UNIT-1: SPUTUM

Sputum is the secretion from the lower respiratory tract i.e.lungs, trachea and bronchi. It comes out when we cough.Salivaand nasopharyngeal secretions are not part of the sputum.

When there is any disease in the respiratory tract, thenormal characteristic of the sputum is altered. We may find blood in it. Bacteria and other causative organisms of a diseaseof this system can also be detected from the sputum. Manydiseases can be diagnosed from the changes which occur in the consistency, colour and smell of the sputum. For example,when we find microbacterium tuberculosis, we diagnose it to bea case of pulmonary tuberculosis. Lung abscess can be detected by the presence of pus and bacteria in the sputum. Thus,laboratory investigation of sputum is extremely helpful for thediagnosis and management of several diseases.

Normally, sputum is clear to slightly opaque, somewhatfrothy and contains a small amount of mucoid material andsome epithelial cells. In bronchiectasis (dilation of the bronchi)the volume of sputum may exceed 100 ml per day. When aninfection is present, the sputum may be opaque, with change of colour and odour and may contain many pus cells as well asblood.

CHEMICAL COMPOSITION OF SPUTUM

It contains approximately 95 percent water and 5 percent total solids. The solids are primarily carbohydrates, proteins, lipids and deoxyribonucleic acid (DNA). These solids increase in amount with increasing inflammation. The consistency of sputum is dependent mainly on the glycoproteins and on the degree of hydration. The important component of sputum viscosity is sialic acid. The lower respiratory tract is maintained virtually sterile by (1) Alveolar macrophage system and by (2) Mucociliary system. The lysozymes and secretory immunoglobulins contribute towards the antimicrobial activity of sputum.

Why Do We Test Sputum?

Cellular examination of the sputum is important particularly for the identification of pus cells, elastic fibres (significant oflung destruction), eosinophils (usually associated with bronchial asthma), bacteria and fungi. An important recentdevelopment in the examination of sputum is a new preparative and staining technique for the microscopical detection of cancer cells in smears of sputum (the **Papanicolaou's stain** technique).

Specimen Collection

- Specimen
  Early morning specimen (or the entire 24 hour specimen).

- Container
  Sterile wide mouth glass bottle with screw cap (50-60 ml capacity).

- Instructions given to the patient
  1 The mouth should be rinsed well by using water.
  2 The sputum must be coughed up from the lungs or bronchi and placed carefully in the container.

Note : It is necessary to avoid smearing any of the sputum on the outside of the bottle.

Procedure of collection

• The patient should remain standing, if possible.
• He should take a deep breath so that his lungs will be filled with air, as far as possible.
• He should empty his lungs in one breath and at the same time he should cough as hard and deeply as he can.
• Whatever he brings up by coughing, he should spit into the cup and collect it there.

Precautions

• Collect the sputum in the morning (The concentration of abnormal materials as well as the volume of sputum is greater in the morning)
• Check that the required amount of sputum has been collected.
• Liquid frothy saliva, nasopharyngeal secretions are not part of sputum. If the patient gives a specimen which consists mostly, of these secretions, ask the patient to produce another specimen.
• To get accurate bacteriological findings, the sputum should always be collected in sterile containers.

ROUTINE EXAMINATION OF SPUTUM

Requirements

1 Glass slides and coverslips
2 Pasteur pipettes
3 Gram staining reagents
4 Acid-fast staining reagents
5 Wright's stain
6 Microscope

The following are the important tests that are carried out in the laboratory from a specimen of sputum.

Test	Normal finding	Abnormal finding	Clinical conditions
■ **Physical Examination**			
1 Quantity	Morning specimen 2–5 ml, 24 hrs. collection below 100 ml	24 hour collection over 100 ml	Pulmonary edema, lung abscess, bronchiectasis, pulmonary hemorrhage, advanced pulmonary tuberculosis
		Over 500 ml (anchovy color)	Amebic abscess, Rupture of amebic abscess of liver in lungs
2 Color	Clear and colorless	Yellow	Pus and epithelial cells as seen in pneumonic process
		Greenish	Pseudomonas infection, Rupture of liver abscess in lungs
		Rust colored	Due to decomposition of hemoglobin as seen in pneumococcal pneumonia or pulmonary gangrene
		Bright red	Recent hemorrhage, which can follow acute cardiac infarction, pulmonary infarction, neoplasm invasion with rupture of a vessel, pulmonary tuberculosis
		Black	Due to inhalation of dirt, coal dust or due to the decomposition of anthracotic tissue
3 Consistency and appearance	Colorless, watery and opalescent	Serous	Frothy colorless or yellow found in pulmonary edema
		Mucoid	Glassy & tenacious, found in acute bronchitis, asthma, lobar pneumonia, whooping cough
		Purulent	Ruptured empyema and some cases of bronchiectasis
		Tenacious	Thick and viscous due to mucus found in lobar pneumonia and in bronchomoniliasis

Test	Normal finding	Abnormal finding	Clinical conditions
		Mucopurulent	Mucus and pus found in lung cavitation and some cases of bronchomoniliasis
		Bloody	Mitral stenosis, pulmonary infarction, carcinoma of the lungs, pulmonary tuberculosis, acute bronchomoniliasis
4 Odor	No odor	Putrid	Lung abscess, bronchiectasis, gangrene of the lung
		Sweetish	Pulmonary tuberculosis with cavities, bronchomoniliasis, bronchiectasis
		Cheesy	Necrosis of malignant tumors and perforating empyemas
5 Layer formation (specimen placed in a test tube for several hrs)	No formation of abnormal layers	Formation of 2 to 3 layers Top- Frothy mucus Middle- Opaque water material Sediment- Pus, tissue, bacteria, etc.	Bronchiectasis, gangrene, abscess of the lung
■ Minute Macroscopic Examination (Figs. 49.1–49.4)			
A portion of the sputum is observed by placing in a petri dish (thin layer of sputum).			
1 Cheesy masses	Absent	Present	Fragments of necrotic tissue found in tuberculosis, pulmonary gangrene, etc.
2 Bronchial casts	Absent	Present as white branching tree like casts (composed of fibrin)	Fibrinous bronchitis, consolidation of pneumonia
3 Dittrich's plugs	Absent	Present (as yellowish white plugs) from bronchi or bronchioles. Size: From pin head to a bean and have a putrid odor	Found in bronchial asthma, putrid bronchitis and bronchiectasis
4 Lung stones or broncholiths	Absent	Present as calcified stagnant contents of cavities or dilated bronchi	Calcified tuberculous material
5 Sulfur granules	Absent	Present as yellow granular structure	Actinomycosis of the lungs
■ Microscopic Examination of Unstained Sputum			
1 Pus cells	Present, few	Present, many	Presence of inflammation in the respiratory tract
2 Red blood cells	May be present, few	Present, many	Presence of inflammation in the respiratory tract, malignant tumor
3 Heart failure cells	Absent	Present	Passive pulmonary congestion due to mitral stenosis, cardiac decomposition, pulmonary infarction, pulmonary hemorrhage
4 Carbon laden cells	Absent	Present	Anthracosis. Those who inhale large amounts of tobacco smoke or live in smoky atmosphere
5 Curschmann's spirals	Absent	Present	Bronchial asthma
6 Myelin globules	May be present	—	No clinical significance
7 Elastic fibers	Absent	Present	Breaking down of the lung parenchyma
• Crystals			
1 Charcot Leyden	Absent	Present	Bronchial asthma
2 Fatty acid	Absent	Present	Chronic tuberculosis, putrid bronchitis, gangrene
3 Hematoidin	Absent	Present	Hemorrhage in the lungs

(Contd...)

Test	Normal finding	Abnormal finding	Clinical conditions
4 Cholesterol	Absent	Present	Empyema, chronic tuberculosis, chronic lung abscess
5 Leucine	Absent	Present	Rupture of an emphysema in lungs
6 Tyrosine	Absent	Present	
• **Parasites**			
1 *Entamoeba histolytica*	Absent	Present	Amebic abscess of lungs (a rare condition), rupture of liver abscess in lungs
2 Larvae of			
a) *Strongyloides stercoralis* and round worm.	Absent	Present	*S. stercoralis* or round worm infection
b) *Paragonimus westermani* (liver fluke)	Absent	Present	Liver fluke infection

Table 49.2: Differential leukocyte count of Wright stained smear

Detection of	Normal finding	Abnormal finding	Clinical conditions
Neutrophils	Present, few	Increased	Pyogenic infections
Lymphocytes	Present, few	Increased	Early or mild cases of tuberculosis
Eosinophils	Absent	Increased	Asthma and eosinophilic lung
Erythrocytes	Absent	Present	Hemorrhage or inflammatory condition

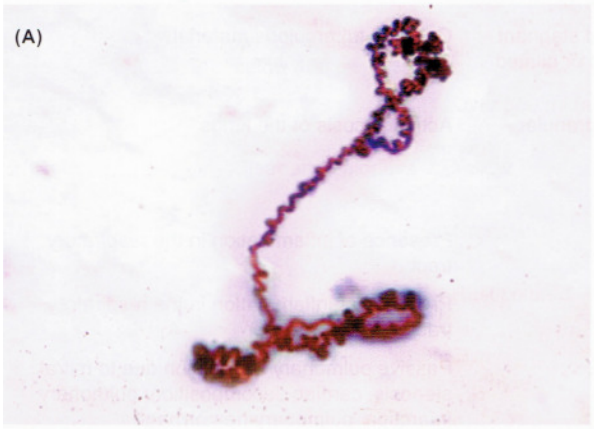

Fig. 49.1: (A & B) Curschmann's spirals.

Fig. 49.2: Bronchial cast.

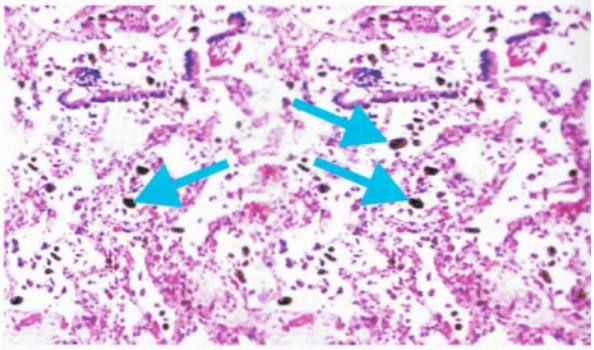

Fig. 49.3: Heart failure cells in chronic passive congestion.

CLINICAL CONDITIONS

Some of the clinical conditions associated with sputum examination are as follows.

Bronchiectasis
Bronchial dilatation of the saccular or cylindrical form. Symptoms may not be present unless a superimposed infection is present. The production of mucopurulent sputum is one of the cardinal symptoms of this disease.

Chronic bronchitis
The bronchioles as well as the bronchi may be inflamed. The inflammation reaction may be either cellular or catarrhal. Macroscopically the sputum is tenacious, white and mucoid in appearance. During superimposed infections, the secretions increase in volume and become purulent yellow green in color.

Lung abscess
Unless the abscess ruptures into a bronchus, there is little or no sputum production. Most abscesses are initiated by tumor, foreign body occlusion, or by bronchial occlusion. These also may originate from a bacterial pneumonia. When rupture occurs, a large amount of bloody, creamy foul smelling pus is suddenly expectorated.

