Clinical Pathology
BML-391

Urine Specimen Collection Procedure:

A urinalysis is a test of your urine. A urinalysis is used to detect and manage a wide range of disorders, such as:

1. urinary tract infections,
2. kidney disease and
3. diabetes.

A urinalysis **involves checking the appearance, concentration and content of urine**. Abnormal urinalysis results may point to a disease or illness.

PRINCIPLE:

Urine is one type of specimen that can be easily collected from a patient. Urinalysis testing can give the doctor valuable information about many body systems especially kidney function. The physician uses the information from urine testing to diagnose and treat many disease states.

Types of urine specimens:

1. **Random sample:** Sample which is collected anytime during the day. Usually used only for routine screening because the composition of urine changes throughout the day.
2. **First voided specimen**: Sample also referred to as a first morning specimen. This sample is collected the first time the patient urinates in the morning. A first voided specimen is the most concentrated and is the preferred specimen for pregnancy testing, bacterial cultures and microscopic examinations.
3. **Timed specimens**: These specimens are used when the physician requires urine samples to be taken at specific intervals during the day. Twenty-four (24) hour urine specimens are required for creatinine clearance tests and many other hormone studies.
4. **Clean-catch midstream specimen**: This sample type is collected if the urine is going to be cultured and examined for bacterial growth or used for cytology.
5. **"Dirty collection:"** This specimen will be used for DNA testing and the FIRST part of the voided stream is collected.

6. **Catheterized specimen**: These specimens are obtained by inserting a catheter or sterile flexible tube into the bladder via the urethra to withdraw urine. This procedure is done only by specially trained personnel

PROCEDURE Specimen collection:

Urine Collection Containers

(cups for collection and transport) Urine collection container cups come in a variety of shapes and sizes with lids that are either snap on or screw on. To protect healthcare personnel from exposure to the specimen and protect the specimen from exposure to contaminants, leak- resistant cups should be utilized.

1. **Routine or random sample**: The patient is given a non-sterile collection container and instructed to collect a midstream specimen in the container. This type of specimen is routinely used for urinalysis and may not be used for a culture and sensitivity.
2. **First voided specimen**: The patient is given a urine container to take home and instructed to collect a sample of the urine the first time he or she urinates in the morning. Because urine is not stable, the specimen should be returned to the laboratory within one (1) hour of collection. If that is not possible, the specimen should be refrigerated until it can be tested.

3. **Timed specimen**: Timed specimens are usually a 24 hour urine collection.

    a. The patient is given a large container (approximately 1 gallon) that is labelled with the patient's name and date. Space is provided to write the time the collection begins and ends.
    b. Before issuing the 24 hour urine container the type of testing ordered is checked for **preservative requirements.** This information can be found in the BVH Laboratory specimen collection manual and any required preservatives addition is done by a tech. Cautionary labels are also often applied to caution patients that the added preservative may be caustic. 24 hour urine specimens are also usually required to be refrigerated during the collection period. This information should be recorded on the label applied to the 24 hour container.

c. The test usually begins in the morning. The patient is told to empty their bladder and **discard** the urine in the toilet and record the time on the label of the urine container. For the next 24 hours, all urine must be collected in the container. The next day at the same time the test began the patient empties their bladder, collects the urine in the container, and records the time the test ended. The patient should be instructed to avoid fecal contamination of the specimen.
d. The 24 hour urine specimen is brought to the laboratory as soon as possible as the 24- hour period is over.

Clean-catch mid-stream specimen: Patients with orders for a urine culture and sensitivity are given the proper mid-stream urine collection kit and the appropriate instruction sheet.

1. Give the patient a sterile urine collection kit. The kits are located in the out-patient lab.
2. Explain to the patient that an instruction sheet is included in the kit. There are two sets of instructions. There are different instructions for males and females. Verify that the patient understands the instructions.
3. *Male urine culture collection instructions*: These are general instructions. BVH periodically changes the company which provides the mid-stream collection kits. Each manufacturer provides very specific instructions to correlate with their specific type of container. These instructions may vary slightly with different manufacturers. The current use kit instructions are the instructions provided to the patient.

    1. Wash hands thoroughly with soap and water, rinse and dry.

    2. Open the collection package but **DO NOT TOUCH INSIDE OF CUP OR RIM**. Open the package of 3 towelettes. Retract foreskin if present. With the first towelette, cleanse the urinary opening of the penis starting at the center and work outward. Repeat the cleansing in the same manner with the two remaining towelletes.
    3. Remove lid carefully from the collection container, **DO NOT TOUCH** the inside of the container or rim. Gently grasp the container.

    4. Begin to void urine, **letting the first 20-25 ml pass into the toilet**. Position the cup in the stream of urine until the container is about half to two-thirds full. Finish voiding into the toilet.

    5. After obtaining the urine specimen, screw the lid on tightly again being careful to avoid touching inside the container or lid.

Bring the specimen to the lab within 1 hour or collection or store refrigerated for up to 24 hours.

D. *Female urine culture collection instructions*: These are general instructions. BVH periodically changes the company which provides the mid-stream collection kits. Each manufacturer provides very specific instructions to correlate with their specific type of container. These instructions may vary slightly with different manufacturers. The instructions included in the kit currently in use are the instructions provided to the patient.

1. Wash hands thoroughly with soap and water, rinse and dry.

2. Open the collection package but **DO NOT TOUCH INSIDE OF CUP OR RIM**. Open the package of 3 towelettes. While seated on the toilet spread labia major (outer folds). With the first towellete, wipe one side of the labia minora (inner fold) using a single downward stroke. Discard towellete. With the second towellete repeat the procedure on opposite side using a single downward stroke. Discard towellete. With the third towelette, cleanse meatus (center area) with a single downward stroke. Discard towellette.

3. Remove lid carefully from the collection container, **DO NOT TOUCH** the inside of the container or rim. Gently grasp the container.

4. Begin to void urine, letting the first 20-25 ml pass into the toilet. Position the cup in the stream of urine until the container is about one-half to two-thirds full. Finish voiding into the toilet.

5. After obtaining the urine specimen, screw the lid on tightly again being careful to avoid touching inside the container or lid.

6. Bring the specimen to the lab within 1 hour of collection or store refrigerated for up to 24 hours.

5. **"Dirty" specimen:** The patient is given a sterile urine cup and told to clean as stated above for a clean-catch specimen. They are then instructed to collect the FIRST part of the voided stream. Fill the container one half to two thirds full and finish voiding into the toilet. Apply the cap tightly and label the cup.

6. **Catheterized specimen**: These specimens are collected by specially trained personnel only.

All urine specimens should be promptly returned to the laboratory. Specimens must be labelled with the patient's **name, DOB, date**, **time of collection and ordering physician.**

Instruction sheets have been developed to be given to patients for common urine collections testing. These forms can be found in Soft Tech as well as in the collection manual.

Another Guidelines for 24 Hour Urine Specimen Collection

Your doctor has ordered a 24 hour urine test. This means you must save all of your child's urine for 24 hours.

- A clean urine collection container. Females will use a toilet hat to catch or collect the urine. Males can use a plastic, portable (easy-to-carry) urinal or the large urine storage container.
- A large urine storage container

How to Label the Specimen

1. Child's full legal name – complete first and last names, correctly spelled
2. **One** of the following unique identifiers:

- Date of birth **or**
- Patient's ID **or**
- Nationwide Children's Hospital medical record number

How to Collect the 24-hour Urine Specimen

1. Decide on a day to do the test when your child will be at home all day.
2. On the day of the test, have your child empty their bladder (urinate or pee) in the toilet right after waking up. Flush the urine down the toilet.
3. **The test begins now with the bladder empty. Write this date and the start time on the storage container's label.**
4. For the next 24 hours, your child will need to pee into a collection container every time they go to the bathroom. Females can use a toilet hat. Males can use a plastic urinal or pee right into the large storage container. If you do not have a toilet hat or urinal at home, you may use some other clean plastic container.
5. Before using the plastic container for the first time, wash it with dish soap and then rinse at least 10 times with tap water. Allow it to air dry completely.
6. Do not let feces (poop) mix with the urine or else the test will need to be restarted.
7. Pour the urine into the large storage container and close the lid tightly. Be very careful not to spill any urine.
8. If using a collection container, rinse it with **water only**. Put it back by the toilet to remind you to use it the next time. Allow it to air dry completely.
9. Put the large storage container in the refrigerator. The urine must be kept cool at all times. If you do not have space in the refrigerator, you can store it in a cooler on top of Add more ice as needed to keep the urine cold.

