of heat forms a strong complex that can not be easily washed off with solvents. The acid-fast bacilli take up the red color of the primary dye, carbol-fuschin. While non-acid-fast bacteria easily decolorize on the addition of the acid-alcohol and take up the counterstain dye of methylene blue and appear blue. This technique has been used in the identification of *Mycobacterium tuberculosis* and *Mycobacterium leprae*.

Reagents:

4. Carbol-Fuschin (Primary dye)
5. 20% sulphuric acid or acid-alcohol (Decolorizer)
6. Methylene Blue dye (counterstain) or malachite green

Preparation of reagents

Carbol fuschin

- Distilled water- 100ml
- Basic fuschin- 1g
- Ethyl alcohol (100% ethanol)- 10ml
- Phenol crystals- 5ml

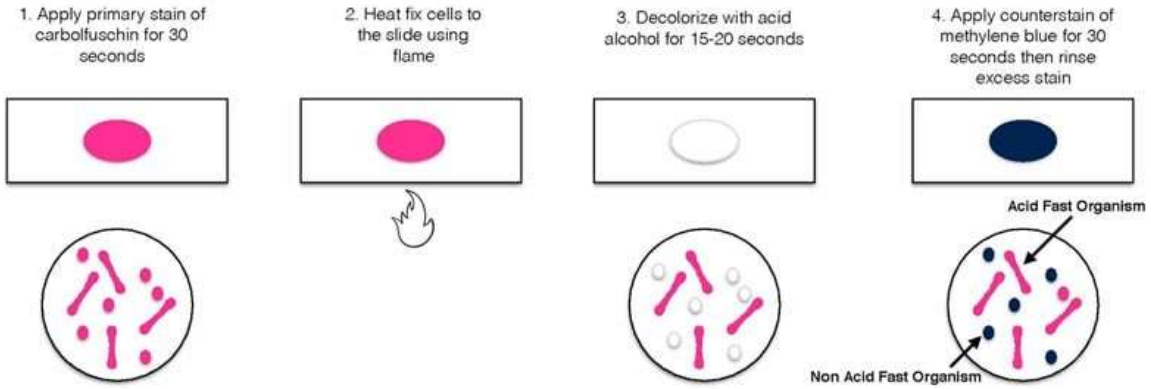
Acid alcohol (3% hydrochloric acid in 95% ethyl alcohol)

- Ethyl alcohol- 95 ml
- Distilled water- 2 ml
- Concentrated hydrochloric acid- 3 ml

0.25% methylene blue in 1% acetic acid

- Methylene blue- 0.25g
- Distilled water- 99ml
- Acetic acid- 1ml

Procedure for Ziehl-Neelsen Staining



1. On a clean sterile microscopic slide, make the smear of the sample culture and heat fix the smear over blue heat.
2. Over the smear, pour and flood the smear with carbol fuschin and heat gently until it produces fumes.
3. Allow it to stand for 5 minutes and wash it off with gently flowing tap water.
4. Add 20% sulphuric acid and leave it for 1-2 minutes. Repeat this step until the smear appears pink in color.
5. Wash off the acid with water.
6. Flood the smear with methylene blue dye and leave it for 2-3 minutes and wash with water.
7. Air dry and examine the stain under the oil immersion lens.

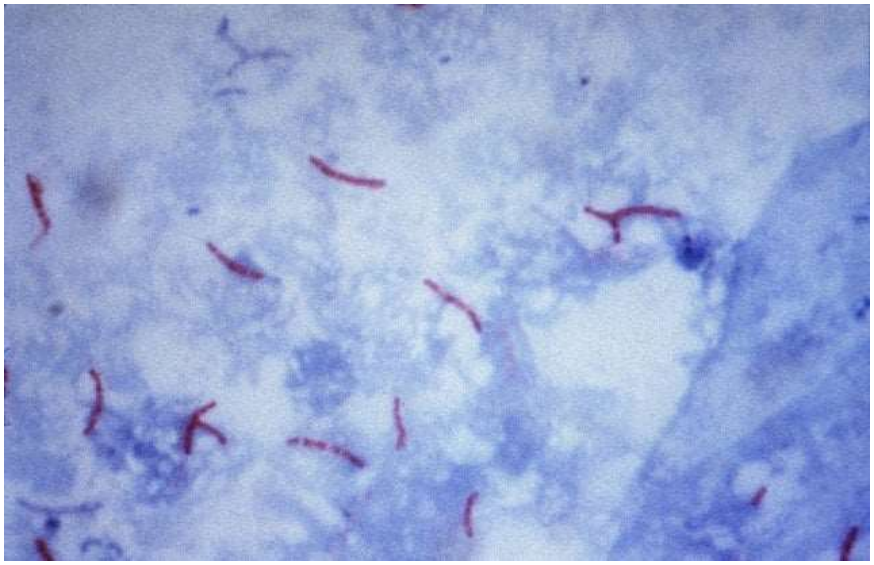


Figure: *Mycobacterium tuberculosis* visualization using the Ziehl–Neelsen stain.