Pneumoconiosis
This term refers to a fibrosis of the lung secondary to inhalation of an organic or inorganic dust. The disease is primarily occupational and its severity differs according to the type of inhaled dust. Macroscopically the sputum is tenacious and can sometimes display the color of the dust inhaled. Pneumonia Initial considerations in the management of pneumonia involve determining whether the infection is bacterial or viral and deciding on the appropriate antimicrobial therapy. The leukocyte count, sputum smear and chest X-ray together with historical data, physical findings and appearance of the patient all contribute to these decisions.

Pulmonary embolism
When the emboli are small the symptoms may be minimum initially. However, with time, dyspnea and pulmonary hypertension develop, and the condition may progress to disabling symptoms. Sputum examination shortly after pulmonary infarction reveals the presence of bright red blood in a very tenacious, mucoid background. As the infarction resolves, the sputum becomes progressively darker in color.

Heart disease
Sputum examination has characteristic findings in some types of heart disease. In acute edema the sputum is abundant, frothy and pink (since the serous exudate pass from capillaries to alveoli). Mi¬croscopically the sputum contains numerous erythrocytes and large hyaline masses. In mitral heart disease the sputum is tenacious and blood is present.
In chronic congestive heart failure the sputum is frothy and rust colored. Microscopic examination reveals, presence of erythrocytes and heart failure cells.

Bronchomoniliasis
It is a disease of the bronchi caused by infection with Candida species and showing signs of bronchitis

and bronchopneumonia.

Asthma

It is a syndrome characterized by narrowing of the smaller bronchi and bronchioles due to spasmodic contraction of the circular muscle of the bronchi. Due to this swelling of the mucous membrane an exudation of mucus takes place. It is a manifestation of the allergic state and occurs in persons who have become sensitized to some foreign substances of protein nature or a bacterial toxin. These substances may enter by inhalation, ingestion or injection. The common air borne allergens are (1) Pollens of grasses and trees (2) Mixed house dust (3) Hairs of animals (4) Face powder (5) Flour etc.

UNIT-2: ROUTINE SEMEN EXAMINATION

GENERAL CONSIDERATION

The analysis of semen is one of the important clinical parameters of gonadal function. A normal semen is practically a guarantee of normal androgenicity. Inadequacies on the part of the male contribute to a significant percentage of infertility problems. Since semen examination is simple to perform, it is often requested before more complicated and expensive examination of the female. It is a part of a comprehensive infertility investigation involving both parents of a barren marriage. In addition to infertility studies, as a part of forensic studies, semen examination may be requested to examine vaginal secretions or clothing stains for the presence of semen in alleged or suspected rape.

The semen specimen collected for routine examination should contain all above mentioned fractions. The use of semen samples obtained from the male urethra following coitus interruptus may result in only a minor part of the total volume of ejaculate, due to the loss of middle sperm rich portion.

The semen analysis is obtained following at least 3 days of abstinence from coitus. Freshly ejaculated normal semen is a highly viscid, opaque and white or greyish white. It has distinct musty or acrid odor. Semen liquefaction takes place within 10 to 30 minutes of collection.

SEMEN ANALYSIS

Following are the various important purposes of routine semen analysis:

1. Evaluation of infertility.
2. Routine follow up of patients who have undergone vasectomy.
3. Artificial insemination.
4. Examination of stored semen specimen. (May be in the case of a husband away from home for a long period and when the wife is undergoing complicated infertility therapy).
5. For men whose future fertility is threatened may be by the need for radiotherapy or chemotherapy in the treatment of cancer.

Specimen Collection

- Length of abstinence
  2-3 days is an adequate length of abstinence for the proper assessment of semen quality.

- Site of production of the semen specimen
  The patient should be allowed (if necessary), to choose where he wishes to produce the specimen. It may be at home or in a room of laboratory or hospital which is quiet, secluded and which will guarantee total privacy.

- Time of production of semen specimen
  A semen specimen is best produced in the morning for an assessment of motility 6 hrs after production. The presence of any'deadline' for semen production should be avoided.

Note

1. It is very important to relax the patient and allow the production of the specimen to take place in surroundings which are the most comfortable both emotionally and physically for the patient.
2. The production of semen for analysis in a situation of stress can lead to inadequate ejaculation. Thismay lead to misdiagnosis towards the evaluation of infertility problem of a couple.

3. The collection of semen specimens must be organized by experienced staff.

Method of Production of Semen Specimen

- The production of semen by masturbation usually results in a specimen that is complete and uncontaminated.
- For those patients who are reluctant to produce semen by masturbation, specimens may be collected by coitus interruptus.

Note

Ordinarily condoms must never be used to collect semen specimens. Most condoms contains spermicidal powder which swiftly obliterates all semen motility.

Containers used for Semen Specimen

All patients should be provided with clean and dry wide-mouth glass bottle or plastic containers (50 ml).

Note

1. Detergents are highly toxic to sperm. Hence there should not be even trace of soap or detergent in the container.
2. Water is equally lethal to sperm (hence the container should be dry).
3. There must not be a rubber lining to the lid as contact between the rubber lid and the semen may result in sperm death.
4. The container should be correctly labeled.

Storage and Transport of the Specimen

- Sperms are easily damaged by either excessive heat or excessive cold.
- Semen specimen should not be left for any length of time in direct sunlight.
- The semen specimen should be brought to the laboratory at close to body temperature.
- It is best but not essential that the semen specimen is delivered to the laboratory within two hours of production.

Number of Semen Specimens that May be Sent forAnalysis

- A reasonable accurate evaluation of only the fertility status of the patient can usually be achieved by assessing at least three samples of semen (collected on different days).

Note

1. Great care must be taken both by the clinician and u by the laboratory to ensure that semen specimens are an accurate reflection of the patient's fertility and not rendered invalid by stress, abnormal or incomplete ejaculation or by the action of physical or chemical damage during their transport to the laboratory.
2. Crystals of spermicidal powder in condom (if it is used by the patient without caring for instructions) and that of talcum powder may be seen in semen (as artifact).

Aspects of Physical Examination of Semen

- Volume of semen and presence of spermatozoa. To express this aspect, following are the related terms used:
    - Aspermia: Means the total absence of ejaculate (a rare phenomenon).
    - Azoospermia: Absence of spermatozoa in semen.
    - Oligospermia (or hypospermia): A reduction in the volume of the ejaculate.
    - Hyperspermia: Increase in semen volume (a very rare phenomenon).

Note

1. There are many causes of infertility that are associated with a low volume ejaculate. As the majority of the seminal fluid comes from the seminal vesicles, or prostate, abnormalities of these glands such as infective and inflammatory disorders will reduce their secretory powers and thus results in oligospermia.
2. The condition of congenital absence of the vas deferens is almost always associated with absence of the seminal vesicle. The semen samples of these patients do not contain sperms and fructose.
3. Abnormalities of ejaculation may also result in oligospermia. Such problems may be the results of stress and nervousness. It can also result from many neurological disorders such as those associated with diabetes mellitus, multiple sclerosis and spinal cord abnormalities.
4. Adequate testosterone production is also needed for normal sexual function and changes in semen volume may also result from endocrine disorders.
5. Ejaculation can also occur in a retrograde manner so that most of the semen ends up in bladder, a phenomenon known as retrograde ejaculation. In this situation, only the secretions of the bulbourethral glands will end up in the semen container resulting in a very low volume ejaculate.

Measurement of Semen Volume

- Semen volume can be measured by using a 10 ml graduated measuring cylinder or a 10 ml graduated centrifuge tube.
- Normal semen volume = 2-6 ml

Note

Volumes as low as 1.0 ml and as high as 10 ml may be regarded as normal.

Color of the Seminal Fluid

Semen is normally pale grey-yellow opalescent fluid. The color of semen may change due to the following reactions:

- Presence of urine
  Urine may occasionally contaminate semen and this may occur in men with disturbances of bladder neck function and ejaculation.
  The presence of urine can be detected by the consistency of semen and by uriniferous odor of semen. A high urea content of the sample may confirm the presence of urine.

Note

Urine has a direct and lethal effect on spermatozoa. The specimens of semen contaminated with urine will contain sperms which are either poorly motile or even completely immotile.

- Presence of blood
  Traces of fresh blood will color semen pink. Larger amounts of blood may impart brighter color to semen.

Note

1. Infection of the seminal vesicles or prostate and in particular infection by tuberculosis are the most common causes of hematospermia.
2. Blood may be present in semen due to trauma and malignancy of the testis.
3. In older men hematospermia is associated with prostatic carcinoma.
4. In situations where bleeding has occurred into genital tract some hours or even days previously, such old blood may color the semen brown.

- Presence of bilirubin
  Presence of bilirubin may impart yellow color to semen. Just as it will color many other tissues and body fluids, bilirubin will also color semen.

Note

The presence of bilirubin in seminal fluid have little effect on semen quality but the associated liver disease can severely disturb spermatogenesis and may affect both the sperm count and motility.

- pH of semen
  The pH of normal fresh semen lies between 7.9 and 8.1. It may slowly fall as the specimen ages (after storage). It is measured by using pH paper, with a range of 6.0-9.0 (The use of pH meter is not essential as such precision is not necessary).

Note

Semen is a very powerful buffer as it contains large amount of protein. However, inflammatory conditions, particularly of the prostate or seminal vesicles may alter the pH of semen.

- Liquefaction of semen
  In the humans, semen is ejaculated in a liquid form and it forms a gel like clot (after ejaculation) within 5-20 minutes.
  The coagulation of semen is dependent on the presence of a fibrinogen-like substance (substrate) manufactured by the seminal vesicle which is acted upon by the enzyme vesiculase produced by the prostate.

Note

1. Absence of the seminal vesicular or of prostatic secretions may, therefore, be associated with absence of coagulation of the semen.
2. Breakdown of the clot and the associated fibrin takes place as a result of the activities of a series of proteolytic enzymes secreted by the prostate. These include proteases, pepsinogen, amylase and hyaluronidase. Further breakdown can occur due to the concomitant presence of transaminase enzymes.
3. If a specimen of semen is brought into the hospital or laboratory from outside, liquefaction by this time should be complete.

- Determination of liquefaction
  Liquefaction is usually assessed visually. Unliquefied semen forms a gel like coagulum. Partially

liquefie semen may contain many small gel like clots. In fully liquefied semen no such clots are seen and the semen appears completely fluid.

Note

a-amylase is used to liquefy unliquefied semen. This can be then used for artificial insemination.

- Viscosity of semen

  Normal viscosity is defined as that which will allow semen to be poured drop by drop out of a container. Viscosity can be quantified by measuring the time it will take for 1drop of semen to leave a standard pipette. Usually a capillary tube of 10 cm in length and which contains 0.1 ml of semen may be used.

Note

1. It has been suggested that hyperviscid semen may fail to coat the cervix and may easily drain out of the vagina after intercourse.
2. Theoretically, a very viscid seminal fluid may impair the ability of the spermatozoa contained within it to escape into the cervical mucus. However, sperm from many hyperviscid semen samples show no difficulty in entering cervical mucus at microscopy.
3. Reduction of viscosity may be achieved by mixing the viscid semen with sperm free seminal fluid from another patient.