10. Each time your child pees during the day and night, follow Steps 4 through 9.
11. The next day (close to the same time that you started on the first day), have your child pee into the collection container one last time. Add it to the large storage container. This ends the 24-hour collection.
12. **Write the date and time of this last urine collection on the label.**
13. Attach a list of all medicines, including over-the-counter medicines, vitamins and herbal remedies, your child took during the 24-hour urine collection.

Other Information

- Take the urine to a Laboratory (Lab) Service Center as soon as possible, **within 24 hours after ending the collection.** Keep the urine cool.
- Make sure the urine does not freeze for these tests: amylase, arylsulfatase, immunoelectrophoresis, micro-albumin, pregnanetriol, protein or uric acid.
- You will need to start the test over if any of the urine spilled, you forgot to save some or it has poop in it. If you must restart the test, it is okay to use the same collection and storage containers. Pour out the urine, clean the containers well and allow them to air dry. Then follow Steps 1 through 13.

Preservation 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, nor- adrenaline, 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.

Specimen Evaluation Urinalysis

Preanalytical Assessment • Before proceeding for examination, specimen must be evaluated in terms of its acceptability.

1. Minimum Labelling Requirements: Patient's full name, Date and Time of collection

2. Intactness: There shouldn't be any leakage, spillage and damage to container.

3. Timing of collection: First voided morning urine is the best for Routine analysis.

4. Preferences: If multiple investigations are to be done from a single specimen, bacteriologic examination should be performed first. Hence, volume of urine should be noted properly.

Physical Examination of URINE

1. Volume
2. Appearance
3. Oduor
4. Specific gravity

Volume

Total volume can be evaluated only from 24-hour urine sample.

Normal individual: – 24-Hour urinary output: 600 to 2000 ml.

Exceptions: Pregnancy- diurnal variation may be reversed Young children- 3-4 times more urine than adults (ml/per body weight).

Increase Urine Volume	Decrease Urine Volume
1. >2000 ml/24 hrs- Polyuria 2. >500 ml during night- Nocturia. **Causes:** a. Diabetes mellitus (Osmotic diuresis) b. Diabetes insipidus (Failure to secrete ADH) c. Chronic renal failure (Loss of concentrating ability of renal tubules) d. Diuretic therapy e. Polydypsia f. Caffeine/ alcohol intake	1. <500 ml/ 24 hours- Oliguria. 2. <100 ml/ 24 hours or complete cessation- Anuria **Causes:** a. Oliguria: High grade febrile states Acute glomerulonephritis (decreased glomerular filtration) Congestive cardiac failure or dehydration (Renal hypoperfusion) b. Anuria: Acute tubular necrosis Complete urinary obstruction

1. **Oliguria and anuria**

Oliguria is a term for urine volume < 400ml/24 hours; while **anuria** stands for volume < 100ml/24 hours.

Oliguria and anuria are ***basic symptoms of kidney failure***. One of the causes might be dehydration resulting from insufficient water intake, or its excessive loss (diarrhoea, sweating). A low diuresis can happen also due to water retention (oedemas, transsudates in body cavities); or the cause may lie in primary damage to the renal parenchyma.

Oliguria/anuria can also result from a mechanical obstruction of the urinary tract (prostatic hypertrophy, wedged concrement, tumors in pelvic area). If the obstacle is located below the urinary bladder, we refer to the condition as ***retention of urine***.

Polyuria

Polyuria denotes increase of daily diuresis above 2,500 ml. Two types of polyuric conditions can be distinguished:

• Polyuria caused by ***water diuresis***

Water diuresis results from ***decreased tubular reabsorption of water*** in distal part of the nephrone. The tubular absorption as well as excretion of osmotically active substances are normal. Osmolality of urine is lower that osmolality of serum; and is always below 250 mmol/kg H_2O. The water diuresis comes physiologically as a result of high water intake; pathologically e.g. due to impaired secretion of adiuretin (diabetes insipidus).

• Polyuria caused by ***osmotic diuresis***

It results from either increased filtration of osmotically active substances due to their high concentration in the ECT (e.g. hyperglycemia), or from their decreased tubular absorption. The unabsorbed osmotically active substances "drag" water, leading in decrease in water reabsorption. Osmolality of urine is higher than 250 mmol/kg H_2O. The osmotic diuresis is characteristic e.g. for diabetes mellitus (glucosuria), polyuric phase of renal failure, or comes as an effect of diuretic drugs.

Appearance:

Normal: Urine is AMBER coloured and CLEAR Colour

Colour	Causing substance	Occurrence
Yellow to colorless		• increased diuresis in excessive drinking • diuretic drugs • diabetes mellitus • diabetes insipidus • polyuric phase of renal failure
Brown	bilirubin	diseases of liver and biliary tract
Green-brown	biliverdin (originates from bilirubin by oxidation air) – old in urine	diseases of liver and biliary tract
Meat red (without turbidity)	Hemoglobin myoglobin porphyrins beetroot	• intravascular hemolysis • burns • necrosis of muscles • inflammation of muscles • porphyrias • exogenous intake
Dark brown (turns black upon standing on air)	melanin homogentisic acid	melanoma alkaptonuria
Light red	urates	hyperuricosuria

Smell of urine

Normal- Aromatic

Odor

Freshly voided urine has a typical aromatic odor due to volatile organic acids. After standing, urine develops ammoniacal odor (formation of ammonia occurs when urea is decomposed by bacteria). Some abnormal odors with associated conditions are:

- Fruity: Ketoacidosis, starvation
- Mousy or musty: Phenylketonuria
- Fishy: Urinary tract infection with Proteus, tyrosinaemia.
- Ammoniacal: Urinary tract infection with Escherichia coli, old standing urine.
- Foul: Urinary tract infection
- Sulfurous: Cystinuria.

Specific Gravity (SG)

This is also called relative mass density. It depends on the number of solutes in the solution. It is basically a comparison of the density of urine against the density of distilled water at a particular temperature.

The specific gravity of distilled water is 1.000.

Normal SG of urine is 1.003 to 1.030 and depends on the state of hydration.

Clinical Significance:

SG of normal urine is mainly related to urea and sodium. SG increases as solute concentration increases and decreases when the temperature rises (since volume expands with rising in temperature). **SG of urine is a measure of concentrating ability of kidneys and is determined to get information about this tubular function.** SG, however, is affected by proteinuria and glycosuria.

Causes of increase in SG of urine are diabetes mellitus (glycosuria), nephrotic syndrome (proteinuria), fever, and dehydration.

Causes of decrease in SG of urine are diabetes insipidus (SG consistently between 1.002-1.003), chronic renal failure (low and fixed SG at 1.010 due to loss of concentrating ability of tubules), and compulsive water drinking.

Methods for measuring SG are the urinometer method, refractometer method, and reagent strip method.

Urinometer method

This is a bulb shaped instrument that has a cylindrical stem ,which contains a scale calibrated in sp. gravity reading. This instrument is floated in a cylinder containing urine. The depth to which it sinks in the urine indicates the sp. gravity of urine .

Principle: The method of measuring sp. gravity of urine is based on the principle of buoyancy. An increased solute concentration or increased sp. gravity increases the upthrust of the solution correspondingly.

Procedure:

1. Mix the urine & pour into the cylinder of 25ml capacity.
2. Carefully float the urinometer by grasping the stem of urinometer at the top and inserting slowly into the urine.
3. Swirl the urinometer slightly as it is inserted. make sure the instrument floats freely away from the sides of container.
4. Take the reading from the graduation gives on the stem at lower meniscus formed at eye level.

ADVANTAGES:

1. Results are directly obtained and depend on the solutes dissolved in urine. 2. Easily performed and quick assessment of results

DISADVANTAGES:

1. Requires minimum of 15 ml for measuring specific gravity.
2. Correction of 0.001 should be made for each 3° C rise or fall in temperature.
3. Correction for every g/dl of protein and sugar is required (0.003 for 1 g/dl of protein and 0.004 for 1 g/dl of glucose).

Refractometer Method:

Urinalysis has been shown to be the most valid and reliable method for determining moderate changes in fluid balance. Here is a method of analyzing urine specific gravity using a refractometer.

Purpose: monitoring hydration levels to prevent dehydration is important for optimizing performance. Urine specific gravity is a scientific measure of hydration by measuring the density (concentration) of a urine sample.

Equipment required: a refractometer (a simple hand-held version is illustrated here), urine specimen containers for urine collection, distilled water, cleaning cloth / disposable tissues, fridge or ice cooler for urine storage, gloves.

procedure:

1. **Collecting the urine.** The first part of the urine stream is discarded, then a small sample of urine is collected into a container. The sample can be measured immediately or stored for later measurement.
2. **Calibrating the refractometer.** Calibrate the refractometer by placing distilled water on the glass as the sample, and adjusting the scale to read 1.000. This should be done before you begin testing, and after every ten samples or so to ensure that the calibration remains accurate.