- Acid-fast bacteria retain the primary dye, carbol-fuschin, and stain pink.
- Non-acid fat bacteria take up the methylene blue dye and appear blue.

Applications:

- Used for examination and identification of *Mycobacterium* species.
- Used to differentiate between acid-fast and non-acid fast bacilli
- Used for the identification of some fungal species such as *Cryptosporidium*.

Limitations:

- It can only be used to identify acid-fast bacilli.
- The physical morphology of the organism is distorted.

Montouxe test.

AIMS: To rapid detect of tuberculosis.

INTRODUCTION: The Mantoux test or Mendel–Mantoux test (also known as the **Mantoux** screening **test**, **tuberculin** sensitivity **test**, Pirquet **test**, or PPD **test** for purified protein derivative) is a tool for screening for tuberculosis (TB) and for tuberculosis diagnosis. It is one of the major tuberculin skin tests used around the world. The Mantoux test is endorsed by the American Thoracic Society and Centers for Disease Control and Prevention. It was also used in the USSR and is now prevalent in most of the post-Soviet states.

PRINCIPLE:

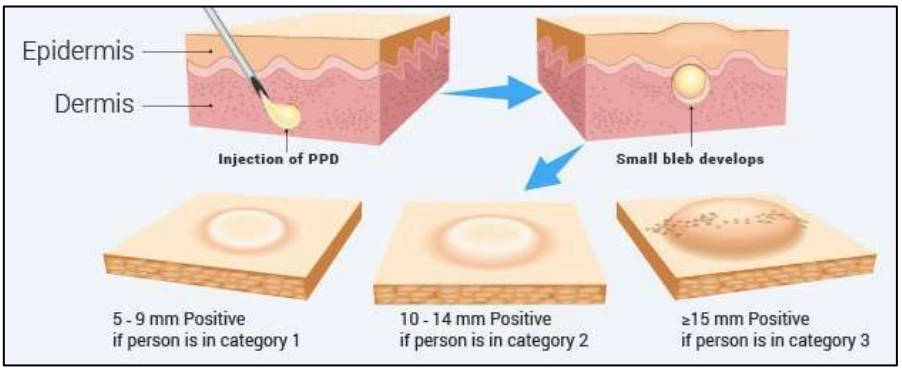
Tuberculin is a glycerol extract of the tubercle bacillus. Purified protein derivative (PPD) tuberculin is a precipitate of species-nonspecific molecules obtained from filtrates of sterilized, concentrated cultures. This active agent is tuberculin, a protein. A person who has been exposed to the bacteria is expected to mount an immune response

in the skin containing the bacterial proteins. The response is a classical example of delayed-type hypersensitivity reaction (DTH), a type IV of hypersensitivities. T cells and myeloid cells are attracted to the site of reaction in the timeframe of 1-3 days and generate local inflammation. The reaction is read by measuring the diameter of induration (palpable raised, hardened area) across the forearm (perpendicular to the long axis) in millimeters. If there is no induration, the result should be recorded as "0 mm". Erythema (redness) should not be measured. In the Pirquet version of the test tuberculin is applied to the skin via scarification.

MATERIALS: Tuberculin, Alcohol swab, Syringe, Scale.

PROCEDURE:

1. In the Mantoux test, a standard dose of 5 tuberculin units (TU - 0.1 ml), according to the CDC, or 2 TU of Statens Serum Institute (SSI) tuberculin RT23 in 0.1 ml solution, according to the NHS, is injected intra-dermally (between the layers of dermis) on the flexor surface of the left forearm, mid-way between elbow and wrist.
2. The injection should be made with a tuberculin syringe, with the needle bevel facing upward. Alternatively, the probe can be administered by a needle-free jet injector.
3. When placed correctly, injection should produce a pale wheal of the skin, 6 to 10 mm in diameter.
4. The result of the test is read after 48-96 hours but 72 hours (3rd day) is the ideal.
5. This intradermal injection is termed the **Mantoux technique**.



RESULTS & INTERPRETATION:

The person's medical risk factors determine at which increment (5 mm, 10 mm, or 15 mm) of induration the result is considered positive. A positive result indicates TB exposure.

According to the guidelines published by Centers for Disease Control and Prevention in 2005, the results are re-categorized into 3 parts based on their previous or baseline outcomes:

- Baseline test: ≥10 mm is positive (either first or second step); 0 to 9 mm is negative
- Serial testing without known exposure: Increase of ≥10 mm is positive
- Known exposure:
  - ≥5 mm is positive in patients with baseline of 0 mm
  - ≥10 mm is positive in patients with negative baseline or previous screening result of >0 mm

Diameter 5 mm or more is positive: An HIV-positive person; Persons with recent contacts with a TB patient; Persons with nodular or fibrotic changes on chest X-ray consistent with old healed TB; Patients with organ transplants, and other immunosuppressed patients.