ROUTINE EXAMINATION OF SEMEN

Clinical Significance

Low sperm counts are observed when there is sup¬ pression of endogenous gonadotropin production by exogenous estrogens or androgens or by anabolic agents. Such suppression of endogenous gonadotropin is also seen in men with estrogen secreting tumors. Hypothyroidism and hyperthyroidism also cause oligozoospermia. Trauma, infections (mumps, viral, leprosy), irradiation and antimitotic chemotherapy can damage the testis. These patients often have oligospermia or azoospermia. In secondary hypogonadism, serum FSH and LH are decreased. Loss of libido, testicular atrophy and azoospermia can result from a pituitary and hyperthalamic tumor.

Requirements

1. Glass slides and coverslips
2. Test tubes (15 x 125 mm)
3. Pasteur pipettes
4. 1ml and 5 ml serological pipettes
5. Neubauer counting chamber
6. WBC pipette
7. pH papers (Range: 2-12)

Reagents

1. Resorcinol reagent
2. Semen diluting fluid
3. Leishman stain or (0.25 g/dl aqueous basic fuchsin).

Specimen Semen (freshly collected)


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Physical Examination of Semen

Procedure: Note the following

1. Color
2. Volume
3. Viscosity: This is observed by taking the specimen in a Pasteur pipette and by allowing it to pour drop by drop. The specimen of normal viscosity can be poured drop by drop.
4. Note formation of coagulum and the liquefaction time.

Chemical examination

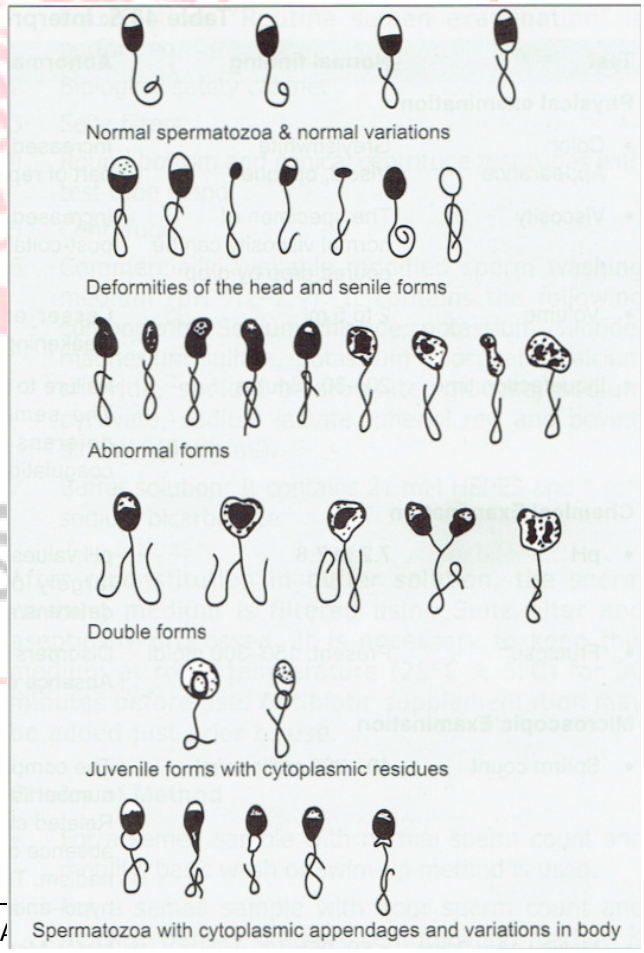
1. Determine pH by using pH paper strip and note down the observed pH.
2. Determination of fructose.

Microscopic Examination of Semen

1. Study of motility of sperms.
2. Determination of sperm count
3. Normal observations

I.	Spermatozoa head caps	Color Light blue
II.	Nuclear posterior	Dark blue
III.	Bodies and tails	Red or pink
IV.	Spermatozoa size	50-70 u
V.	Head size	3-6 p x 2-3 u

4. Observations for other abnormalities
   - I. Abnormally shaped head
   - II. Abnormally sized head (giant or minute)
   - III. Double heads
   - IV. Vacuoles in the chromatin
   - V. Middle section: Absent, bifurcated or swollen
   - VI. Tail: may be rudimentary, double or absent



Test	Normal finding	Abnormal values and clinical conditions
Physical examination		
• Color Appearance	Greyishwhite Viscid, opaque	Increased turbidity may be associated with inflammatory process in some part of reproductive tract.
• Viscosity	The specimen of normal viscosity can be poured drop by drop	Increased viscosity is associated with poor invasion of the cervical mucus in post-coital studies. Absence of viscosity points to reduced cell content.
• Volume	2 to 5 ml	Lesser amounts may arouse suspicion of deficiency and premature weakening by vaginal acidity.
• Liquefaction time	20 –30 minutes	Failure to liquefy within 30 minutes may be associated with infertility. The semen from males with bilateral congenital absence of the vas deferens and seminal vesicles fails to coagulate due to absence of coagulation substrate.
Chemical Examination		
• pH	7.2 to 7.8	pH values less than 7.0 are frequently associated with semen consisting largely of prostatic secretion due to congenital aplasia of the vas deferens and seminal vesicles.
• Fructose	Present, 150–300 mg/dl	Disorders of seminal vesicle may lead to reduction in fructose concentration. Absence of fructose results in immobile sperm formation.
Microscopic Examination		
• Sperm count	40– 300 millions/ml	The complete absence of spermatozoa is called azoospermia. A reduced number is termed oligozoospermia. Related clinical conditions: Mumps orchitis, prostatitis, occlusion or absence of efferent ducts, hypopituitarism, hypogonadotropic hypogo–nadism. The sperm count may be low in estrogen secreting tumors and in hypo–and hyperthyroidism.
• Motility after 2 Hrs. 3 Hrs., 6 Hrs.	= 60–95%	Note: Motile forms decrease by about 5% per hour after the fourth hour following collection. Motility less than 60% may be associated with infertility.
• Abnormal forms	0–20%	More than 20% abnormal forms may be associated with infertility. Following abnormalities in spermatozoa are observed. Heads: Too small, too large, double heads, pointed heads, ragged heads Middle piece: Absent, bifurcated or swollen Tails: Double, curved, rudimentary or absent
Other Findings		
• Pus cells	1 to 2/hpf	Increased number of pus cells: Inflammation due to infection in some part of reproductive system. Infection of seminal vesicle.
• Epithelial cells	1 to 2/hpf	Increased number: Not significant
• Red blood cells	Absent	Tuberculosis of seminal vesicles, rupture of blood vessel, infection of prostate, vitamin C deficiency
• Trichomonas	Absent	Trichomonas infection (motile flagellate with pus cells)

UNIT-3: ROUTINE EXAMINATION OF CEREBRO SPINAL FLUID

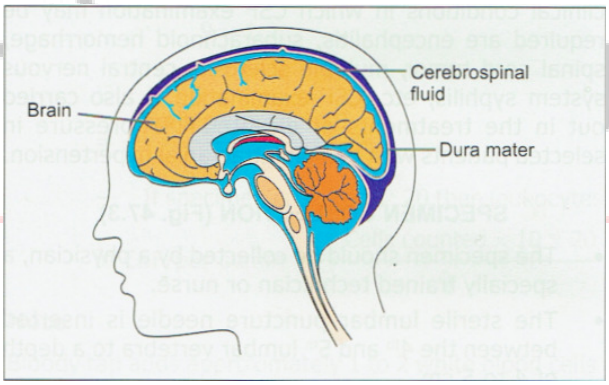
INTRODUCTION

The cerebrospinal fluid (CSF) is formed by selective dialysis of plasma by the choroid plexus of the ventricles of the brain. Through the foramina in the fourth ventricle it then passes into subarachnoidal cisterns at the base of the brain and travels over the surfaces of the cerebral hemispheres. It is finally absorbed into the blood in the cerebral veins and dural sinuses. CSF is present in the cavity that surrounds the brain in the skull and the spinal cord in the spinal column. The volume of CSF (adults) is about 150 ml. CSF performs following functions.

1. It helps to protect the brain and spinal cord from injury by acting like a fluid buffer.
2. It also acts as a medium for the transfer of substances from the brain tissue and spinal cord to blood.

Normal Composition of CSF

1. Color : Colorless
2. pH : 7.3-7.4
3. Appearance : Clear
4. Clot formation : No clot formation on standing
5. Specific gravity : 1.003-1.008
6. Total solids : 0.85-1.70 g/dl
   - Protein : 15 to 45 mg/dl
     (albumin = 50-70%)
     (globulins = 30-50%)
   - Glucose : 40-80 mg/dl
   - Chlorides : 700-750 mg/dl
   - Sodium : 144-154 mEq/1
   - Potassium : 2.0-3.5 mEq/1
   - Creatinine : 0.5-1.2 mg/dl
   - Cholesterol : 0.2-0.6 mg/di
   - Urea : 6-16 g/dl
   - Uric acid0 : 5-4.5 mg/dl
7. Cells : 0-8 lymphocytes/per cumm (pi)
   (Neutrophils: Absent)



Clinical Significance

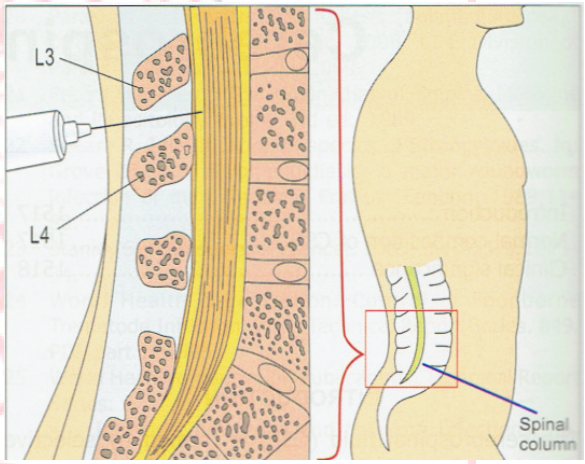
CSF examination is carried out in the laboratory mainly for the diagnosis of meningitis. It is inflammation ofthe meninges, the lining of the skull and covering ofthe brain and spinal column. Meningitis causesdisturbance in the central nervous system. The otherclinical conditions in which CSF examination may berequired are encephalitis, subarachnoid hemorrhage,spinal cord tumor, multiple sclerosis, central nervoussystem syphilis, etc. CSF examination is also carriedout in the treatments of elevated CSF pressure inselected patients with benign intracranial hypertension.

SPECIMEN COLLECTION

- The specimen should be collected by a physician, aspecially trained technician or nurse.
- The sterile lumbar puncture needle is insertedbetween the 4th and 5th lumbar vertebra to a depth of 4 to 5 cm.
- After the withdrawal of stylet the fluid is collectedthrough the needle into two test tubes.
  1. Tube 1: (sterile tube): About 0.5 ml or fewdrops of CSF.
  2. Tube 2: About 3 to 5 ml of CSF.

Note

1. The specimen in tube 1 may be used for bacterial culture (if necessary).
2. Specimen in the second tube is centrifuged.
   a) Supernatant is used for the biochemical tests such as glucose, protein, globulin and chlorides.
   b) Use the sediment for the following purposes,
   i. Prepare 3 smears.
   — Smear 1: for Gram's staining
   — Smear 2: for acid-fast staining
   — Smear 3: for differential leukocyte count
   ii. Prepare wet mount for trypanosoma
   iii. Proceed for India ink preparation in case of cryptococcus infection



Important Precautions

1. The collected CSF specimen must be examinedimmediately (at least within one hour of thecollection).
2. The specimen collected for bacterial culture shouldnot be stored in the refrigerator. (The commonsought pathogen *Neisseria meningitidis*is killed byexposure to cold). The specimen meant for biochemical tests only, may be stored at 2-8°C for 2 to 3 hrs.
3. Cells and trypanosomes are rapidly lysed after thecollection of CSF. Hence urgent analysis of CSF isnecessary.
4. The specimen is difficult to collect, hence once it iscollected it is necessary to analyze the specimen carefully and economically.
5. The specimen may contain virulent organisms,henceit is necessary to handle it carefully.