3. **Measurement.** Open up the flap at the end of the refractometer. Clean with distilled water and dry with a soft non-abrasive cloth. Place a drop of urine on the glass plate and close the flap. Hold the refractometer up towards an area of natural light, look though the eye piece and read the specific gravity level off the scale - the point where the contrast line (difference between light and dark areas) crosses the scale.

Results: The measurement may be done immediately after collection, or the specimen can be stored in refrigeration for later analysis. The specific gravity results will range from 1.000 (which is equivalent to water) up to 1.035 (very dehydrated). There are several levels that are used in the literature to indicate dehydration, such as a value of 1.15 or greater.

comments: The sample is usually collected first thing in the morning. It may also be of interest to collect samples prior to or post exercise, though there may be a time delay for the effect of dehydration to show in the specific gravity measure.

Precautions:

- Certain medicines, vitamins or the presence of glucose may cause the urine specific gravity to change and give incorrect readings of dehydration. If any of these situations occur then the test is unreliable.
- The refractometer should be calibrated before you begin testing, and after every ten samples or so to ensure that the calibration remains accurate.

Advantages: The hand held refractometer is very easy to operate.
Disadvantages: This test requires the collection of urine (which is sometimes difficult) and the purchase of a specific apparatus for measurement. For a more simple test of hydration you can use urine color.
Comments: There is a minimal difference in the accuracy of the related measures of urine specific gravity, urine osmolarity, and urine color.

Chemical Examination of URINE

Performing the Chemical Test by Reagent Strip

The urine must be collected using appropriate technique and be tested within one hour of collection. If tests cannot be performed within this time, the specimen may be refrigerated for up to 8 hours. Refrigerated specimens should be allowed to return to room temperature prior to testing.

Chemical testing is performed by dipping a reagent strip into a fresh urine. The color changes on the reagent pads should be visually compared to the color chart after the appropriate time period. This can also be done on an electronic instrument which reads the color and displays it on a lighted panel. Automation eliminates technician error due to differences in timing or interpretation of the colors.

Principles of Chemical Tests

The pH is a measure of the degree of acidity or alkalinity of the urine. A pH below 7 indicates and acid urine; pH above 7 indicates an alkaline urine. Normal, freshly-voided urine may have a pH range of 5.5 - 8.0. The pH of urine may change with diet, medications, kidney disease, and metabolic diseases such as diabetes mellitus. Colors on the pH reagent pad usually range from yellow-orange for acid pH to green-blue when pH is alkaline.

Protein. Protein in the urine is called proteinuria. This is an important indicator of renal disease, but can be caused by other conditions as well. At a constant pH, the development of any green color on the protein reagent pad is due to the presence of protein. Colors range from yellow for negative to yellow-green or green for positive.

Glucose. The presence of glucose in urine is called glycosuria. This condition indicates that the blood glucose level has exceed the renal threshold. This condition may occur in diabetes mellitus. The reagent strip is specific for glucose and uses the enzymes glucose oxidase and peroxidase, which react with glucose to form colors ranging from green (low concentration) to brown (high concentration.

Ketone. When the body metabolizes fats incompletely, ketones are excreted in the urine resulting in ketonuria. The ketone test is based on the development of colors ranging from light pink to maroon when ketones react with nitroprusside. Ketonuria may be present in diabetes and starvation or fasting. Since ketones will evaporate at room temperature, urine should be tightly covered and refrigerated if not tested promptly.

Bilirubin. Bilirubin is a breakdown product of hemoglobin which produces an extremely yellow color in urine and may be an indication of liver disease, hepatitis or bile duct obstruction. Samples suspected of containing bilirubin should be handled cautiously because of the possibility of hepatitis. These samples should also be protected from light until testing is completed, since direct light will cause decomposition of bilirubin. The test for bilirubin is based on the coupling of bilirubin with a dye to form a color.

Blood. Presence of blood in the urine may indicate infection or trauma of the urinary tract or bleeding in the kidneys. Hemoglobin (hemoglobinuria) and red blood cells (hematuria) may be detected by the formation of a color due to the enzyme peroxidase (in red cells) reacting with orthotolidine, a chemical which is in the reagent pad. The resulting color ranges from orange through green to dark blue.

Protein Detection

Normally, kidneys excrete scant amount of protein in urine (up to 150 mg/24 hours)

Salfosalicylic acid test:

Principle of tests:

Protein gets precipitated after adding organic acid like sulphosalicylic acid.

Reagents:

1. 3g/dl sulfosalicylic acid
2. Glacial acetic acid

Method:

1. 2ml of clear urine in a test tube.
2. If urine neutral or alkaline- add one drop of glacial acetic acid.
3. Add 2-3 drops of 3-5% sulphosalicylic acid.
4. Check turbidity against dark background.

Observations:

Negative : No cloudiness.

Trace: Barely visible cloudiness.

1+ : definite cloud without granular flocculation.

2+ : heavy and granular cloud without granular flocculation.

3+ : dense cloud with marked flocculation.

4+ : Cloudiness with precipitation

Glucose test of urine

Method

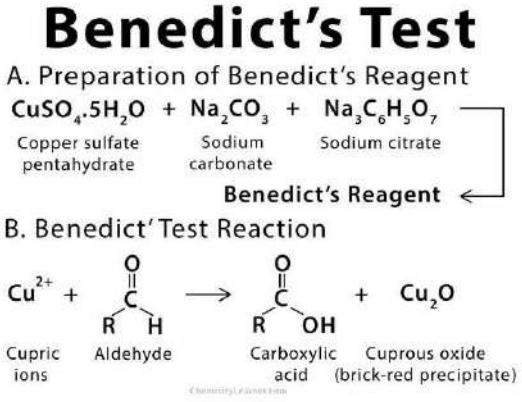
Benedict's Test

Objectives of Benedict's Test

- To determine the presence or absence of reducing sugar in the solution.
- To determine the glucose concentration in the solution quantitatively.

Principle of Benedict's Test

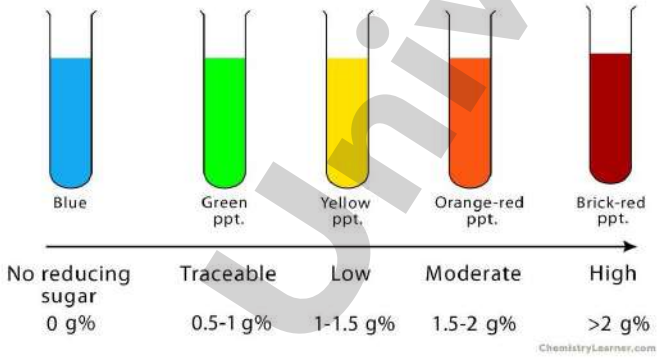
Benedict's test is performed by heating the reducing sugar solution with Benedict's reagent. The presence of the alkaline sodium carbonate converts the sugar into a strong reducing agent called enediols. During the reaction, enediols decrease the cupric particles (Cu^{2+}) present in Benedict's reagent to cuprous particles (Cu^{+}) which appear as red copper oxide (Cu_2O) which is insoluble in water. When Benedict's reagent solution and reducing sugars are heated together, the solution changes its color to orange-red/ brick red precipitate. The red-colored cuprous oxide is insoluble in water and hence, separate out from the solution. When the concentration of the reducing sugar is high in the solution, then the color becomes more intense (reddish) and the volume of the precipitate increases in the test tube making it clearly visible.



The procedure of Benedict's Test

1. Pipette out 2 ml (10 drops) of Benedict's reagent and placed it in the clean test tube
2. Approximately 1 ml of sample (urine) is added to Benedict's reagent.
3. The test tube is placed over the boiling water bath for 3-5 minutes (can be heated directly over flame).
4. Observe for color change in the solution of test tubes or precipitate formation.

Observation:



Ketone test of urine

Method name :Rothera's test

Rothera's test Definition

- Rothera's test is a type of laboratory test which is performed for the qualitative detection of ketone bodies in urine.
- During the "ketosis" three ketones bodies or acetone bodies are found in the urine which are the products of fat metabolism, known as acetone (2%), acetoacetic acid (20%) and beta-hydroxybutyrate (78%).
- During starvation and uncontrolled diabetes, the Ketone bodies are synthesized within the liver to re-utilized energy. These are types of acid that can results in metabolic acidosis in uncontrolled diabetes.

- If the rate of ketone bodies production exceeds, the excess amounts of ketone bodies will be eliminated through the urine and the condition is termed as ketonuria. Acetone is volatile and also excreted in breath.
- Ketosis may be correlated with diabetes mellitus termed Diabetic ketoacidosis, or it may be due to starvation, persistent vomiting and high fat and low carbohydrate diet.
- There are present different methods for the detection of ketones in urine such as Rothera's test, Gerhardt's test, Lang's test, Lindeman's test, Han's test, and Tablet test. All the tests used for the detection of ketonuria are based on the principle of Rothera's nitroprusside test.