Diameter 10 mm or more is positive: Recent arrivals (less than five years) from high-prevalence countries; Injection drug users; Residents and employees of high-risk congregate settings (e.g., prisons, nursing homes, hospitals, homeless shelters, etc.); Mycobacteriology lab personnel; Persons with clinical conditions that place them at high risk (e.g., diabetes, prolonged corticosteroid therapy, leukemia, end-stage renal disease, chronic malabsorption syndromes, low body weight, etc.); Children less than four years of age, or children and adolescents exposed to adults in high-risk categories.

Diameter 15 mm or more is positive: Persons with no known risk factors for TB.

A tuberculin test conversion is defined as an increase of 10 mm or more within a two-year period, regardless of age. Alternative criteria include increases of 6, 12, 15 or 18 mm.

BMLT(VU) 2nd semester

Fundamental Biochemistry lab manual

MI – 2P

Qualitative analysis of Glucose, Albumin, Ketone bodies, Fructose, Protein in Urine

Qualitative analysis of glucose in urine:

Introduction: Glucose is the most important of the sugars which may appear in urine. Normally, approximately 130 mg of glucose in urine is passed per 24 hours which is undetectable by qualitative tests. Above that level is called glycosuria that may be detected or estimated by qualitative or quantitative analysis.

Qualitative tests:

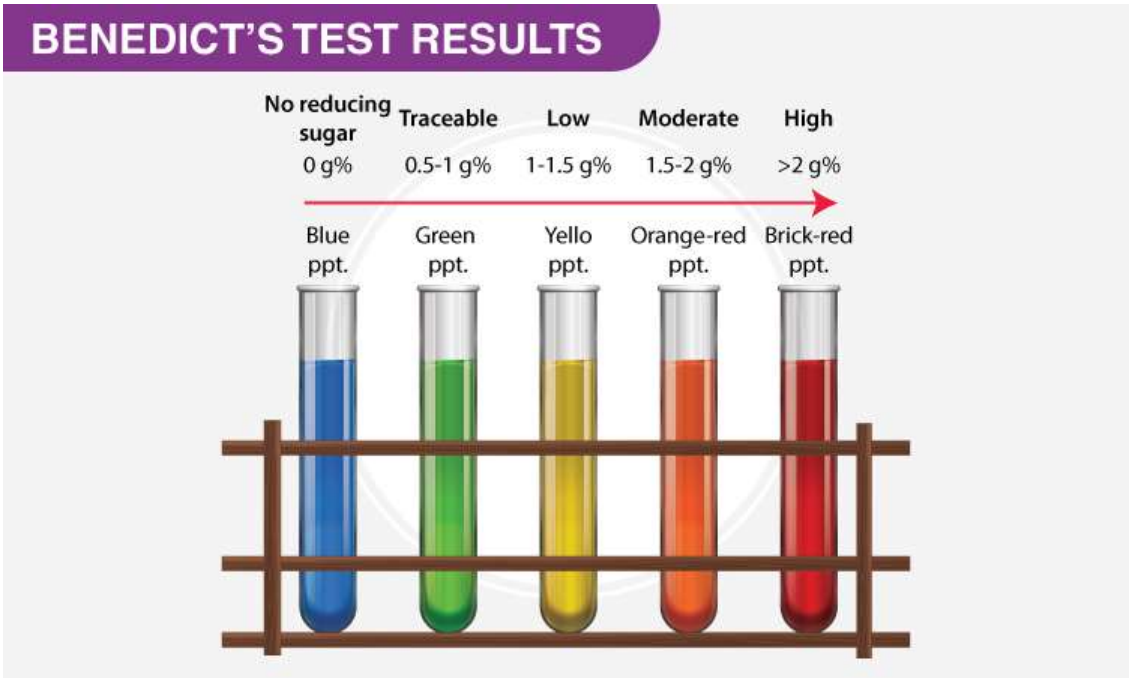
Benedict's test:

Principle:

Cupric ion is reduced by glucose to cuprous oxide and a coloured precipitate is formed.

Procedure: Benedict's test:

1. Take 5 ml of Benedict's qualitative reagent in a test tube.
2. Add 8 drops (or 0.5 ml) of urine.
3. Heat to boiling for 2 minutes.
4. Cool in water bath or in running tap water and look for colour change and precipitation.



Results & interpretation: Benedict's test:

No change of blue colour= Negative

Greenish colour= Traces (< 0.5 g/dl)

Green/cloudy green ppt= + (0.5-1 g/dl)

Yellow ppt= ++ (1-1.5 g/dl)

Orange ppt= +++ (1.5-2 g/dl)

Brick red ppt = ++++ (> 2 g/dl)

Note: Benedict's test is for reducing sugars excreted in the urine, the test is positive for all reducing sugars (glucose, fructose, maltose, lactose but not for sucrose which is a nonreducing sugar) and other reducing substances (e.g. ascorbic acid, salicylates, antibiotics, L-dopa).