Routine Examination of CSF

It is carried out by (1) Physical examination (2) Microscopic examination and by (3) Chemical examination.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Physical Examination

1. Observe the specimen and note down observations for the following aspects:
   (a) Color
   (b) Appearance
   (c) Presence of blood
   (d) Presence of clot or fibrin web
2. Use pH paper (range 2 to 10.5) to determine pH.
3. If necessary determine specific gravity by the weight method (weight of CSF/volume of CSF) or by using a hand refractometer.

Microscopic Examination

1. Total leukocyte count
2. Determination of differential leukocyte count

   Additional tests: Wet mount of CSF is prepared in the case of suspected sleeping sickness (trypanosomiasis) and Cryptococcosis. A drop of CSF sediment is observed under the high power objective for motile flagellates. Positive findings may correlate with increased lymphocyte count, presence of mott cells (mononuclear vacuolated large cells) and increased globulins (positive Pandy's test)

CHEMICAL EXAMINATION OF CSF

1. QUANTITATIVE DETERMINATION OF GLUCOSE
2. PROTEINS
3. CHLORIDES

Table 47.1: Laboratory observations of routine CSF examination in various clinical conditions

Clinical condition	Appearance	Cells/cumm (µl)	Glucose	Chlorides	Proteins
Bacterial infection	Cloudy	> 500 neutrophils	Low values (0-40 mg/dl)	Marked decrease 600-700 mg/dl	High values (45-500 mg/dl), increase in globulins
Viral infection	Clear	(10-200) mostly lymphocytes	Slightly low or normal	Moderate decrease	High values (45-300 mg/dl)
Fungal infection (very rare) *Cryptococcus neoformans*	Clear	(0-5) lymphocytes	Low values (0-40 mg/dl)	Normal or slight decrease	Normal
Acute purulent meningitis	Cloudy to purulent clot	Very high count (500-20,000) per cumm, mostly neutrophils	Very low values (0-40 mg/dl)	Low values (600-700 mg/dl)	Very high (45-1000 mg/dl), increase in globulins
Tuberculous meningitis	Cloudy, fibrin web	High count (10-500) mostly lymphocytes	Very low values (0-40 mg/dl)	Very low values (500-600 mg/dl)	High values (45-500 mg/dl), increase in globulins
Acute syphilitic meningitis	Clear or turbid	High count (20-2000) mostly lymphocytes	Low values (0-40 mg/dl)	Normal or slightly decreased	Normal, globulin: Normal
Brain tumor	Clear	0-5	Increased	Normal	Increased, globulin: Increased
Cerebral hemorrhage	Xanthochromic	0-5	Variable	Normal	Increased, globulin: Normal
Encephalitis lethargica	Clear	10-100 all lymphocytes	Slightly increased 80-120 mg/dl	Normal	Normal or increased

UNIT-4: ROUTINE EXAMINATION OF BODY FLUIDS

INTRODUCTION

The other commonly examined body fluids in the laboratory are:

(1) Serous fluids such as
- (a) Pleural (around the lungs)
- (b) Pericardial (around the heart)
- (c) Peritoneal fluids (around the abdominal and pelvic cavities) and

(2) Synovial fluids (around the joints).

These fluids are grouped as extravascular fluids as they exist outside the body vessels. These specimens are collected by the attending physician or the registered nurse or the technician.

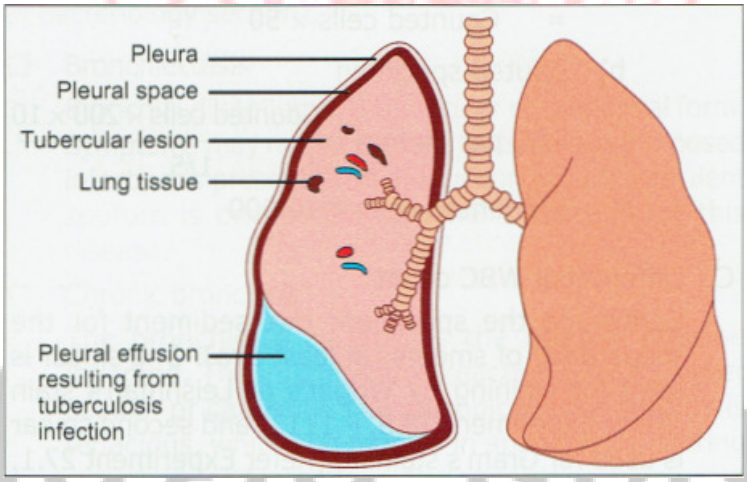
1. **Serous fluids-**

(a) ROUTINE EXAMINATION OF PLEURAL FLUID

Introduction

The pleura is a serous membrane which folds back onto itself to form a two-layered, membrane structure. The thin space between the two pleural layers is known as the pleural cavity, which surrounds the lungs. It normally contains a small amount of pleural fluid, which allows the pleurae to slide effortlessly against each other during ventilation.

Pleural effusion is excess fluid that accumulates in the pleura, the fluid-filled space that surrounds the lungs. Excessive amounts of such fluid can impair breathing by limiting the expansion of the lungs during respiration.



Clinical Significance

Abnormal pleural fluid accumulation or pleural effusion may be caused by

1. Increased capillary permeability
   This may occur due to inflammation. (This condition is associated with increase in protein content over 3.0 g/dl.)

2. Increased hydrostatic pressure
   This may occur due to increased systemic or pulmonary venous pressure (as in congestive heart failure).
3. Decrease in plasma colloid osmotic pressure
   This occurs in hypoproteinemia (protein concentration of fluid may be about 1.0 g/dl).
4. Decrease in lymphatic drainage
   This may occur due to inflammation, fibrosis or tumors (involving mediastinal lymph nodes) and also due to systemic venous hypertension. This condition is also associated with increased pleural fluid protein over 3.0 g/dl.

Specimen Collection

The pleural fluid is collected in the following three aseptic test tubes.

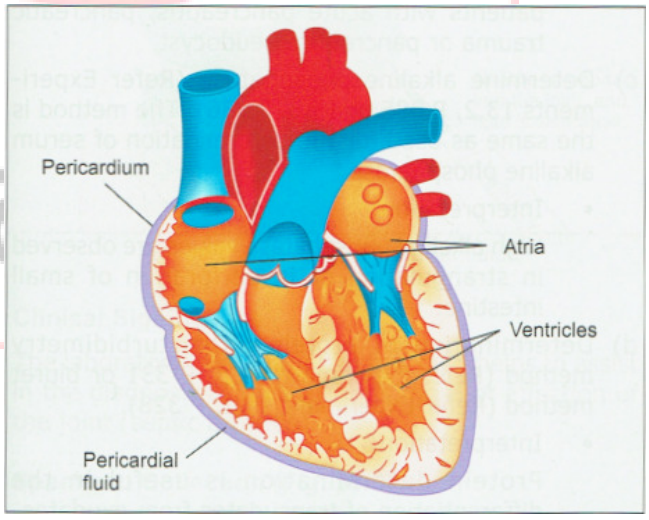
1. Tube containing 15 mg fluoride-oxalate (one part of sodium fluoride and three parts of potassium oxalate).
   Collect 5 ml of the fluid for sugar and protein determination.
2. Tube containing 15 mg EDTA Collect 5 ml of the fluid for microscopic examination.
3. A plain tube (without anticoagulant) Collect about 5 ml for the observation of clot and for bacteriological tests.

Note

1. These fluids are obtained by percutaneous punctures.
2. Since there is risk of accidentally introducing infection into these closed spaces it is necessary to take aseptic precautions.
3. The specimen should be examined as early as possible to prevent (a) Chemical changes, (b) Growth of bacteria and (c) Disintegration of cells.
4. The puncture should be done as atraumatically as possible to avoid mixing of fresh, whole blood with the collected fluid.

(b) EXAMINATION OF PERICARDIAL FLUID

Surrounding the heart is a sac known as the pericardium, which consists of two membranes- The outer layer(the fibrous parietal pericardium) and the inner layer (the serous visceral pericardium). It is the serous visceral pericardium that secretes the pericardial fluid into the pericardial cavity, (the space between the two pericardial layers). The function of pericardial fluid is to reduce friction within the pericardium by lubricating the epicardial surface by allowing the membranes to glide over each other with each heart beat.



Clinical Significance

Under normal circumstances the pericardial sac contains 20-50 ml of clear, straw colored fluid. The

various changes observed in related clinical conditions
are as follows:

- Increased amounts of normal appearing pericardial fluid may be observed in
    a) Congestive heart failure
    b) Inflammation (early stage)
    c) Idiopathic pericarditis.
- Cloudy appearance may be associated with —
    a) Chronic effusions of any etiology (myxedema, post-myocardial infarction syndrome, etc.)
    b) Septic condition (bacterial Inflammation)
    c) Rheumatic or rheumatoid inflammation.
- Blood tinged fluid is seen in traumatic tap.
- Grossly bloody fluid may be caused by —
    a) Bacterial pericarditis
    b) Postmyocardial infarction syndrome
    c) Tuberculosis
    d) Systemic lupus erythematosus
    e) Leaking aortic syndrome

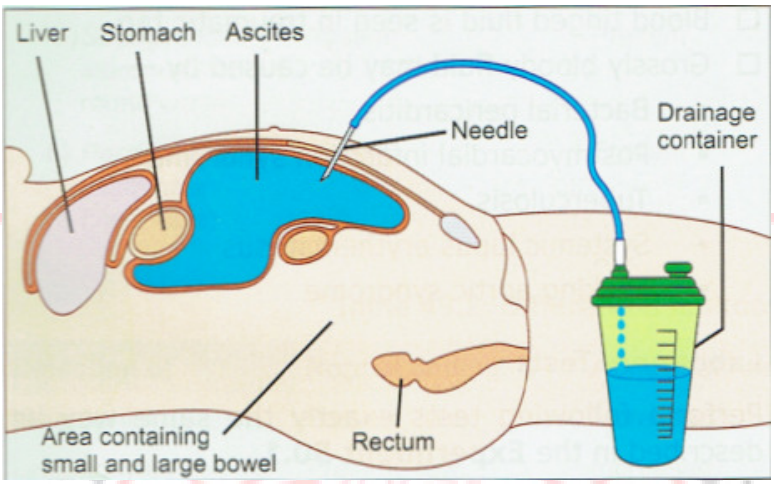
Laboratory Tests

1. Appearance and color
2. Ability to clot
3. Specific gravity
4. Microscopic examination
    a) WBC count
    b) RBC count
    c) Differential WBC count
5. Chemical examination
    a) Protein determination
    b) Glucose determination

(c) EXAMINATION OF PERITONEAL (ASCITIC) FLUID

Introduction

The peritoneum is the serous membrane that forms the lining of the abdominal cavity. It covers most of the intra-abdominal organs. It is composed of a layer of mesothelium supported by a thin layer of connective tissue. The peritoneum supports the abdominal organs and serves as a channel for their blood and lymph vessels and nerves. The outer layer, called the parietal peritoneum, is attached to the abdominal wall and the inner layer, the visceral peritoneum, is wrapped around the internal organs that are located inside the intraperitoneal cavity. The space between these two layers is the peritoneal cavity, which is filled with a small amount (about 50 ml) of slippery serous fluid that allows the two layers to slide freely over each other.