Aim of Rothera's Test

To detect the presence of Ketone bodies within the supplied urine sample.

Rothera's Test Principle

Acetoacetic acid and acetone react with an alkaline solution of sodium nitroprusside to form a purple-colored complex. This method can detect above 1-5 mg/dl of acetoacetic acid and 10-20 mg/dl of acetone. Beta-hydroxybutyrate is not detected.

Requirement for Rothera's Test

- **Specimen:** Urine
- **Glassware:** Test tubes, pipette.
- **Rothera's powder:** Sodium nitroprusside = 0.75 gm, Ammonium sulphate = 20gm (Mix and pulverize).
- Liquor Ammonia (Ammonium hydroxide)

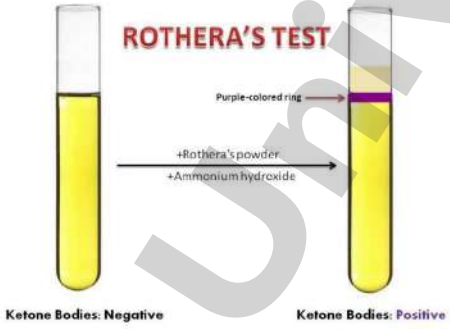
Rothera's Test Procedure

1. Take a clean test tube and add 5 ml of urine to it.
2. Transfers 1 gm of Rothera's powder mixture within the test tube and mix well.
3. Add 1-2 ml of concentrated ammonium hydroxide to the urine sample within the test tube. It will create a thin layer over the urine sample.

4. Observe the pink-purple ring at the interface.

Results

- **Positive Test:** If a purple permanganate-colored ring is immediately formed at the interface, means Ketone bodies are present.
- **Negative Test:** If no permanganate colored ring is formed at the interface, means ketone bodies are absent within the test sample.



Precautions

- Wash the apparatus before and then after the experiment.
- Carefully manage all the chemicals within the laboratory.
- Don't touch the urine sample during the experiment.
- Holds the test tubes by using test tube holders.
- Use a clear and clean test tube, make sure the test tubes are free of any dirt and chemicals because this will give a false result.
- Place all the apparatus in their respective place after the completion of the experiment.

Occult blood test of urine

Haematuria:

Denotes the presence of red blood cells in urine. It is seen in various renal disorders, infectious or neoplastic or trauma related to any part of urinary tract.

Hemoglobinuria: is the presence of blood pigments in the urine without the presence of red blood cells. It is associated with certain hemolytic anemia's that cause hemolytic anemia, transfusion reactions, malaria, and paroxysmal nocturnal hemoglobinuria

Test name : Benzidine Test

PRINCIPLE : Heme acts as a catalyst when hydrogen peroxide is mixed with benzidine.

REAGENTS A: Saturated solution of benzidine in glacial acetic acid
B: Hydrogen peroxide

PROCEDURE

1. Mix equal parts of A and B in a test tube and an equal amount of the mixed reagent.

RESULTS:

A blue color indicates the presence of hemoglobin.
No colour change indicates absent occult blood.

Stool Examination

PRECAUTION BEFORE COLLECTION

1) Patient should avoid the following things for at least 48 hours before collection of stool:
2) Mineral oils, bismuth, non absorbable anti diarrhoeal drugs, antimalarial drugs, antibiotics, etc.
3) Should not have barium swallow examination before stool R/E
4) Avoid iron containing drugs, meat, fish etc for atleast 48 hours before stool for occult blood.
5) In constipated patients use only non residual purgative Stool examination

COLLECTION

1. Pt. is asked to pass stool in a clean container.
2. Stool should be collected in a steralized, wide mouthed container.
3. Loose/last/portion containing mucus, blood etc is to be collected in a wide mouthed bottle.
4. Should be uncontaminated with urine or any other body secretions.
5. >2gm is required.
6. Properly named and always a fresh sample should be tested.
7. Liquid stool to be examined within ½ hour
8. Solid stool to be examined within 1 hour.
9. If delayed store in a refrigerator.
10. samples of stool within 10 days to exclude false negatives.
11. samples to be examined on alternate days after normal defaecation and 1 sample after a purgative for certain worms.
12. Formalin is the best preservative. It kills the bacteria but ptreserves the protozoa and helminthes.
13. For culture no preservatives to be used Stool examination

Preservation:

- **For Stool Culture (enteric pathogens):**

o Cary-Blair vial (green lid with pink liquid) is preferred for this test and preserves the stool for culture.

- CarefullyopentheCary-Blairvialsothatnoliquidisspilledandnotethelidhasanattachedscoop.
- Use the scoop to break up the stool. Add small portions of the stool to the container with the scoop until

  the level of the liquid reaches the fill line note on the vial's label. Do not overfill.

- Recap the vial tightly and shake well to mix the stool thoroughly in the liquid.
- Store at room temperature until delivered to the lab within 3 days.

o If you do not have a Cary-Blair vial, fresh unpreserved stool is also acceptable. Place stool in a clean, dry container that seals tightly. The provided collection container is acceptable.

Note the collection date and time on the container.
Store at room temperature until delivered to the lab on day of collection. Do not refrigerate or freeze.

For Ova and Parasite (O and P):

o Make sure to read the above preparation information carefully.

o PVA vial (blue lid with blue liquid) and Formalin vial (pink lid with clear liquid) are used to preserve stool for ova and parasite testing.

o Open and fill one vial at a time to prevent mixing of liquids or spilling of vials.

o Carefully open the first vial so that no liquid is spilled and note the lid has an attached scoop.

o Use the scoop to break up the stool. Add small portions of the stool to the container with the scoop until the level of

the liquid reaches the fill line note on the vial's label. Do not overfill.

o Recap the vial tightly and shake well to mix the stool thoroughly in the liquid.

o Note the collection date and time on the container.

o Repeat process with the second vial.

o Store at room temperature until delivered to the lab within 3 days.

o Note: This Formalin vial can also be used for Giardia EIA and/or Cryptosporidium EIA.

• **For Giardia EIA and/or Cryptosporidium (Crypto) EIA:**

o Formalin vial (pink lid with clear liquid) is used to preserve stool for both of these tests.

o Carefully open the Formalin vial so that no liquid is spilled and note the lid has an attached scoop

o Use the scoop to break up the stool. Add small portions of the stool to the container with the scoop until the level of

the liquid reaches the fill line note on the vial's label. Do not overfill.

o Recap the vial tightly and shake well to mix the stool thoroughly in the liquid.

o Note the collection date and time on the container.

o Store at room temperature until delivered to the lab within 3 days.

o Note: 1 Formalin vial is sufficient for both of these tests.

o Note: If Ova and Parasite testing is also requested, the 1 Formalin vial can be used for both.

• **For Clostridium difficile Toxin (C. diff or CDT):**

o This test requires fresh, unpreserved stool. Formed stool is not acceptable.

o Place stool in a clean, dry container that seals tightly. The provided collection container is acceptable. o Note the collection date and time on the container.

o Store at refrigerated temperature until delivered to the lab within 3 days. Do not freeze.

• **For Helicobacter pylori Stool Antigen (HPSA or H. pylori):**

o Make sure to read the above preparation information carefully.

o This test requires fresh, unpreserved stool. Watery, diarrhea stools are not acceptable.

o Place stool in a clean, dry container that seals tightly. The provided collection container is acceptable.

o Note the collection date and time on the container.

o Store at refrigerated temperature until delivered to the lab within 3 days.

o If the specimen cannot be delivered to the lab within 3 days, the specimen may be frozen for up to 1 month.

For Fecal Occult Blood:

o Make sure to read the above preparation information carefully.

o If requested 2 or 3 times, collect only 1 specimen per day on consecutive days. o Occult blood test cards are preferred for this test.

- Open the flap on the patient name side.
- Place a small amount of stool on one end of the applicator stick.
- Apply at hinsmearinsideboxA.
- Reuse the applicator stick to obtain a second sample from a different area of the stool.
- Apply at hinsmearinsideboxB.
- Letcardairdrybeforeclosingthecover.
- Use tab closure to secure cover over sample area.
- Store the card at room temperature, protecting from heat and light, until delivered to the lab within 7 days.

o If you do not have an occult blood test card, fresh unpreserved stool is also acceptable. Place stool in a clean, dry container that seals tightly. The provided collection container is acceptable. ♣ Note the collection date and time on the container.

♣ Store at refrigerated temperature until delivered to the lab within 3 days. Do not freeze.