Qualitative analysis of Albumin

Heat coagulation test:

Procedure- 3ml of urine is taken in a dry and clean test tube. Then few drops of glacial CH3COOH is added to it and heated.

Results & interpretation:

If no coagulation is observed after heating, then the test will be negative (Albumin is absent). If coagulation is observed after heating, then the test will be positive (Albumin is present), called albuminuria.

Esbach's test:

Procedure- 3ml of urine is taken in a dry and clean test tube. Then 1.5 ml of Esbach solution is added to it.

Result:

If precipitate appears then the test will be positive (Presence of albumin) and absence of precipitate indicates absence of albumin.

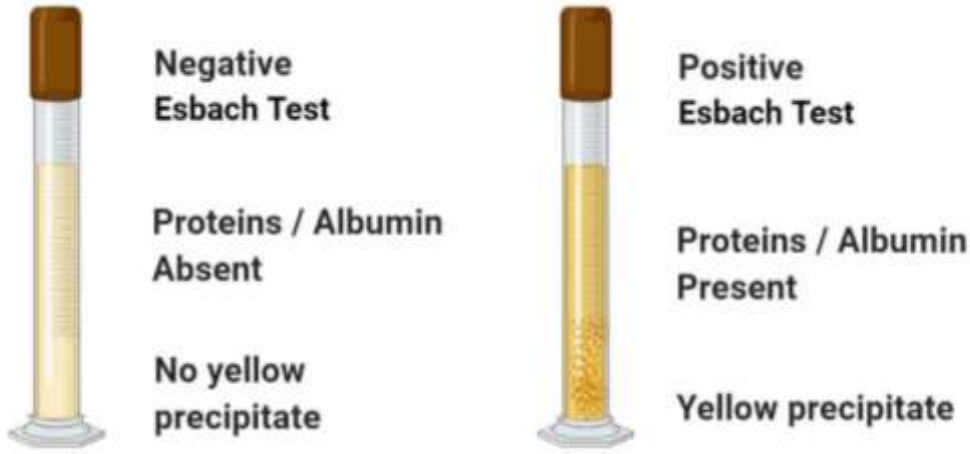


Fig: Esbach's test

Qualitative analysis of Protein in urine

Urine may be tested for proteinuria by qualitative tests

Heat coagulation test:

Principle: Heat causes coagulation of proteins.

Procedure:

i. Take a 5 ml test tube.

ii. Fill 2/3rd with urine.

iii. Acidify by adding a few drops of 3% acetic acid if urine is alkaline.

iv. Boil upper portion for 2 minutes (lower part acts as control).

v. If precipitation or turbidity appears, add a few drops of 3% acetic acid.

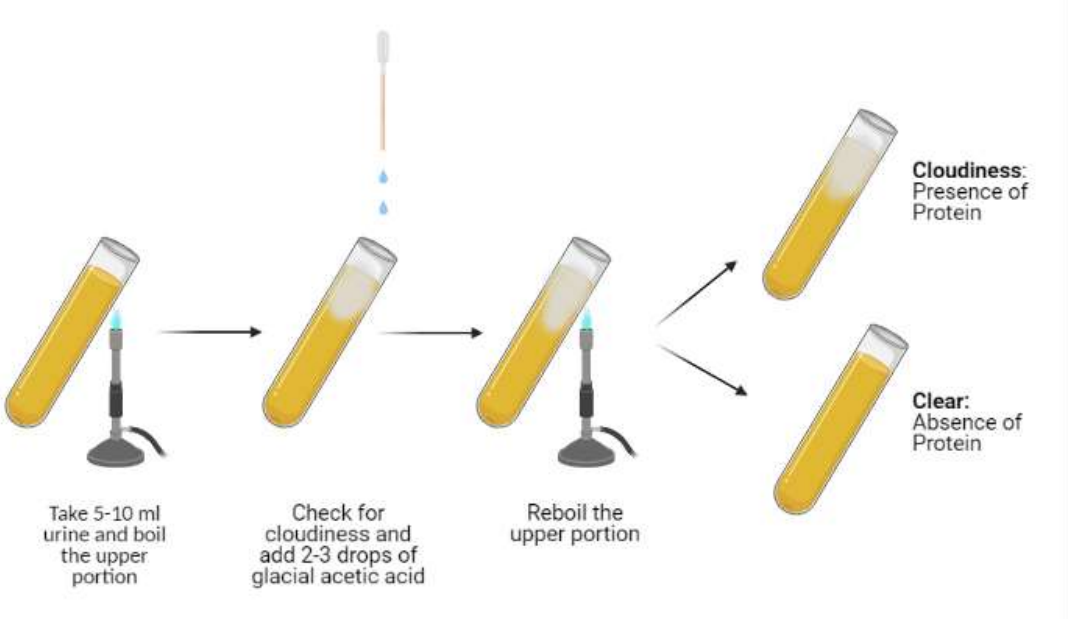


Fig: Heat coagulation test

Interpretation:

Turbidity or precipitation disappears on addition of acetic acid, it is due to phosphates; if it persists after addition of acetic acid, then it is due to proteins.