Clinical Significance

Normally the peritoneal cavity contains less than 100 ml of clear and straw colored fluid. Chief complications of abdominal paracentesis is intestinal perforation. The indications of abdominal paracentesis are

(a) Possible ruptured viscous or intraabdominal hemorrhage due to trauma
(b) Acute abdominal pain of unknown etiology
(c) Post-operative hypotension
(d) Ascites of unknown etiology and
(e) Instillation of cytotoxic drugs in ascites due to malignancy.

Specimen Collection

The specimen is collected in fluoride oxalate tube, EDTA tube and in plain tube. (same as pleural fluid)

Physical Examination

A. Note: appearance and color: Following are the various observations and related clinical conditions.

a) Turbid fluid
- (i) Appendicitis
- (ii) Pancreatitis
- (iii) Ruptured or torn bowel due to trauma and
- (iv) Strangulated or infected intestine.

b) Pale yellow (or amber)
- (i) Hepatic vein obstruction
- (ii) Cirrhosis
- (iii) Nephrotic syndrome
- (iv) Congestive heart failure.

c) Greenish
- (i) Perforated intestine
- (ii) Cholecystitis
- (iii) Perforated gall bladder
- (iv) Appendicitis
- (v) Perforated duodenal ulcer.

d) Milky appearance

(i) Parasitic infections
(ii) Nephrotic syndrome
(iii) Carcinoma
(iv) Lymphoma.

e) Bloody fluid

(i) Hemorrhagic pancreatitis
(ii) Ruptured spleen
(iii) Ruptured liver
(iv) Torn mesenteric vessels (trauma).

B. Examine the specimen for appearance of clot
C. Determine specific gravity

Microscopic Examination

Chemical Examination

a) Determine glucose by glucose oxidase method
• Interpretation
Glucose levels in ascitic (peritoneal) fluid may be reduced below 60 mg/dl in about 30 to 50% patients with tuberculous peritonitis and in peritoneal carcinomatosis.

b) Determine serum amylase. The method used is the same as for serum amylase. Instead of serum, use peritoneal fluid.
• Interpretation
Amylase activity in peritoneal fluid is elevated above normal blood levels in 90% of the patients with acute pancreatitis, pancreatic trauma or pancreatic pseudocyst.

c) Determine alkaline phosphatase. The method is the same as used for the determination of serum alkaline phosphatase.
• Interpretation
High alkaline phosphatase values are observed in strangulation or in perforation of small intestine.

d) Determination of proteins:
• Interpretation: Protein determination is useful in the differentiation of transudates from exudates.

2. EXAMINATION OF SYNOVIAL FLUID

Introduction

Synovial fluid is found around the joints such as knee, ankle, hip, elbow, wrist and shoulder. The chemical composition of synovial fluid resembles with that of other body fluids such as serous fluids and spinal fluid. In addition it contains a mucopoly-saccharide, hyaluronic acid, which acts as a binding and protective agent for the connective tissues.

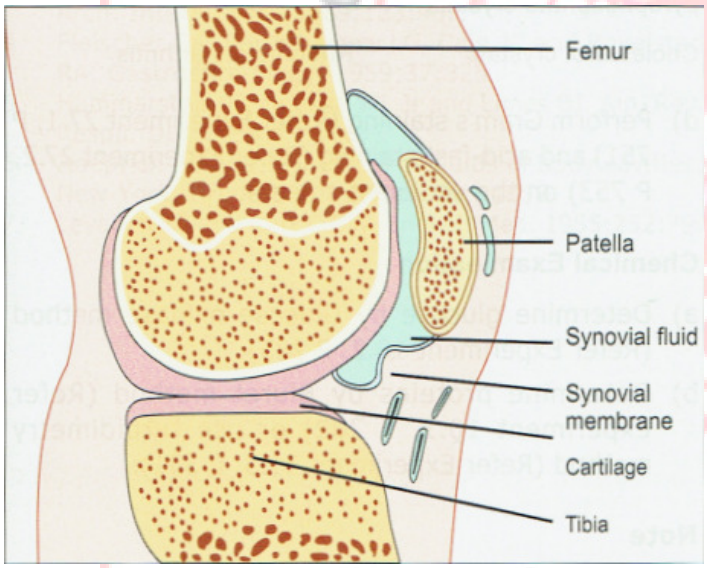
Clinical Significance

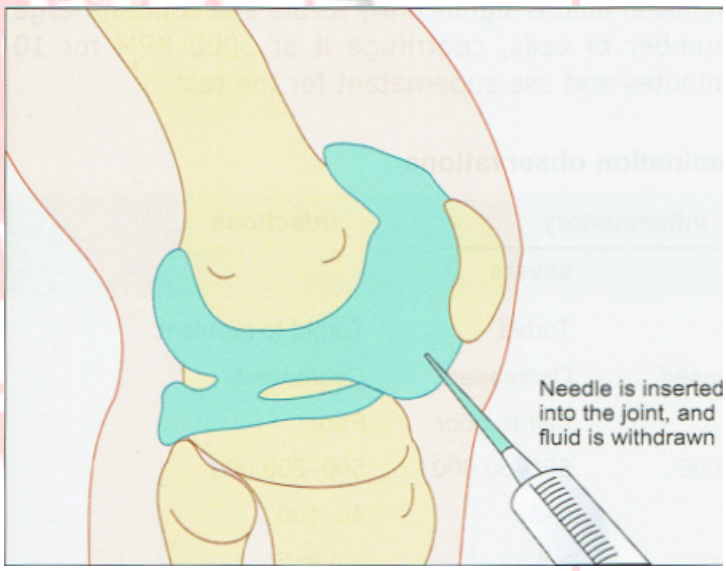
The laboratory examination of this fluid helps to assist in the diagnosis of joint arthritis, gout or infection of the joint (septic arthritis).

Specimen Collection

The specimen is collected in the following sterile tubes.

1. EDTA tube: For cell counts and microscopic examination.
2. Plain tube (without anticoagulant): For gross examination, mucin clot test, evaluation of viscosity and for microbiological and serological tests.
3. Fluoride-oxalate tube for glucose determination.





Physical Examination

A. Note: color and appearance: The various observations are as follows:
   (i) Normal synovial fluid is clear, straw colored and viscous. It does not clot.
   (ii) Turbid appearance: Inflammatory and infected conditions. It may be due to presence of crystals,amyloid and cartilage fragments. (This can be confirmed by microscopic examination.) Highly purulent fluid with increased leukocyte count indicates acute septic arthritis.
   (iii) A gross red or dark brown supernatant (or the presence of black streaks).

Note

Traumatic tap may cause presence of blood in the synovial fluid. In that case the amount of blood will

decrease in the second and the third tube.

B. Viscosity test
Synovial fluid is viscous and the viscosity is due to the presence of hyaluronic acid. The viscosity decreases due to the breakdown of hyaluronic acid by the enzyme hyaluronidase in inflammatory disorders.

Test procedure
Drop the fluid from a syringe and note the length of the tenacious string formed. Use a scale to measure the length.

Note
The normal fluid forms a string of at least 4 cm long. If the string breaks before reaching 3 cm length, the viscosity is lower than normal.

C. Mucin clot test
Hyaluronic acid forms compact clot in the presence of acetic acid. Low concentration of hyaluronic acid does not allow the formation of firm clot.

Microscopic Examination

a) Total leukocyte count
b) Differential WBC Count
c) Prepare a wet coverslip preparation of the synovial fluid and observe first under low power objective and afterwards under high power objective. The various observations are as follows

Observations	**Condition**
Urate crystals	Gouty arthritis
Rhomboid calcium, pyrophosphate crystals	Pseudogout
Cholesterol crystals	Rheumatoid arthritis

d) Perform Gram's staining and acid-fast staining on the sediment smears.

Chemical Examination

a) Determine glucose
b) Determine proteins by Biuret method

Note
For chemical tests, use Synovial fluid instead of serum (plasma or urine). For glucose determination, synovial fluid is significantly turbid and contains large number of cells, centrifuge it at 3000 RPM for 1C minutes and use supernatant for the test.

UNIT-5: ANALYSIS OF URINE EXAMINATION

CLINICAL SIGNIFICANCE:

The oldest of laboratory procedure used in medicine is the inspection of urine for diagnostic purposes.
Routine urine analysis is mainly performed for two purposes.

1. **To find out metabolic or endocrine disturbances of the body.**

For example, a) Presence of bilirubin in urine: metabolic disturbance of bilirubin (hepatic or post hepatic condition).b) Presence of glucose in urine: diabetes mellitus (Deficiency of insulin, an endocrine disturbance).

2. **The second purpose of urine analysis is to detect intrinsic conditions that may adversely affect the urinary tract or the kidneys**.

Diseased kidneys cannot function normally in regulating the volume and composition of body fluids and also in maintaining acid-base balance and homeostasis.Consequently, structural elements, such as leukocytes, red blood cells, urinary tract cells and casts from the lower urinary tract may appear in urine.

THE FORMATION OF URINE

Urine is formed by a summation of these three processes-

(1) Filtration of blood plasma at the glomeruli

(2) Selective reabsorption (sugars, fatty acids, amino acids, saltsand also water).

(3) Secretion of certain substances such as creatinine, potassium, uric acid, organic ions and hydrogen ions by the tubules.

The anatomic unit that carries out these functions is the nephron and each kidney possesses about one million nephrons.A large volume of blood (approximately 1 liter/minute) flows through the kidneys. In 4 to 5 minutes, a volume of blood equal to the total blood volume passes through the kidneys.The glomerular filtration rate in adults is, therefore, about 120 ml/minute.

For a normal adult, the daily average urine excreted is about 1,200-1,500 ml.More urine produced during day than night.

Clinical conditions related to urine excretion:

The clinical conditions related to urine excretion are as follows:

1. **Polyuria** - Abnormal increase in urine volume > 2000 ml/24 hrs.as in diabetes insipidus and diabetes mellitus.
2. **Oliguria** - Decrease in urine volume < 500 ml/ 24 hrs. as occurs in shock and acute nephritis.
3. **Anuria** - Complete suppression of urine formation as in renal failure.

Note: Polyuria can also be observed as a physiological response to increased fluid intake, ingestion of certain diuretic drinks such as coffee, tea and alcohol and ingestion of diuretic drugs, and also due to nervousnessand anxiety.

COMPOSITION OF NORMAL URINE:

- Volume: 600-2000 ml/24 hrs. Average: 1,200 ml.
- Specific gravity: 1.003-1.030
- Reaction: Acidic (pH: 4.7-7.5) Average pH: 6.0
- Total solids: 30-70 g/liter.

Specimen:

Type of specimen: First voided midstream morning urine.

1. The concentration of urine varies throughout 24-hour period. It depends on water intake of a person and partly on his activities. A random urine specimen collected during the day time may be diluted and may not be suitable for detection of certain substances present in low concentration. Hence more concentrated urine is preferred for testing, which can be obtained by collecting first voided morning urine.