• For Fecal WBC (fecal white blood cells or fecal leukocytes):

o This test requires fresh, unpreserved stool.

o Place stool in a clean, dry container that seals tightly. The provided collection container is acceptable. o Note the collection date and time on the container.

o The specimen should be tested as soon as possible, preferably within a few hours.

o Store at refrigerated temperature until delivered to the lab on day of collection.

• For Fecal Fat:

o These tests require fresh, unpreserved stool.

o Place stool in a clean, dry container that seals tightly. The provided collection container is acceptable. o Note the collection date and time on the container.

o Store at refrigerated temperature until delivered to the lab within 3 days. Do not freeze.

• For Fecal Reducing Substances:

o This test requires fresh, unpreserved stool.

o Place stool in a clean, dry storage cup that has a screw cap. The provided collection container is not acceptable. o Note the collection date and time on the container.

o The specimen should be frozen immediately.

o Store frozen until delivered to the lab within 3 days.

Occult Blood test

Test name: Benzidine test

Principle:

Perioxidase action of hemoglobin in blood converts hydrogen peroxide to water and nascent oxygen. This oxygen oxidizes benzidine in acid medium to form green to blue coloured complex

Procedure:

1. Take pinch of benzidine powder in a small test tube
2. 2 to 3 drops of glacial acetic acid and mix well
3. 1.0 ml of hydrogen peroxide and mix well
4. Place small quantity of stool on a clean dry glass slide
5. Observe change in colour

Interpretation:

– Trace- faint blue colour (after 1 minute)

– + : Definite blue green (slowly)

– + + : Green blue (rapidly)

– + + + : Blue (almost immediately)

– + + + + : dark blue (immediately)

Microscopic Examination

Requirements:

Glass slide

Coverslip

Normal saline

Lugol,s iodine solution

Saturated saline solution

Small bottles

Procedure

Method to prepare the stool smears:

Saline and iodine Direct wet preparations:

Saline wet preparation:

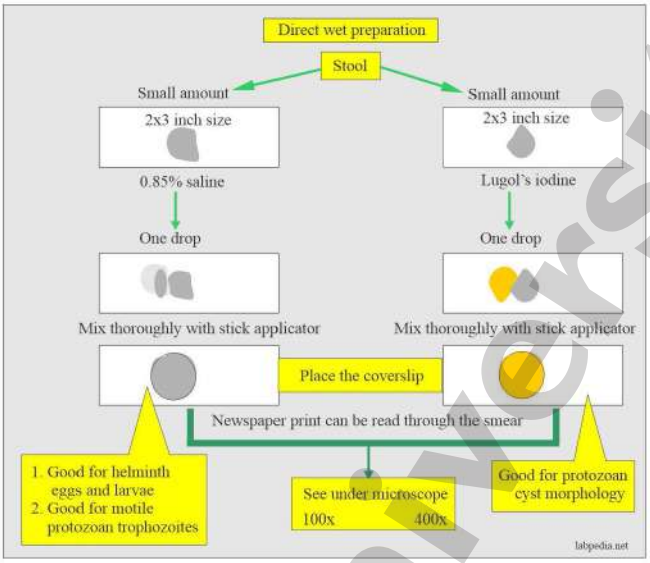
1. Take one drop of 0.85% saline.
2. Take a small amount of stool and mix well.
3. The smear should be thin so that you can see the newsprint under the slide.
4. Put cover glass and see under the microscope 100x and 400x objective.
    1. This is best to see helminth eggs, larva, and trophozoites.

Iodine wet preparation:

This is also called wet preparation.

Iodine solution consists of:

1.
    1. Potassium iodide (KI) = 10 grams
    2. Iodine powder crystals = 5 grams
    3. Distilled water = 100 ml
    4. **Procedure** to make Lugol's iodine solution:
        1. Dissolve KI in D.water.
        2. Slowly add iodine crystals.
        3. Shake the solution gently until they dissolve.
        4. Filter the solution before use, and this is the stock solution.
        5. Dilute the stock solution 1:5 with D. water. Make this working solution before use.
2. Take a drop of Lugol's iodine solution.
3. Take a small amount of stool and mix it well.
4. Make a thin smear.
5. Put the cover glass on it and gently press it to get an evenly thin smear.
6. See under 100x and 400x objective lenses.
    1. Too weak iodine solution; in that case, organisms will not stain properly.
    2. Too strong iodine solution will clump the stool.



Concentration method

The main aim of the concentration method is to remove the debris. Also, when the parasite is low in number.

There are three methods used for the concentration of stool:

1. Formalin-ethyl-acetate concentration method.
2. Zinc-floatation method.
3. Sheather sugar floatation method.

The formalin-ethyl-acetate concentration:

The formalin-ethyl-acetate concentration method is most commonly used.

This method recovers the helminth eggs and larvae, to a lesser extent, trophozoites.

Principle: This is based on specific gravity. After centrifugation, the stool's parasites are heavier and settles down at the bottom as sediments. Debris is lighter and rises to the upper layers.

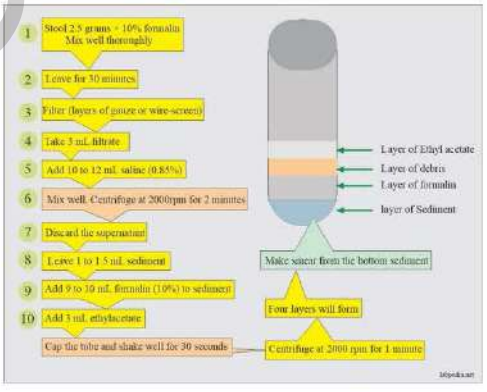
Advantages are:

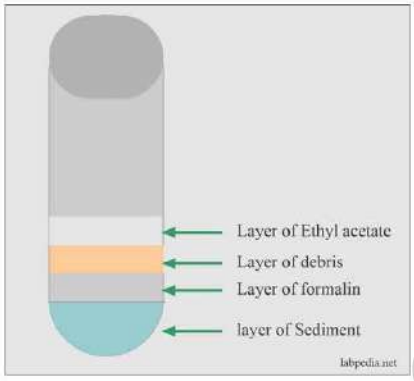
1. It is easy to prepare the solution.
2. This is inexpensive.

3. The procedure is easy to perform.
4. There is a rare distortion of parasite forms (eggs).

The procedure of formalin-ethyl-acetate concentration:

1. The stool should be fixed in formalin for at least 30 minutes.
    1. Take 2 to 5 grams of the stool and mix thoroughly in the 10% formalin.
2. Filter the above stool in the formalin.
    1. This can be done by two layers of gauze or a wire screen and collect around 3 mL.
3. Add 10 to 12 mL of 0.85% saline and mix it well.
4. Centrifuge for 2 minutes at 2000 RPM (or 2500 RPM).
5. Discard the supernatant and leave 1 to 1.5 mL of the sediment.
    1. If the supernatant is cloudy, then repeat the above steps of saline.
6. Add 9 mL of 10% formalin to the sediment.
7. Now add 3 mL of ethyl acetate.
8. Cap the test tube and shake well for 30 seconds.
9. Centrifuge the tubes for 1 minute at 2000 RPM.
10. Four layers will form. The bottom is the sediment that is needed to prepare the smear.





11. Remove the debris with a wooden applicator stick. Decant the upper three layers carefully and leave the sediments in the test tube.
12. Clean the sides of the test tube with a swab.
    1. Giardia cyst may stick to the side of the test tube.
13. Add a few drops of the formalin and mix the sediment thoroughly. This will preserve the sediment.
    1. Now, we can make the smears in saline and iodine wet preparation.
14. Examine under the microscope.

Zinc sulfate floatation method:

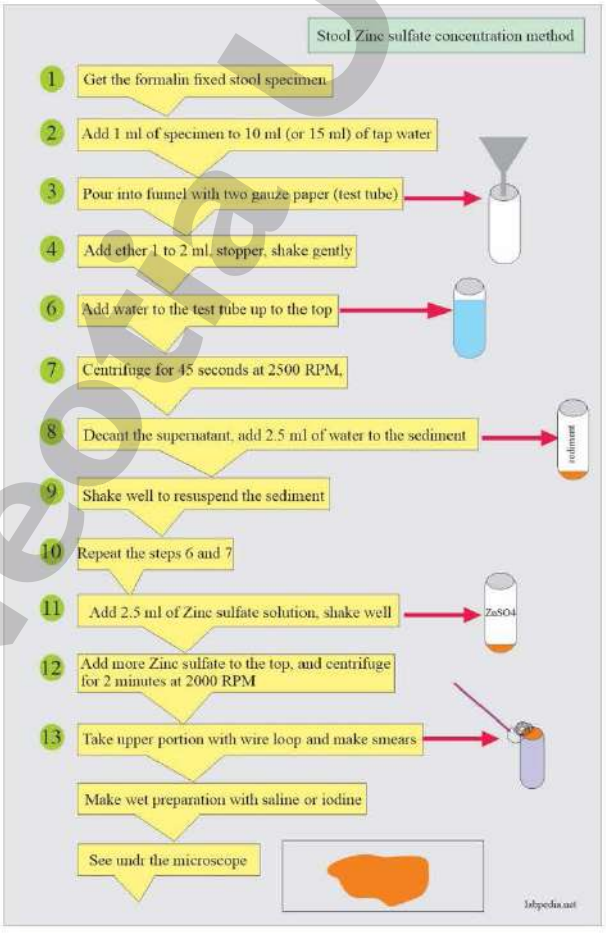
Some authors believe it a superior method for concentration and identifying eggs and protozoan cyst.