No cloudiness= Negative

Faint cloudiness= Traces (less than 0.1 g/dl).

Cloudiness without granularity = +(0.1 g/dl).

Granular cloudiness= = ++(0.1-0.2 g/dl)

Precipitation and flocculation= = +++(0.2-0.4 g/dl).

Thick solid precipitation= = ++++ (> 0.5 g/dl).

<u>Tests for ketonuria</u>:

Aim: To detect the ketone bodies, present in urine.

Introduction:

Ketone present in urine because of incomplete fat metabolism. The three ketone bodies excreted in urine are: acetoacetic acid (20%), acetone (2%), and β-hydroxybutyric acid (78%).

1. Rothera's test:

Principle: Ketone bodies (acetone and acetoacetic acid) combine with alkaline solution of sodium nitroprusside and form purple coloured complex.

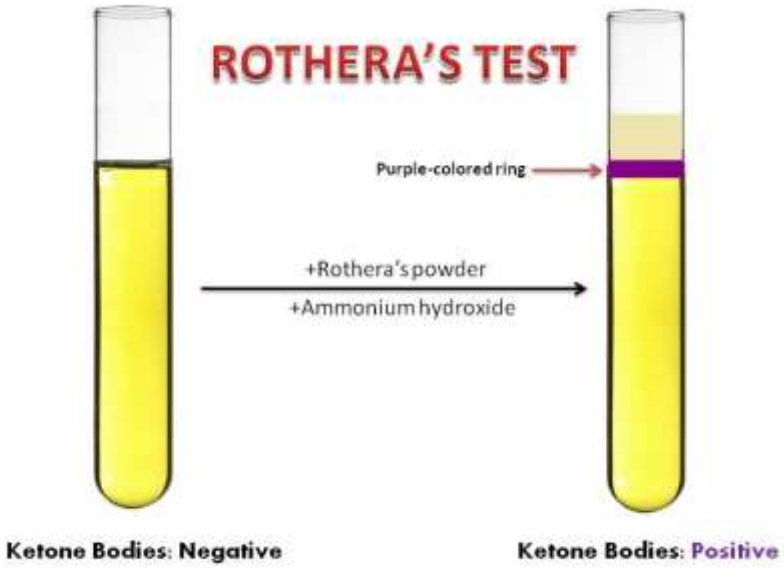
Procedure:

i. Take 5 ml of urine in a test tube.

ii. Saturate it with solid ammonium sulphate salt; it will start settling to the bottom of the tube when saturated.

iii. Add a few crystals of sodium nitroprusside and shake.

iv. Add liquor ammonia from the side of test tube.



Interpretation:

Appearance of purple or permanganate coloured ring at the junction indicates presence of ketone bodies.

Gerhardt's Test:

Procedure:

i. Take 5 ml of urine in a test tube.

ii. Add 10% ferric chloride solution drop by drop.

iii. Filter it and add more ferric chloride.

Interpretation: Brownish-red colour indicates presence of ketone bodies.

Results & inference:

Write your results/observation and interpret as stated below:

Ketone bodies found in urine sample may be due to:

i. Diabetic ketoacidosis

ii. Dehydration

iii. Hyperemesis gravidarum

iv. Fever

v. Cachexia

vi. After general anaesthesia.

Qualitative analysis of Fructose in urine

Principle: Fructose reacts with Seliwanoff's reagent to form a cherry red colour.

Procedure:

i. Take 5 ml of Seliwanoff's reagent in a test tube.

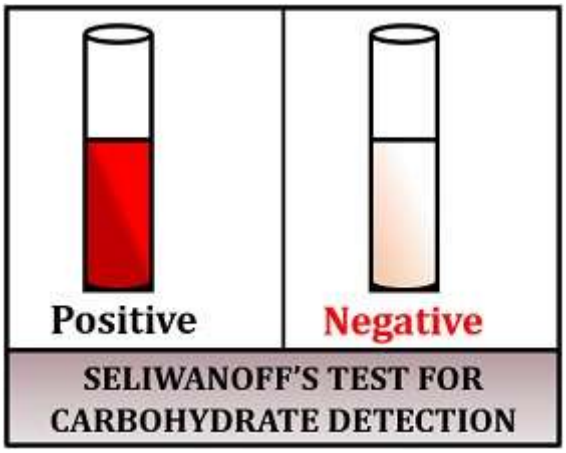
ii. Add 3 ml of urine sample drop by drop.

iii. Heat to boiling for 2 minutes.