2. For urgent routine examination, however, to get general idea of expected pathological condition a random urine specimen may be used.

    - Container used for urine collection
    - Clean and dry wide mouth glass or plastic bottles, with screwcap tops, (capacity, about 250-300 ml).

3. The bottles need not be sterile.

Instructions given to the patient:

1. The patient should be instructed to void directly into the container. During the collection, the initial portion of the urine stream is allowed to escape while the midstream portion is collected.

2. Specimens from infants and young children can be collected in a disposable collection apparatus. It consists of a plastic bag with an adhesive backing about the opening to fasten it to the child so that patient voids directly into the bag. Care must be taken to avoid fecal contamination.

For qualitative tests, the morning urine is useful, however, quantitative tests are performed only on urine specimen collected for 24 hrs.

Preservation:

- All the specimen for routine urinalysis should be examined while fresh (within one hour of the collection).
- When urine is kept for longer than one hour before analysis, to avoid deterioration of chemical and cellular material and to prevent multiplication of bacteria, it should be stored at 2-8°C in a refrigerator.

The expected changes in the composition of urine stored at room temperature are as follows:
— Lysis of red blood cells by hypotonic urine
— Decomposition of casts.
— Bacterial multiplication.
— Decrease in glucose level, due to bacterial growth.

— Formation of ammonia from urea by the action of bacteria (and the nature of urine changes to alkaline).

Urine preservative:

Preservative	Concentration	Limitations
Toluene	2 ml/100 mi urine	It floats on the surface of urine, good for chemical constituents. It is not effective, if bacteria are already present in urine
Formalin	3 drops/100 ml urine	Good for sediments. May precipitate proteins.
Thymol	One small crystal per 100 ml of urine	May interfere with the acid precipitation test for proteins.
Chloroform	5 ml per 100 ml urine	Forms upper layer. It causes changes in the characteristics of the cellular sediment.
Commercial preservative tablets. These release formaldehyde	1 tablet/30 ml urine	Concentration of formaldehyde is controlled, so that it may not interfere.

(1) Urine samples collected randomly during the day are sometimes quite dilute due to increased fluid consumption. Hence tests performed on random urine specimen tend to give a false picture of patient's health.
(2) However, for the detection of glycosuria, postprandial urine (PP sample collected, 2 hrs. after lunch or dinner) is the best.

PHYSICAL EXAMINATION OF URINE

Requirements

1 Pasteur pipettes
2 Ordinary filter papers
3 Measuring cylinder
4 Litmus papers and pH papers (range 2-10.5)
5 Urinometer**:** This is a bulb shapedinstrument that has a cylindrical stem which containsa scale calibrated in specific gravity readings. Thisinstrument is floated in a cylinder containing urine.The depth to which it sinks in the urine indicates thespecific gravity of urine, which is read on theurinometer scale at the junction of the urine withthe air**.**

Procedure

1 Observation of color,appearance,odor and sediment

• Observe the following aspects of the urinespecimen and note down in the note book (a) Color (b) Appearance (c) Odor, and (d) Sediment(if present)

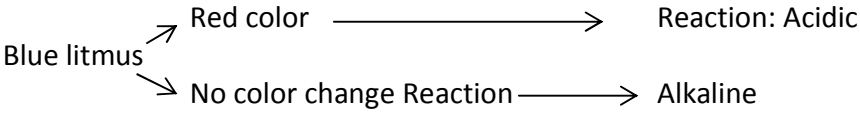
2 Determination of urine volume

• Measure the volume of urine and note it downin the note book.

3 Determination of urine reaction and pH

• Place a drop of urine by using a Pasteur pipette(or a glass rod) on a blue litmus paper and notedown the reaction.

Litmus test



• Place a drop of urine on a pH paper (range 2-10.5) and from the color change, note the pH value.

4 Determination of urine Specific gravity

• Principle of working of urinometer

Urinometer is specifically calibtrated in such away that it sinks in distilled water up to the "0" mark on its stem in level with the surfaceof the water. Thus, specific gravity of distilled water is indicated as 1.000. In dilute urine,when concentration of dissolved ions is significantly low, urinometer sinks more indicatinglow specific gravity compared to concentratedurine (containing more dissolved ions). Urinometer must float centrally in the urine specimen, without touching the sides of the cylinder (or container).

• Procedure:

a) Mix urine well and fill the container threefourth full of urine.
b) Remove all foam by using a filter paper.
c) Float the urinometer in the urine. Rotateit carefully so that it can be prevented from touching the bottom or sides of thecontainer.
d) Note the specific gravity reading from thescale.

CHEMICAL EXAMINATION OF URINE

The routine urinalysis includes chemical testing for (1)Protein (2) Glucose (3) Ketone bodies (4) Occult blood(5) Bile pigments (6) Bile salts and (7) Urobilinogen.

Requirements

Glassware

Centrifuge tubes, Pasteur pipettes, test tubes (10x 75, 15 x 125 and 20 x 150 mm), Beakers (250 or500 ml), graduated pipettes (5 ml) and test tuberacks.

Reagents and chemicals required for chemicalexamination of urine.

1. Benedict's qualitative reagent
   • Use: Determination of urine sugar
   • Preparation
   a) Sodium citrate : 173 g
   b) Sodium carbonate : 100 g
   Place in about 900 ml of distilledwater. Boil for 2 to 3 minutes, cooland add,

c) Cupric sulfate: 17.3 g.
Dissolve and make final volume oneliter. Store in a polythene container.This reagent is stable at roomtemperature (25°C ± 5°C).

2. 3 g/dl sulfosalicylic acid
   • Use: Determination of urine proteins.
   • Preparation
   a) Sulfosalicylic acid : 3.0 g
   b) Distilled water to : 100 ml
   Mix and store in a glass bottle at roomtemperature (25°C ± 5°C)

3. Fouchet's reagent
   • Use: Determination of urine bile pigments
   • Preparation
   a) Trichloroacetic acid : 25 g
   b) Distilled water : 100 ml
   Dissolve trichloroacetic acid and add,
   c) Ferric chloride : 1.0 g
   Dissolve and store in a glass bottle atroom temperature (25°C ± 5°C).

4. Ehrlich reagent
   • Use: Determination of urine urobilinogen.
   • Preparation
   a) Paradimethylaminobenzaldehyde: 2 g
   b) 50% (v/v) hydrochloric acid: 100 ml
   Dissolve and store in an ambercolored bottle.

5. Sulfur powder
   • Use: Detection of urine bile salts

6. a) Benzidine powder
   b) Hydrogen peroxide
   c) Glacial acetic acid
   • Use: Detection of urine occult blood
   Following are the reagents required for theadditional tests:

7. Determination of porphobilinogen
   A) Modified Ehrlich's reagent
   a) Paradimethylaminobenzaldehyde:0.35 g
   b) Cone, hydrochloric acid: 75 ml

Mix well and store in an amber coloredbottle at room temperature.
B) Saturated sodium acetate
Add 100 g of sodium acetate in 100 ml ofdistilled water. Mix well. Store in a plain bottle at room temperature (25°C± 5°C).

8. Determination of phenylpyruvic acid
   A) Magnesium reagent
   a) Magnesium chloride 11 g
   b) Ammonium chloride 14 g
   c) Ammonium hydroxide 20 ml
   Dissolve in distilled water, make finalvolume one liter and store at room temperature (25°C ± 5°C).
   B) IN Hydrochloric acid.
   C) 10 g/dl Ferric chloride.

Nature of the Tests
Qualitative

DETERMINATION OF URINARY PROTEINS

Proteinuria is also found in a wide range of pre-renal conditions which produce it because ofsecondary effects on the kidneys. The proteinuriagenerally disappears when the primary disease iscured. Examples are dehydration, intestinalobstruction, myocardial infarction, intra-abdominaltumors (since there is intra-abdominal pressure onascites) and in fevers.
In postrenal conditions, proteins may be added inurine as it passes along the urinary tract. Lesionsof the renal pelvis, bladder, prostate and urethracan all lead to such a condition

1 The urine should be clear. If it is turbid, it is necessary, to centrifuge it.In that case supernatantis tested for protein and sediment for the microscopic examination. Even after centrifugationif the urine is turbid then filter it.
2 If urine is alkaline, add few drops of glacial acetic acid and make it slightly acidic.

Requirements

1 Test tubes (10 x 75 mm)
2 Pasture pipettes
3 3 g/dl sulfosalicylic acid
4 Glacial acetic acid
5 Bunsen burner

Procedure (A): Sulfosalicylic Acid Test

Principle: If proteins are present in urine, these are present in soluble form. However, when sulfosalicylicacid reagent is added, these proteins appear as white precipitate, due to denaturation by the acidic reagent.Presence of turbidity at the top of the reaction mixture indicates presence of proteins in urine

1 Transfer 3 to 4 ml of centrifuged (or filtered) urine to a small test tube (10 x 75 mm).
2 Add 2 to 3 drops of sulfosalicylic acid on the top ofthe specimen.
3 Observe for turbidity after 5 minutes.

Observation

1 No formation of turbidity at the upper portion of urine: protein absent
2 Formation of turbidity: Protein present
3 If proteins are present grade the result accordingto degree of turbidity as trace, +, + +, + + + and+ + + +.

Procedure (B): Heat Test

Principle: If proteins are present in urine, due to heating, these are denatured and their specific structure gets disrupted. Denatured proteins appearas white precipitate. Presence of tubidity (which persists after the addition of glacial acetic acid),indicates presence of proteins in urine.
1 Place 5 to 10 ml of clear urine in a test tube (20 x 125 mm).
2 Boil the upper portion over a flame.
3 If turbidity develops, add 1 to 2 drops of glacialacetic acid. If the turbidity is due to phosphate precipitation, it will clear.
4 Reboil the specimen.

Observation AfterReboiling the Specimen

1 No turbidity: Proteins absent
2 Presence of turbidity: Proteins present
3 Grade the degree of turbidity as mentioned above.

DETERMINATION OF GLUCOSE

Clinical Significance

A small amount of glucose (2-20 mg/dl) may be present in fasting urine which is not detectable bychemical methods. The presence of chemically detectable amount of glucose in urine is called glycosuria (or glucosuria). The quantity of glucose thatappears in the urine is dependent upon a) Blood sugarlevel b) The rate of glomerular filtration and c) Thedegree of tubular reabsorption

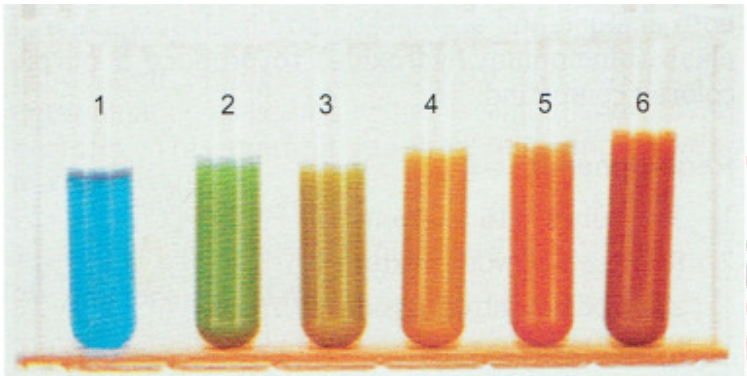
Various clinical conditions related to glycouria are as follows:
1 The normal renal threshold for glucose is 150-170 mg/dl. When the glucose exceeds the renal threshold the tubules cannot reabsorb all of the filtered glucose and then glycosuria occurs. The main reasons for glycosuria is hyperglycemia, i.e. elevatedblood sugar level. Diabetes mellitus is the mostcommon cause of hyperglycemia. Glucose is also found in urine of some hyperglycemia patients withendocrine hyperactivity, that is in hyperthyroidism, in hyperpituitarism and in hyperadrenalism. Injection of the hormones of these glands can also produce glycosuria, the other conditions which may cause glycosuria due to hyperglycemia are myocardial infarction, cerebral hemorrhage, brain tumors, severe liver diseases and whole organ disease of the pancreas.