The parasites are lighter and float on the surface, while the debris settles at the bottom.

Procedure:

1. Fix the stool in the formalin.
2. Make a dilution of the above specimen (1 mL) with tap water as 1:10 to 15.
3. Pour above suspension through a funnel that has two layers of gauze in a small test tube.
4. Add 2 mL of ether to the above test tube with a stopper and gently shake.
5. Now add water to the above test tube to the top just 1 cm from above.
6. Centrifuge at 2500 rpm for 45 seconds.
7. Decant the supernatant.

8. Add 2.5 ml of the water to the sediment, shake well to resuspend the sediment—repeat steps 5 and 6.
9. Add 2.5 ml of the zinc sulfate (ZnSO4) to the sediment to resuspend it.
10. Add zinc sulfate solution to the top of the test tube, leaving only 1 to 0.5 cm open top.
11. Centrifuge the test tube for 2 minutes at 2000 RPM.
12. Take the surface material with a wire loop; this is the material where you can see the parasites.
13. Make wet preparation with saline and iodine.
14. Examine under the microscope.



Semen analysis

Collection Procedure:

1. The semen specimen should be collected after 2-7 days of sexual abstinence unless otherwise instructed by your physician.
2. The specimen **must** be collected at one of the Patient Service Centers listed above.
3. Collect the specimen by masturbation into a sterile container (containers are provided by the
   laboratory). Since the volume of semen produced may be significant to diagnosis, it is important to submit the entire specimen. Be sure the container lid is closed tightly.
4. Label the container with your name, date of collection and exact time of collection.

In addition the information below is needed to properly evaluate the results of this test. If you have any question about how to answer the questions, please ask your doctor or a Sonora Quest Laboratories employee. Please mark down the number of days of abstinence (days since last ejaculation)

Method of collection was masturbation (REQUIRED)

Other

1. A period of abstinence is important as it affects both the quantity and motility of spermatozoa.
2. Ordinarily abstinence for 3-5 days is adequate.
3. The specimen should be collected in the morning to allow sufficient time for its analysis.
4. Masturbation is the ideal method for producing the semen specimen.
5. Condoms must never be used for collection of semen by intercourse
6. Note the volume, colour, appearance and the pH.
7. A clean, dry, wide-mouth glass or plastic jar should be used as semen container.
8. Its lid must not be rubber lined. Detergents, water and rubber are injurious to sperms.
9. Specimen should be transported to the laboratory at a temperature as close to 37°C as possible and delivered to the laboratory in less than 2 hours.

SPERM COUNTING

1. Place a drop of semen on a clean glass slide.
2. place a cover slip over it.
3. Examine the slide under the high power objective of a microscope.
4. make a visual assessment of the sperm count and to determine the need for any dilution.
5. Dilution
6. The diluent used is 3.5% buffered formal saline
7. Normally 1 in 20 dilution is made by adding 50 µl of well-mixed and liquefied semen to 950 µl of diluent .
8. Improved Neubauer chamber (Haemocytometer) is used for counting
9. The diluted semen is carefully mixed and the chamber is charged using a Pasteur pipette.
10. The chamber is then examined by using x10 objective of microscope.
11. Sperms are counted in the four large corners and one large central square.
12. It is important that loose tails and germinal cells are not counted.
13. At least 200 spermatozoa must be counted If these are not available in these 5squares, more squares must be counted.
14. Calculation:- • Where • C = Count in 5 large squares • D = Dilution factor • V = Vvolule of 5 large s1ures • 1000 = To convert mm3 into ml

ASSESSMENT OF SPERM MOTILITY

1. Assessment of motility must be performed soon after production of sample, 3 and 6 hours later.
2. Both motile and immotile sperms are counted at least in 5 fields with a minimum count of 200.
3. The count should be performed in duplicate and the average recorded.
4. Only forward movement of the sperms is taken as positive.
5. Percentage motility is then calculated.

6. The sperm count can be calculated using the

Formula:

Motile sperm count= Sperm count/ml % motility semen vol/100.

More objective results can be obtained by following procedure.

About 30 min after collection transfer the semen in a capped tube.

Gently mix by inverting the tube several times.

Pipette one drop of semen onto a clean glass slide; place a clean cover slip over it.

Observe with a x40 objective and estimate the percentage of spermatozoa moving at following speeds:

Grade 0: No movement at all

Grade 1: Moving with no forward progression.

Grade 2: Moving with slow and wandering movement.

Grade 3: Moving rapidly in almost straight line

Grade 4: Moving with high speed in straight line

Calculate a motility score by adding up the product motility grade and percentage of spermatozoa in that grade. Example is as under

Normal motility score for spermatozoa is ≥150.

Motility depends upon temperature. At 37°C only 50% sperms are motile after 3 hours. Exposure to cold: Return of sperm motility after placing the semen sample for 30 min in the incubator is diagnostic of reduced motility due to cold. Infection: Manifested by the presence of excess white cells or bacteria. Bacterial culture will help.

Fructose in semen (colorimetric method)

FRUCTOSE IN SEMEN

Colorimetric Method **25 Tests**

PRINCIPLE :

Fructose forms a pink color when heated with resorcinol in the presence of hydrochloric acid, which can be directly measured photometrically .

SAMPLES :

Proceed immediately with the sample since the fructose decomposes. If the determination is to be performed a few days later , freeze the sample . (stability : several days) or add sodium fluoride (stability at room temperature : 24 hours)

REAGENTS :

1.	Standard Fructose	300 mg / dL (16.6 mmol/L)
2.	Trichloroacetic acid (TCA)	1 mol / L
3.	Resorcinol	9.0 mmol / L
4.	Hydrochloric acid	9.0 mol / L

-Reagent 3 causes severe burns. Keep container tightly closed. Keep away from sources of ignition Avoid contact with skin and eyes.
-Use safety pipettes or pipette aids when pipetting hydrochloric acid and resorcinol solution

STABILITY :

Store in dark place. The reagents are stable at + 15°C to + 25°C until the expiry date stated on the kit .

PROCEDURE :

Deproteinization

Pipette into centrifuge tubes :

	Standard ml	Sample ml
Standard (R1)	0.05	-
Sample	-	0.05
TCA (R2)	0.5	0.5

Mix well. Let stand for 10 min. at room temp . Centrifuge tubes at 3000 r . p. m for 10 min. Take an aliquotes from each as follow :

	Blank ml	Standard ml	Sample ml
Standard mixture	-	0.05	-
Sample supernatant	-	-	0.05
Resorcinol (R3)	0.1	0.1	0.1
Hydrochloric (R4)	1.0	1.0	1.0

Mix well, place into a boiling water bath for exactly 5 min. Allow to cool in cold water. Measure the absorbances of the standard ($A_{Standard}$) and the sample (A_{Sample}) against the blank. at 495 nm (490 – 500 nm). Color stable for 2 hours . Linearity up to 1000 mg / dl .

CALCULATION :

Fructose Concentration

$$= \frac{A_{Sample}}{A_{Standard}} \text{ X Standard Conc.}$$

Conversion in mmol / L : mg / dL x 0.055 = mmol/L

NORMAL RANGES :

200 – 400 mg / dl (11.1 – 22.2 mmol / L)

REFERENCE :

Foreman, D. et al., Analytical Chem.1973. 56, 584-590

Analysis of CSF

Clinical Significance:

1. CSF examination is carried out in the laboratory mainly for the diagnosis of meningitis.

Specimen Collection:

1. The specimen should be collected by a physician a special trained or nurse

2. The sterile lumber puncture needle is inserted between the 4th and 5th lumber vertebra ta a depth of 4 to 5 cm
3. After the withdrawal of stylet the fluid is collected through the needle into test tubes
    a. Tube-1- About 0.5 ml or few drops of CSF
    b. Tube-2 About 3 to 5 ml of CSF
4. The specimen is difficult to collect, hence once it is collected it is necessary to analyze the specimen carefully and economically.
5. The specimen may contain virulent organisms hence it is necessary to handle it carefully.