Interpretation:

No change of colour: Negative

Development of red colour indicates: Presence of fructose in urine **(Fructosuria).**



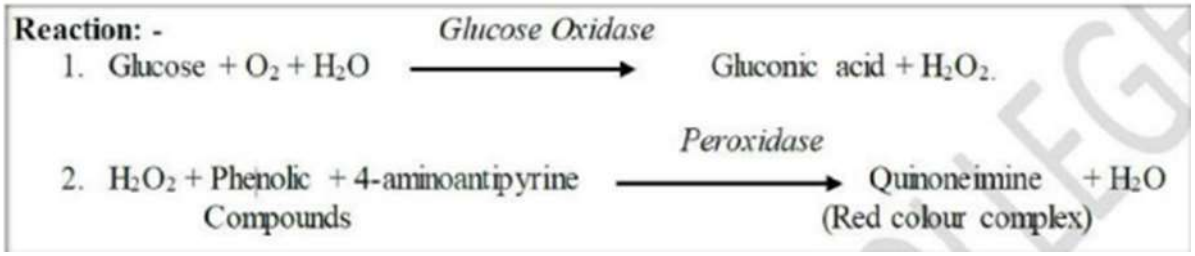
Estimation of glucose

Estimation of plasma glucose by GOD-POD Method

Principle:

Glucose oxidase (GOD) oxidizes the specific substrate β-D- glucose to gluconic acid and hydrogen peroxide (H2O2) is liberated. Peroxidase (POD) enzyme acts on hydrogen peroxide to liberate nascent oxygen (O2), then nascent oxygen couples with 4-amino antipyrine and phenol to form red quinoneimine dye. The intensity of the colour is directly proportional to the concentration of glucose present in plasma. The intensity of colour is measured by colorimeter at 530 nm or green filter and compared with that of a standard treated similarly. Final colour is stable for at least 2 hours if not exposed to direct sunlight.

Reaction:



Reagents:

1. Glucose colour reagent; it contains GOD, POD, 4- amino antipyrine, phenol & phosphate buffer (pH 7.5).

2. Glucose standard solution, Concentration = 100 mg/dl. 100 mg of anhydrous glucose is dissolved in 100 ml of distilled water.

Procedure:

Pipette into clean, dry test tube labelled as Blank (B), Standard (S) and Test (T).

Then add the solution in each of test tubes separately as shown in table below

	BLANK	STANDARD	TEST
Glucose colour Reagent	1000 µl	1000 µl	1000 µl
Distilled Water	10 µl	----------	---------
Standard		10 µl	
Plasma	----------		10 µl

Mix thoroughly and keep the tubes at 37˚C for 15 minutes and measure the OD at 530 nm.

Calculation:

Concentration of Glucose = (OD of Test/OD of standard) × Concentration of standard

Normal Range

	Fasting	After eating	2-3 hrs after eating
Normal	80 - 100	170 - 200	120 - 140
Pre Diabetic	101 - 125	190 - 230	140 - 160
Diabetic	126+	220 - 300	200+

Clinical Significance:

Increased glucose (Hyperglycemia): i) Diabetes Mellitus

ii) Hyperthyroidism

iii) Hyperpituitarism

iv) Adrenocortical hyper activity

Decreased glucose: (Hypoglycemia): Hypothyroidism

Glucometer

A glucometer is a medical device used for determining the approximate amount of glucose in a drop of blood obtained by pricking the skin with a lancet.

Typical features common to most of the glucose meters are given below:

- The average size is now approximately the size of the palm of the hand. They are battery powered.
- A consumable element containing chemicals, which react with glucose in the drop of blood, is used for each measurement. For most models this element is a plastic test strip with a small spot impregnated with glucose oxidase and other components.
- Each strip can only be used once and is then discarded.
- The glucose value in mg/dL or mmol/L displayed in a small window.
- Glucose levels in plasma are generally 10-15% higher than glucose measurements in whole blood (and even more after eating). This is important because home blood glucose meters measure the glucose in whole blood while most laboratory tests measure the glucose in plasma.
- Current "count times" range from 5 to 60 seconds for different models.
- The size of the drop of blood needed by different models currently varies from 0.3 to 10 uL
- All meters now include a clock, which must be set for date and time, and a memory for past test results. The memory is an important aspect of diabetes care, as it enables the person with diabetes to keep a record of management and look for trends and patterns in blood glucose levels over days. Most memory chips can display an average of recent glucose readings.