Name of the test
Benedict's qualitative test

Principle of the test
When Benedict's qualitative reagent (5 ml) is heated with eight drops of urine (about 0.5 ml), glucose present inurine reduces cupric ions present in the reagent to cuprous ions. Alkaline medium is provided to the reactionby sodium carbonate present in the reagent. The original color of Benedict's reagent is blue. It changes togreen, yellow, orange or red, according to the concentration of glucose present in urine.
The test is nonspecific for glucose since the reaction may be brought by other carbohydrates such asfructose, galactose, lactose, and pentoses and also by noncarbohydrates such as ascorbic acid, salycylates,creatine and uric acid. Hence if Benedict's test is positive it is necessary to perform glucose oxidase(uristix) test to confirm whether it is due to only glucose.

Requirements
1 Test tubes (20 X 150 mm)
2 5.0 ml, 10.0 ml graduated pipettes
3 500 ml beaker
4 Pasteur pipettes
4 Benedict's qualitative reagent
5 Bunsen burner.

Procedure
1 Pipette 5.0 ml of Benedict's reagent in a test tube (20 x 150 mm).
2 By using Pasteur pipette, add eight drops (0.5 ml of urine.
3 Heat carefully on the flame of a gas burner (or spirit lamp) or place in a boiling water (in a 500 ml beaker) for 5 to 10 minutes.
4 Cool under tap water or by placing in a beaker containing tap water and observe color of the reagent.
If Benedict's test is positive then it is necessary confirming it by using glucose-oxidase uristix. If glucoseis present then use the above mentioned tabular observations to grade the results.If glucose is absent then it may be necessary to identify the reducing substance present in urine.



1) Sugar absent; 2) Sugar present trace; 3) Sugar present +; 4) ++; 5) +++; 6) ++++

DETERMINATION OF KETONES

Whenever there is inadequate carbohydrate in the diet or a defect in carbohydrate metabolism the body tendsto metabolize increasing amounts of fatty acids. Due to this the otherintermediary products such as ketonebodies also increase in blood. These are (1) Acetone (2) Diacetic acid (acetoacetic acid) and (3) (3-hydroxybutyric acid. (Relative percentage: acetone =24%, diacetic acid = 18-20% and p-hydroxybutyricacid = 76-78%.

Clinical Significance

Diabetes mellitus is the most important disorder in which ketonuria occurs. Detection of ketonuria in apatient with diabetes mellitus is of great significance since a change in insulin dosage or other managementis often indicated. During the periods of gastrointestinal disturbances, acute infections, stress or surgery and whenever the routine management does not controlthe disease, the urine of all diabetic patients shouldbe tested for ketone bodies. Ketonuria also accompanies the other conditions suchas anorexia, fasting, starvation, fever and prolongedvomiting Since in most instances of ketonuria, acetone, diaceticacid and p-hydroxybutyric acid, all are excreted inurine, a test procedure which determines one of these components is generally satisfactory for the diagnosisof ketonuria.

Rothera Test

Principle: Nitroprusside used in this test reacts withboth acetone and acetoaceticacid in the presence ofalkali (ammonium hydroxide) to produce a purplecolored compound.

Requirements

1 Test tubes: (15 x 125 mm)
2 Rothera's powder mixture
a) Sodium nitroprusside : 0.75 g
b) Ammonium sulfate : 20 g

— Mix and pulverize. Store in a wide mouth glasscontainer at room temperature (25°C ± 5°C)

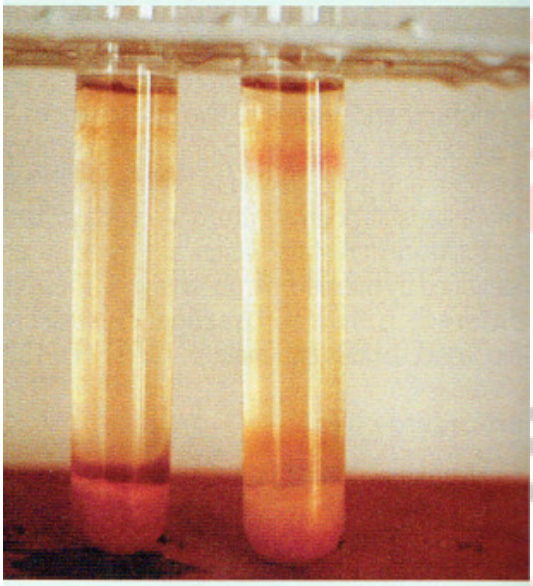
3 Liquor ammonia solution
4 Pasteur pipettes

Procedure

1 Transfer about 5 ml of urine to a test tube using aPasteur pipette,
2 Add about 1.0 g of Rothera's powder mixture andmix well.
3 Layer over the urine 1 to 2 ml of concentratedammonium hydroxide (allow it to flow gently downthe side of the inclined test tube).
4 Observe for pink-purple ring at the interface.

Observations

1 No appearance of pink-purple ring: Ketone bodies absent.
2 Appearance of pink-purple ring: Ketone bodiespresent.



Determination of acetone (Left: Negative, Right: Positive)

Note

Grade the results according to the intensity of the formation of colored ring as trace, +, ++, +++ and++++

Dumn and Shipley's method for the qualitativedetermination of ketone bodies is simple and easy toperform. This method requires following powder mixture.

1 Sodium nitroprusside (fine powder) : 1.0 g
2 Ammonium sulfate : 20 g
3 Anhydrous sodium carbonate : 20 g
Mix the powders and use morter and pestle to prepare fine powdered mixture.

Procedure

1 Take two test tubes (10 x 75 mm) and label them as T and C respectively.
2 Add pinch of powder mixture in both the test tubes.
3 In the tube labeled as T, add one drop of urine.
4 In the tube labeled as C, Add one drop of distilled Water.
5 Observe color of the reaction mixture after 5minutes.

Observations

1 T : No violet color: Ketone bodies absent

: Violet color: Ketone bodies present
2 C : No violet color
Color of the reaction mixture T should be compared with that of C.

DETERMINATION OF BILE PIGMENTS, BILE SALTS AND UROBILINOGEN

Clinical significance

Determination of bile pigments, bile salts and urobilinogen is useful in the diagnosis of jaundice. Icterus or jaundice refers to the yellowpigmentation of the skin,conjunctivae and mucous membrane by the abnormal increase of bilirubin inblood (serum). This may be observed in prehepatic, hepatic and posthepatic conditions.Following observations are useful for the differential diagnosis of jaundice.

Bile pigment metabolism

Bilirubin is formed from the breakdown of hemoglobin in the reticuloendothelial system (mainly spleen, liver and bone marrow). This indirect bilirubin (water insoluble) then bound to serum albumin gets transported through the

Clinical condition	Bile pigments	Bile salts	Urobilinogen
Prehepatic	Absent	Absent	Very high, + + + to + + + +
Hepatic	Present, trace to + + + +	Present	Increased + +
Posthepatic	present, + + to + + + +	Present	Present or may be absent

blood to the liver. The free indirect (or unconjugated) bilirubinbeing insoluble in water, cannot be filtered throughthe glomerulus. In the liver, bilirubin is removedby parenchymal cells. It is then converted to, water soluble (conjugated) bilirubin by the action ofenzyme glucuronyltransferase, by conjugation withglucuronic acid. This, conjugated bilirubin is excreted by the liver through the bile duct and intothe duodenum. Normally very small concentration (0.2-0.4 mg/dl of conjugated bilirubin is presentin blood due to regurgitation, back from the bileduct. Since conjugated (direct or water soluble) bilirubin is not bound to protein, it is easily filteredthrough the glomerulus and excreted in urine. But since the concentration of this normally excreteddirect bilirubin is very small,itcan not be detectedchemically.

In the intestine, bilirubin is converted by bacterialenzymes to several related compounds, collectivelycalled as urobilinogen. Most of urobilinogen is lost in feces as stercobilinogen. (and after exposure toair as stercobilin). About 10 to 15% of urobilinogen is reabsorbed into the blood stream and returns to liver.It is re-excreted in the intestine and also excreted by the kidneys into the urine. Excretion rate is 1to 4 mg in 24 hours. Freshly collected normal urine (fasting specimen) usually gives positive reaction for urobilinogen.

The normal level of total serum bilirubin is upto1.0 mg/dl (which contains about 0.5 mg of directbilirubin and nearly equal amount of indirect bilirubin). In Jaundice when the level of total bilirubin exceeds approximately 2.5 mg/dl the tissue of the body take on the yellow color of bilirubin.

• In pre-hepatic jaundice (hemolytic jaundice) whichaffects the red blood cells, there is an increase inunconjugated bilirubin while conjugated bilirubin is normal (below 1.0 mg/dl). Hence no bilirubinwill be excreted in the urine because unconjugated bilirubin can not be filtered at the glomerulus.

Due to the increased formation of bilirubin, therate of formation of urobilinogen increases considerably and it is excreted in urine in high proportion.

The diseases related to prehepatic conditions are(a) Sickle cell disease (b) Thalassemia major (c)Acquired hemolyticanemias (d) Incompatible blood transfusion, etc.

Principles of the Tests

1 Bile pigment determination (Harrison spot test): When barium chloride reagent is added to urine, itcombines with sulfate radicals in urine and precipitate of barium sulfate is formed. If bilepigments are present in urine, they will adhere tothese large molecules. Ferric chloride present inFouchet's reagent then oxidizes yellow bilirubin, in the presence of trichloroacetic acid to greenbiliverdin. Appearance of green color indicates presence of bile pigments in urine

2 Urobilinogen determinationUrobilinogen reacts with pdimethylaminobenzaldehyde in acidic medium (in Ehrlich reagent) toform pink colored compound. Appearance of pink color indicates presence of urobilinogen in urine.

DETERMINATION OF BILE PIGMENTS AND UROBILINOGEN

Requirements

1 Centrifuge tubes or test tubes 10 x 75 mm and

Procedure

1 Place 3 to 4 ml of urine in a centrifuge tube (about 1/3 full) by using a Pasteur pipette.
2 Add equal amount of 10 g/dl barium chloride, mixwell.
3 Centrifuge at 1,500 RPM for 10 minutes (or filterby using Whatman No. 1 filter paper).
4 Place supernatant in another test tube forurobilinogen test.
5 Add one to two drops of Fouchet's reagent to thesediment (or to the precipitate on a filter paper).
6 Add about 0.5 ml of Ehrlich reagent to thesupernatant.