Microscopical examination of CSF

Microscopic examination

Normal CSF has no or very few cells present and appears clear. If the CSF sample appears clear, a small drop of undiluted CSF is examined under a microscope and cells are counted manually. If the number of cells present are very few (for example, 5 or less), the laboratory may or may not perform a cell differential (see below). If cells are numerous (such as greater than 5), a differential will most likely be done. To perform a differential, laboratories will often use a special centrifuge (cytocentrifuge) to concentrate the cells at the bottom of a test tube. A sample of the concentrated cells is placed on a slide, treated with special stain, and an evaluation of the different kinds of WBCs present is performed.

However, if the CSF is very cloudy or bloody, which can indicate the presence of many cells, the specimen may be run on an automated cell counter to count the different types of cells present. These samples may be cytocentrifuged, but if there are too many cells present in the centrifuged sample, an accurate differential may be difficult to perform. In those cases, the specimen may be diluted, cytocentrifuged, and then stained.

If cancer is suspected or has been previously diagnosed, the sample is usually cytocentrifuged regardless of the number of cells counted, and a differential is performed.

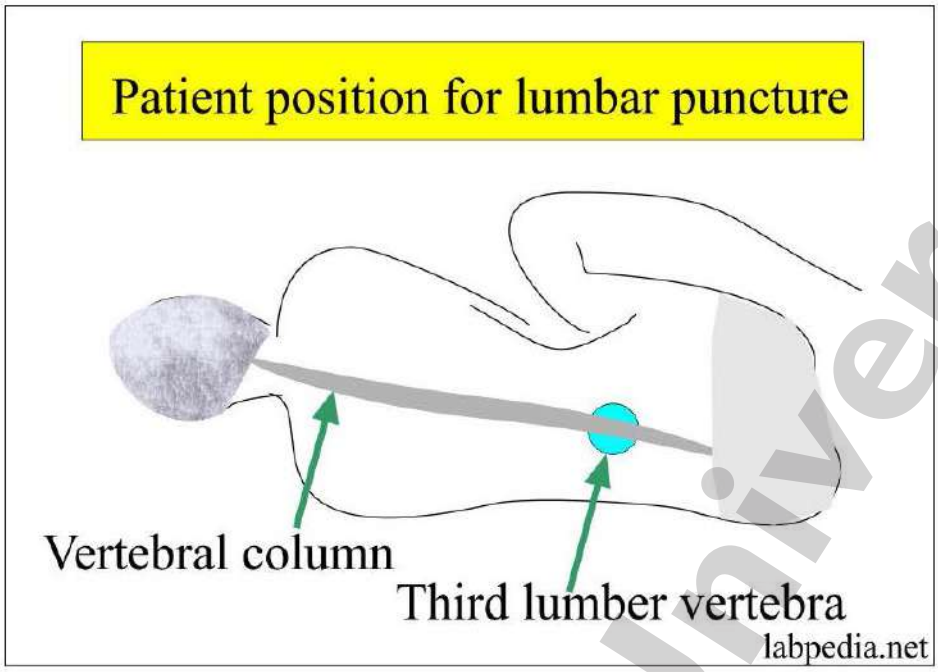
CSF total cell counts

- Red blood cell (RBC) count. Normally no red blood cells are present in the CSF. The presence of red blood cells may indicate bleeding into the CSF or may indicate a "traumatic tap" - blood that leaked into the CSF sample during collection.
- White blood cell (WBC) count. Normally less than 5 cells/μL are present in the adult. A significant increase in white blood cells in the CSF is seen with infection or inflammation of the CNS.
- **CSF WBC differential.** Small numbers of lymphocytes, monocytes (and, in neonates, neutrophils) are normal in a sample of CSF. There may be:
    - an increase in neutrophils with a bacterial infection
    - an increase in lymphocytes with a viral infection
    - sometimes an increase in eosinophils with a parasitic infection
    - abnormal and increased numbers of WBCs may be seen with leukaemia that is present in the CNS
    - abnormal cells may be present with cancerous tumours
    - immune disorders of the CSF, such as multiple sclerosis, may also cause a slight increase in lymphocytes.

There may be an increase in the different types of WBCs with a variety of other conditions, including brain abscess, following seizures or bleeding within the brain or skull, metastatic tumour, Guillain-Barré syndrome, and inflammatory disorders such as sarcoidosis.

Sample

1. The sample is CSF fluid.
2. Three tubes are collected.
    - Don't use the first tube for culture because this is mostly contaminated.
    - The last tube is best for chemistry and microscopy because it is less hemorrhagic or contaminated.
3. The sample is taken from the spinal canal, the most common position is a lumbar puncture.



Position of lumbar puncture for CSF aspiration

Purpose of the test (Indications):

1. To Diagnose the type of meningitis.
2. To diagnose the cause of hemorrhage.
3. This is part of the patient's workup in a coma.
4. This can diagnose cerebral malaria in infants and children.
5. CSF electrophoresis is done to diagnose multiple sclerosis where there is an oligoclonal band.

CSF Examination includes:

1. CSF pressure.
2. Volume.
3. Appearance
4. Biochemical tests include:
    1. Glucose.
    2. Protein.
5. The microscopic examination gives the idea about:
    1. A total number of cells.
    2. Type of cells, Neutrophils, Lymphocytes, or RBC.

3. To rule out the presence of malignant cells.

6. Special stains to find bacteria (Gram stain).
7. Culture.
8. Special studies include:
    1. CSF electrophoresis for the oligoclonal band.
    2. lactate dehydrogenase (LDH).
    3. Lactic acid.
    4. Chloride.
    5. Serology to rule out syphilis.
    6. Glutamine.

CSF pressure

1. **Normal** pressure is 50 to 180 mm of water.
    1. CSF pressure is increased in:
        1. Congestive heart failure.
        2. Obstruction of superior vena cava.
        3. Cryptococcal meningitis.
        4. Intracranial tumors.
        5. Meningitis, of all types.
        6. Cerebral edema.
        7. Subarachnoid hemorrhage.
        8. Thrombosis of venous sinuses.
    2. CSF pressure is decreased in:
        1. Circulatory collapse.
        2. Leakage of spinal fluid.
        3. Severe dehydration.
        4. Spinal subarachnoid block.

Appearance

1. **Normal** CSF is crystal clear like water.
    1. The initial color of CSF is due to:
        1. Inflammatory diseases.
        2. Traumatic tap.

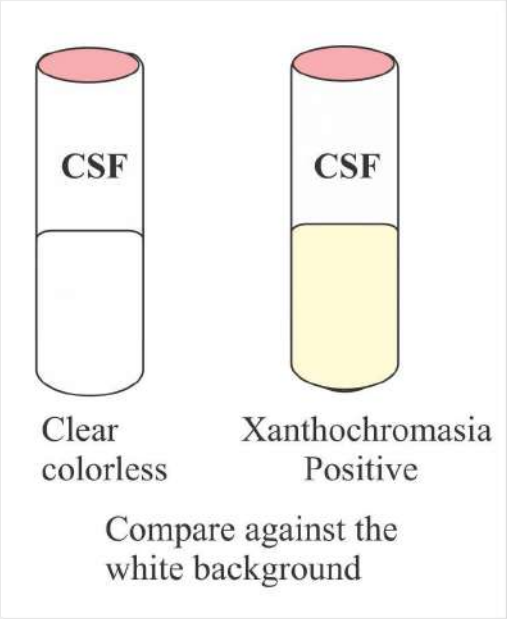
3. Hemorrhage.
4. Tumors.

How to assess the appearance

1. Compare with water.
2. Can read the paper through CSF in a test tube in normal condition.

Causes of various appearances of CSF

1. **Blood** like appearance:
    1. Subarachnoid hemorrhage. If the sample collected in three tubes, then all the tubes will show the same color.
    2. Traumatic tap. Now the third tube will be clear or less in color.
2. **Cloudy** (Turbid) may be due to:
    1. The presence of WBCs.
    2. Increased protein.
    3. The presence of the microorganism.
    4. RBCs.
    5. Contrast media.
3. **Xanthochromia** is pale pink to yellow color and it depends upon the presence of protein. This may be due to:
    1. Increased protein when more than 150 mg/dL.
    2. Bilirubin when > 6 mg/dL.
    3. The presence of methemoglobin.
    4. Systemic carotenemia.
    5. Oxyhemoglobin due to hemolysis of RBCs.
    6. Melanin in meningeal melanoma.
    7. The yellow color may be seen in hemorrhage if this occurs 10 hours to 4 weeks prior to tap.
    8. The yellow color may also be seen if bilirubin > 10mg/dL.