- Many meters have now had more sophisticated data handling capabilities. Many can be downloaded by a cable or infrared to a computer which has software to display the test results in a variety of formats. Some meters allow entry of additional data throughout the day, such as insulin dose, amounts of carbohydrates eaten, or exercise.
- A number of meters have been combined with other devices, such as insulin injection devices, PDAs. A radio link to an insulin pump allows automatic transfer of glucose readings to a calculator that assists the wearer in deciding an appropriate insulin dose.
- Special glucose meters for multi-patient hospital use are now used. These provide more elaborate quality control records and the data handling capabilities are designed to transfer glucoses into electronic medical records and the laboratory computer systems for billing purposes.

Principle

- Most of the glucometers are based on electrochemical technology which use electrochemical test strips to perform the measurement.
- Each glucometer test strip contains an enzyme called glucose Oxidase. This enzyme then reacts with the glucose in the blood sample and creates an acid called gluconic acid.
- The gluconic acid thus formed then reacts with another chemical in the testing strip called ferricyanide. The ferricyanide and the gluconic acid then combine with each other and forms ferrocyanide.
- As soon as the ferrocyanide has been formed the device (i.e., glucometer) runs an electronic current through the blood sample on the strip.
- This current thus generated is able to read the ferrocyanide and identify the amount of glucose present in the blood sample on the testing strip.
- That number is then displayed on the screen of the glucometer.

Procedure:

1. Clean the finger and proceed as per manufacturer's instructions.

2. A small drop of the blood is to be tested is placed on a disposable test strip of the glucometer for glucose measurement.

3. Accuracy of glucose meters is a common topic of clinical concern. Nearly all of the meters have similar accuracy (+10-15%) when used optimally. However, a variety of factors can affect the accuracy of a test. Factors affecting accuracy of various meters have included calibration of meter, ambient temperature, pressure used to wipe off strip, size of blood sample, high levels of certain drugs in blood etc.

Fig: Glucometer

SGPT (ALAT) KIT

(Modified IFCC Method)

Summary:

SGPT is found in a variety of tissues but it is mainly found in liver. Increased levels are found in hepatitis, cirrhosis, obstructive jaundice and other hepatic diseases. Slight elevation of the enzymes is also seen in myocardial infarction.

Principle:

SGPT (ALAT) catalyses the transfer of amino group between L Alanine and α-Ketoglutarate to form Pyruvate and Glutamate. The Pyruvate formed reacts with NADH in the presence of Lactate Dehydrogenase to form NAD. The rate of oxidation of NADH to NAD is measured as a decrease in absorbance which is proportional to the SGPT (ALT) activity in the sample.

SGPT

L-Alanine + α-Ketoglutarate → Pyruvate + L-Glutamate

LDH

Pyruvate + NADH + H+ → Lactate + NAD+

Expected values:

Serum (Males): upto 40 U/L at 37°C

(Females): upto 31U/L at 37°c

Procedure:

Wavelength/filter: 340 nm

Temperature: 37°C/30°C/25°C R.T.

Light path: 1 cm

Substrate Start Assay:

Pipette into a clean dry test tube labelled as Test (T):

Addition Sequence	(T) 25°C/30°C	(T) 37°C
Enzyme Reagent (L 1)	0.8 ml	0.8 ml
Sample	0.2ml	0.2ml

Incubate at the assay temperature for 1 min. and add

Starter Reagent (L2)	**0.2ml**	**0.2ml**

Mix well and read the initial absorbance A, after 1 min. and repeat the absorbance reading after every 1, 2, & 3 mins. Calculate the mean absorbance change per min. (ΔA /min).

Calculations

Substrate /Sample start

SGPT (ALAT) Activity in U/L 25°C/30°C = ΔA/min x 952

SGPT (ALAT) Activity in U/L 37°C = ΔA/min. x 1746

SGOT (ASAT) KIT

((Modified IFCC Method)

Summary:

SGOT is an enzyme found mainly in heart muscle, liver cells, skeletal muscle and kidneys. Injury to these tissues results in the release of the enzyme in blood. Elevated levels are found in myocardial infarction, Cardiac operations, Hepatitis, Cirrhosis, acute pancreatitis, acute renal diseases, primary muscle diseases. Decreased levels may be found in Pregnancy, Beri Beri and Diabetic ketoacidosis**.**

Principle:

SGOT (ASAT) catalyzes the transfer of amino group between L-Aspartate and α-Ketoglutarate to form Oxaloacetate and Glutamate. The Oxaloacetate formed reacts with NADH in the presence of Malate dehydrogenase to form NAD. The rate of oxidation of NADH to NAD is measured as a decrease in absorbance which is proportional to the SGOT (ASAT) activity in the sample.

SGOT

L-Aspartate + α-ketoglutarate → Oxaloacetate + L-glutamate

MDH

Oxaloacetate + NADH + H+ → Malate + NAD+

Expected values:

Serum (males): upto 37 U/L at 37°C

(Females): upto 31 U/L at 37°C

Procedure:

Wavelength/filter: 340 nm

Temperature: 37°C/30°C/25°C R.T.

Light path: 1 cm

Substrate Start Assay

Serum Pipette into a clean dry test tube labelled as Test (T)

Addition Sequence	**(T)** **25°C/30°C**	**(T)** **37°C**
Enzyme Reagent (L1)	0.8 ml	0.8 ml
Sample	0.2 ml	0.2 ml

Incubate at the assay temperature for 1 min. and add		
Starter Reagent (L2)	0.2ml	0.2ml

Mix well and read the initial absorbance A, after 1min. & repeat the absorbance reading after every 1, 2, & 3 mins. Calculate the mean absorbance change per min. (ΔA /min.).

Sample Start Assay:

Pipette into a clean dry test tube labelled as Test (T)

Addition Sequence	**(T)** 25°C/30°C	**(T)** 37°C
Working Reagent	1.0 ml	1.0 ml

Incubate at the assay temperature for 1 min and add

Sample	0.2ml	0.1ml

Mix well and read the initial absorbance A, after 1 min. & repeat the absorbance reading after every 1, 2, & 3 mins. Calculate the mean absorbance change per min. (ΔA/min.)

Calculations:

Substrate/Sample start

SGOT (ASAT) Activity in U/L 25C/30°C = ΔA /min. x 952

37C °C = ΔA /min. x 1746

Electrolytes Test Kit

Principle for sodium:

The sodium and the proteins are Precipitated Simultaneously by means of a reagent containing magnesium uranyl acetate containing alcohol. The precipitate is separated by centrifugation. The content of sodium is calculated from the loss in the concentration of magnesium uranyl acetate in the reagent solution in comparison to a standard sodium solution treated similarly. The residual amount of magnesium uranyl acetate is estimated by forming brown (dark) ferrous uranyl acetate. Which is read in a colorimeter.

Principle for potassium:

Potassium can be determined by a number of different methods. It Can be directly estimated by flame photometry, colorimetry. It can also be measured by the use of ion selective electrode. The method is based on the measurement of turbidity of the reaction mixture containing Sodium Tetraphenyl Boron, Alkaline EDTA, Formaldehyde and sample containing potassium or standard potassium salt. The method is accurate within the concentration of 2.0 to 7.0 mmol/L. There is a good agreement with flame photometry.

Reagents:

1. Sodium Precipitating Reagent	33 ml.
2. Standard Sodium /Potassium	3 ml.
3. Sodium Colour Reagent	10 ml.
4. Potassium Reagent	45 ml.

The reagents are ready to use and usable to the expiration date when stored at 2-8°C, if contamination is avoided.

Sample:

Serum (Haemolysed sera should not be used)

1. Serum should be separated from the clotted blood without delay to prevent any leakage of potassium from the RBC, which contains 23 times higher concentration of potassium than the serum.

2. Lipemic samples should be avoided. Turbid or icteric samples produced falsely elevated potassium results.

3. Serum urea level higher than 150 mg% will produce elevated potassium results.

Expected range:

Potassium: 3.5 to 5.5 mmol/L

Sodium: 135 to 155 mmol/L

LINEARITY:

Potassium: This method is linear between 2 to 7 mmol/L

Sodium: This method is linear between 100 to 200 mmol/L

Sodium assay:

Step I - Precipitation of sodium and proteins.

Pipette into two clean dry test tubes labelled standard (S) and test (T)

	S	T
Sodium PPT Reagent(l)	1.0 ml	1.0 ml
Standard Sodium/Potassium (2)	0.02 ml	0.02 ml
Serum		0.02 ml

Mix well on vortex for one minute and wait for five minutes at room temperature. Centrifuge for one minute at 3000 rpm.

Step II - Colour Development.

Pipette into three clean dry test tubes labelled blank (B), standard (S) and test (T)

	B	S	T
Distilled Water	3ml	3ml	3ml
Supernatant from step I	_____	0.05ml	0.05ml
Sodium PPT Reagent (1)	0.05ml	_____	_____
Sodium Colour Reagent (3)	0.2ml	0.2ml	0.2ml

Mix well and allow it to stand at room temperature for five minutes. Then measure absorbance of B, S, T against distilled water on a photocolorimeter at 540 nm within 10 minutes.

Calculation:

Sodium in mmol/L = (Absorbance of T/ Absorbance of S) X 150 (Concentration of standard)

Potassium assay:

Pipette into two clean dry test tubes labelled standard (S) and test (T)

	S	T
Potassium Reagent (4)	1.0 ml	1.0 ml
Standard Sodium/Potassium (2)	0.05 ml	----------
Serum	----------	0.05 ml

Mix gently wait for five minutes at room temperature and read the absorbance of standard and test against distilled water on a photo colorimeter at 620 nm within 10 minutes.

Calculation:

Potassium in mmol/L = Absorbance of T/Absorbance of S x 5 (Concentration of standard)