CSF with xanthochromia

4. **The difference between Subarachnoid hemorrhage(SH) and Traumatic tap:**
    1. The traumatic tap may form clots while SH does not form a clot.
    2. Traumatic tap negative for xanthochromia while SH is positive.
    3. An immediate repeat at a higher level will show blood in SH while clear in the case of the Traumatic tap.

CSF Glucose

1. CSF glucose level correlates with blood glucose.
2. This is 60% of the blood glucose.
3. Always advise blood glucose whenever there is a CSF examination.
4. There is a lag time in the blood glucose and CSF, which is roughly one hour.
5. Glucose is utilized by the bacteria more than T.bacilli.
6. There is no effect of viruses on the glucose level.
    1. **Normal CSF Glucose**
        1. Adult = 40 to 70 mg/dL.
        2. Child = 60 to 80 mg/dL.
        3. CSF : Plasma ratio = < 0.5
        4. CSF glucose is less than blood glucose = 60 to 70%.
7. ***Decreased glucose is seen in:***
    1. Acute bacterial meningitis.

2. Tuberculous meningitis.
3. Subarachnoid hemorrhage.
4. Diabetes with hypoglycemia.
5. Malignant tumors with metastases to meninges.
6. Non-Bacterial meningoencephalitis.

8. *Increased glucose level is seen in:*
    1. Diabetic hyperglycemia.

CSF Protein

1. CSF protein is a nonspecific test because it is raised in so many diseases.
2. CSF has a very small quantity of protein because of the blood-brain barrier.
3. Increased CSF protein is caused by:
    1. Increased permeability of the blood-brain barrier.
    2. Decreased resorption by the arachnoid villi.
    3. Obstruction of CSF flow.
    4. Increased synthesis of immunoglobulin in the intrathecal space.
        1. **Normal CSF Protein**
            1. Adult (Lumbar area) = 15 to 45 mg/dL.
            2. Adult (Cisternal area) = 15 to 25 mg/dL.
            3. Adult (Ventricular area) = 5 to 15 mg/dL.
            4. Neonates (Lumbar area) = 15 to 100 mg/dL.

4. **Increased CSF protein is seen in:**
    1. Traumatic tap.
    2. Bacterial meningitis may increase even up to >1000 mg/dL.
    3. Tuberculous meningitis leads to a mild increase which maybe 50 to 300 mg/dL.
    4. Fungal meningitis, the increase maybe 50 to 300 mg/dL.
    5. Viral meningitis, increase is mild < 200 mg/dL.
    6. Subarachnoid hemorrhage.
    7. Nonbacterial conditions like Uremia, and Hypercalcemia,
    8. Dehydration.
    9. Hypercapnia.
    10. Cerebral thrombosis.

11. Diabetic neuropathy.
12. Myxedema.
13. Hypoparathyroidism.
14. Drug toxicity e.g. Phenothiazine, ethanol, and phenytoin.
15. Guillain-barre syndrome.
16. Autoimmune diseases.

5. **Decreased CSF protein seen in:**
    1. Leakage of CSF due to trauma.
    2. Intracranial hypertension.
    3. Hyperthyroidism.
    4. Removal of the large volume of CSF.
    5. Young children between 6 months to 2 years of age.

CSF Gamma globulin

1. The albumin is smaller than the globulins, so the globulins cannot cross the blood-brain barrier.
    1. Any alteration in the permeability leads to the leakage and these globulins are found in the spinal fluid.
    2. Raised level of IgG and increase the ratio of IgG to other proteins (albumin), detection of the oligoclonal band are highly suggestive of inflammatory and autoimmune diseases.
2. This is increased in:
    1. Infections or inflammatory processes like meningitis, encephalitis, or myelitis.
    2. A demyelinating disease like multiple sclerosis.
    3. Neurosyphilis.
    4. Other immunologic degenerative diseases.
    5. Guillain-Barre syndrome.

CSF Chloride

1. The concentration of the chloride in the CSF is higher than the serum because protein concentration in the CSF is low.
    1. The normal concentration is 120 to 132 meq/L.

2. It falls in the CSF in case of bacterial meningitis due to increased proteins in the CSF.

2. This test is not done in routine unless requested.
3. Decreased Chloride is seen in:
    1. Bacterial meningitis.
    2. Tuberculous meningitis.
    3. In low blood chloride level.
4. Its raised level is not neurologically significant, it correlates with the blood chloride level.

CSF lactate dehydrogenase (LDH)

1.
    1. The source of LDH is neutrophils, which fight with the bacteria.
    2. LDH is helpful to diagnose bacterial meningitis, particularly isoenzyme 4 and 5.
    3. It is raised in CNS leukemia, where there is increased cell count.
        1. The nerve tissue in the CNS is also high in the LDH isoenzyme 1 and 2.
    4. It is also raised in Stroke.

CSF Lactic acid

1.
    1. CSF lactic acid does not readily pass through the blood-brain barrier, elevated blood lactic acid levels are not reflected in the CSF.
        1. Chronic cerebral hypoxemia or cerebral ischemia is associated with elevated CSF lactic acid levels.
        2. Raised in cerebral hypoxia or ischemia.
    2. CSF level of lactic acid increases in bacterial and fungal meningitis.
    3. CSF level of lactic acid is normal in viral meningitis.
    4. The lactic acid level can also be increased in patients with some forms of mitochondrial diseases that affect the CNS.

CSF protein electrophoresis

1.
    1. **Indication:**
        1. Electrophoresis is done to find any abnormality of the proteins and immunoglobulins.

2. This is helpful to diagnose:
    1. Multiple sclerosis.
    2. Neurosyphilis.
    3. Autoimmune diseases.
3. **In Myelosclerosis** typical findings are:
    1. Increased total proteins and this is mainly gamma globulins.
    2. There is a discrete sharp band in the gamma region called the **oligoclonal band**.
4. The oligoclonal band may be seen in HIV.
5. Electrophoresis differentiates CSF from serum, where there is an extra band of transferrin in CSF and not in the serum.

CSF Microscopic examination

1. Normal CSF has very few mononuclear cells. Essentially free of cells.
2. Normal cell count :
    1. Adult = 0 to 5 / cmm.
    2. Newborn = 0 to 30 / cmm.
    3. Child = 0 to 15 / cmm.
3. Neutrophils = 0 to 6% of the total cell count.
    1. Lymphocytes = 40 to 80 % of the total cell count.
    2. Monocytes = 25 to 45 % of the total cell count.
4. Neutrophil in bacterial meningitis may increase from 1000/cmm to > 20,000 /cmm.
5. **Increased Neutrophils are seen in:**
    1. Bacterial meningitis.
    2. Viral meningitis.
    3. tuberculous meningitis.
    4. Fungal meningitis.
    5. Amoebic encephalomyelitis.
    6. Abscess in an early stage.
    7. Metastatic tumors.
    8. reaction to repeated lumbar puncture.
6. **Increased Lymphocytes are seen in:**

1. Viral meningitis.
2. Syphilis with CNS involvement.
3. Tuberculous meningitis.
4. Multiple sclerosis.
5. Guillain-barre syndrome.
6. Sarcoidosis of meninges.
7. HIV.
8. Fungal meningitis.
9. Polyneuritis.

7. **Increased Monocytes are seen in:**
    1. Chronic bacterial meningitis.
    2. Multiple sclerosis.
    3. Rupture of brain abscess.
8. **Plasma cells were seen in:**
    1. Multiple sclerosis.
    2. Sarcoidosis.
    3. Acute viral infection.
    4. Infiltrate by multiple myeloma.
    5. Tuberculous meningitis.
    6. Parasitic infestation.
    7. Guillain-barre syndrome.
9. **Eosinophils are seen in:**
    1. parasitic infestation.
    2. Fungal infection.
    3. Sarcoidosis.
    4. Rocky Mountain spotted fever.
10. **Macrophages** may be seen in TB or viral meningitis.

Sputum Examination

Procedure:

1. The cup is very clean. Don't open it until you are ready to use it.
2. As soon as you wake up in the morning (before you eat or drink anything), brush your teeth and rinse your mouth with water. Do not use mouthwash.
3. If possible, go outside or open a window before collecting the sputum sample. This helps protect other people from TB germs when you cough.
4. Take a very deep breath and hold the air for 5 seconds. Slowly breathe out. Take another deep breath and cough hard until some sputum comes up into your mouth.
5. Spit the sputum into the plastic cup.
6. Keep doing this until the sputum reaches the 5 ml line (or more) on the plastic cup. This is about 1 teaspoon of sputum.
7. Screw the cap on the cup tightly so it doesn't leak.
8. Wash and dry the outside of the cup.
9. Write on the cup the date you collected the sputum.
10. Put the cup into the box or bag the nurse gave you.
11. Give the cup to your clinic or nurse. You can store the cup in the refrigerator overnight if necessary. Do not put it in the freezer or leave it at room temperature.