Observations

1 Sediment (in the test tube or on filter paper)
a) No change in color: Bile pigments absent
b) Color change to green: Bile pigments present

Notes Grade the positive results as trace, +, ++, + + + and + + + +, according to the intensity of the color of the sediment,
2 Supernatant
a) Development of pale pink color

: Urobilinogen, present normal.
b) Development of cherry red color

DETERMINATION OF BILE SALTS

Principle

Bile salts when present lower the surface tension of urine. When sulfur powder is added on the surface ofurine, sulfur particles sink to the bottom of the test tube. In the case of a normal urine sample sulfurparticles float on the surface of urine.

Procedure

1 Place about 10 ml urine in a test tube (15 x 125 mm)
2 Sprinkle a little dry sulfur powder on to the surfaceof urine.
3 Observe the sulfur particles.

Observations

1 Sulfur particles sink to bottom : Bile salts present.
2 Sulfur particles remain floating: Bile salts absent.

DETERMINATION OF OCCULT BLOOD

The term "occult" means hidden. The tests are capable of detecting even minute of blood (which is not visibleto eyes) in urine. These procedures actually detect the free hemoglobin from lysed red blood cells. In caseswhere all the red cells stayed intact, the test results may be negative (although red blood cells are seen inthe microscopic examination).
The chemical methods detect free hemoglobin andmyoglobin. Since the urine is normally free of all thesesubstances, a positive test for occult blood should be followed by exact cause and origin of the abnormalfindings. Positive test result may be obtained due tohemoglobinuria and myoglobinuria. A correlation mustbe made with the microscopic examination. Hematuria, hemoglobinuria and myoglobinuria can occur eitherindividually or together.

Clinical Significance

• Hematuria
Generally, red blood cells are not found in the sediment of centrifuged normal urine. But the finding of 1 to 2 red blood cells, per high powerfield should not be considered abnormal. However, presence of more number of red bloodcells (or presence of blood) in urine is called hematuria. A urine that has low specific gravity (1< 1.007) or highly alkaline urine can cause the red blood cells to lyse. This leads to the release ofhemoglobin in urine and still considered to behematuria as far as the origin is known. Hematuria can occur in the following clinical conditions.
• Renal diseases
— Acute infections, chronic glomerulonephritis, tuberculosis of the kidney, nephrotic syndrome,toxic damage to glomerulus, malignant hypertension, infarction, renal calculi, traumato kidneys, acute cystitis, calculi and tumors in the ureter or bladder.
Other clinical conditions

— Bleeding disorders such as leukemia, thrombocytopenia, coagulation factor deficiency, sickle disease or trait, scurvy.

— Use of anticoagulant drugs.
— Hemoglobinuria
Hemoglobinuria is the presence of free hemoglobin in the urine as a result of intravascular hemolysis. Hemoglobinuria without hematuriaoccurs as a result of hemoglobinemia (i.e. presence of free hemoglobin in the blood). Thehemoglobin released from destroyed red blood cells quickly binds to a special plasma globulin called haptoglobin. The haptoglobin binding capacity is exceeded when excessiveamount of hemoglobin is released in circulation in many conditions associated with intravascular hemolysis. The free plasma hemoglobin is readily filtered through the glomerulus.
Some of the hemoglobin is reabsorbed intothe tubular epithelial cells and remaining unabsorbed hemoglobin is lost in urine. The conditions associated with intravascular hemolysis which results in hemoglobinuria are as follows:
— Hemolytic anemias
— Poisoning from snake venom, spider bites and bacterial toxins.

Principle of Occult Blood Test

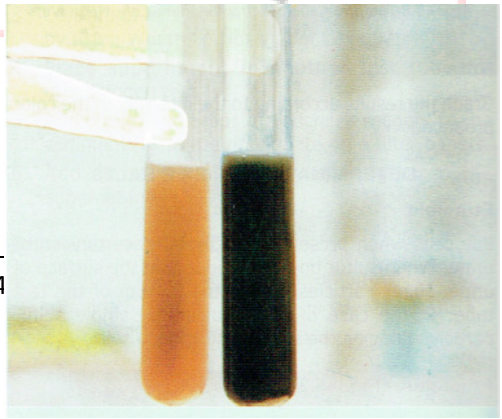
The peroxidase activity of hemoglobin (present in urine) decomposes hydrogen peroxide and theliberated oxygen oxidisesbenzidine to form a greenbluecolored complex.

Requirements

1 Test tubes (15 x 125 mm)
2 Graduated pipettes of 5 ml
3 Pasteur pipettes
4 Benzidine powder
5 Glacial acetic acid
6 Hydrogen peroxide (30%, v/v).
7 Pasteur pipettes

Procedure

1 Place pinch of benzidine powder in a test tube.
2 Add 2 to 3 drops of glacial acetic acid and mix well.
3 Add about 2 ml of hydrogen peroxide solution andmix well. Transfer 1.0 ml of supernatant to a testtube labeled as T.
4 Add 0.5 ml of urine and mix well.
5 Observe the color of the mixture after 5 minutes.



Observations

1 No change in color: Occult blood absent.

2 Color changes to green or blue: Occult blood present. Report presence of blood as follows

PHYSICAL EXAMINATION:

NO.	DETERMINATION	NORMAL FINDING	ABNORMAL FINDING	PATHOLOGIC	NON-PATHOLOGIC
1	Volume (of first voided mid-stream urine sample)	50 ml to 200 ml (some-times up to 300 ml) when properly collected	> 500 ml < 20 ml	Polyuria, diabetes mellitus Diabetes insipidus Oliguria, anuria Renal conditions Post renal conditions	Quantity of first voided morning urine volume may not give idea of change in the urinary output, which may be associated with a clinical condition. A urine sample collected for 24 hours is a satisfactory sample for such purpose
2	Color	Pale yellow	Yellow, dark yellow, brownish yellow toorange White Pink to red Brownish black	Presence of- Water soluble (direct) bilirubin, hepatic, post-hepatic conditions Presence of- - Pus (many WBCs) Presence of- - Hemoglobin (hemoglobinuria) - Red blood cells (hematuria), renal disease	Intake of the following: Food (yellow) color, vitamin B-complex, and concentrated urine Excretion of red urine after eating beets Intake of iron

			Blue to green	Presence of- - Biliverdin - Pseudomonas infection	compounds Intake of the following: Methylene blue,
3	Appearance	Usually clear sometimes cloudy	Turbid Hazy Smoky Milky	Presence of abnormal number of leukocytes, epithelial cells, bacteria Mucus Red blood cells Chyle	Precipitation of amorphous phosphates in alkaline urine and amorphous urates in acid urine.
4	Reaction	Usually acidic, pH range 4.8-7.5	pH less than 4.8 more acidic urine pH more than 7.5 (alkaline) urine	fevers, Severe vomiting, urine retention, obstructive, gastriculcers	Protein rich diet, Urine collected after taking large quantities of citrus fruits.
5	Odor	Characteristic aromatic	Fruity Ammoniacal Foul smelling	Acidosis, ketosis in severe diabetes mellitus (due to the presence of acetone). Urinary tract infections, especially due to coliform bacteria	Decomposition of urea to ammonia by bacterial action, due to storage at room temperature
6	Sediment formation at the bottom of the container after collection	Usually there is no formation of sediment (or formation of very little	Sediment present (moderate to high proportion)	Presence of leukocytes (pus cells), epithelial cells, red blood cells, casts, etc.	Precipitate of amorphous phosphates (in alkaline urine) appearance white and

		sediment)			amorphous urates (in acidicurine) appearance pinkish white
7	Specific gravity	Varies from 1.003 to 1.060	Low sp. gr. High sp. gr.	diabetes insipidus Diabetes mellitus, fevers,	

CHEMICAL EXAMINATION

NO.	**Determination**	**Normal finding**	**Abnormal finding**	**Pathologic**	**Non-pathologic**
1	Protein	Absent	Present	Dehydration, heart disease severe diarrhea. All forms of renal diseases.	Excessive muscular exercise Prolonged cold bath Excessive protein ingestion False proteinuria: Due to pus, blood and vaginal discharge,
2	Glucose	Absent	Present	Diabetes mellitus	
3	Ketone bodies	Absent i.e. chemically not detected	Present	Severe diabetes mellitus, Fevers Certain nervous disorders, After prolonged diarrhea and vomiting	Starvation
4	Bile pigments	Absent	Present	Hepatic conditions Post	

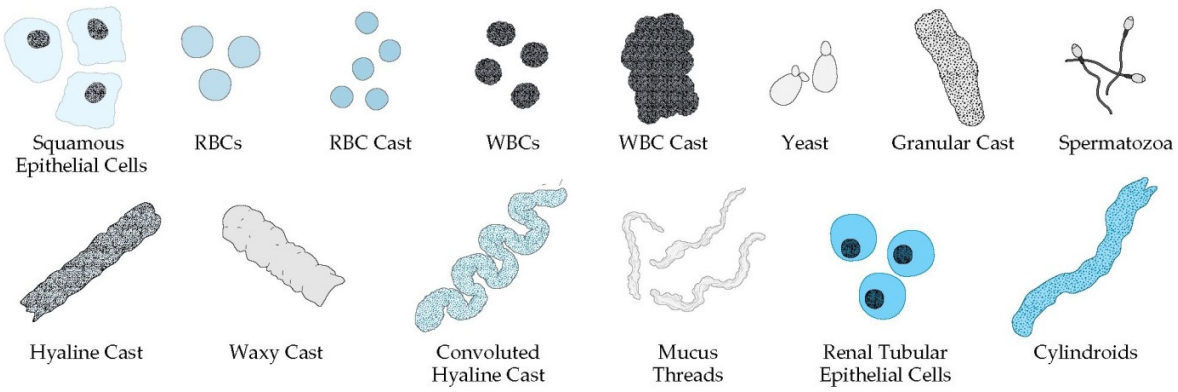
				hepatic conditions	
5	Bile salts	Absent	Present		
6	Urobilinogen	Present (very low concentration)	Increased Very high	Hepatic and posthepatic conditions Pre-hepatic conditions	May increase by constipation
7	Blood	Absent	Present	Hematuria: calculi, renal carcinoma, tuberculosis of kidneys, chronic infections Hemoglobinuria: Hemolytic, poisons, severe burns, hemolytic transfusion reaction	

MICROSCOPIC **EXAMINATION:**

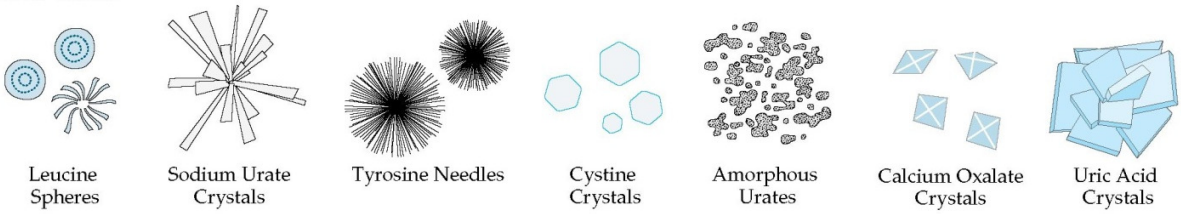
No	**Determination**	**Normal finding**	**Abnormal finding**	**Pathologic**	**Non-pathologic**
1	Pus cells (leukocytes	2 to 3 per HPF	> 5 pus cells per HPF	Urinary tract infections. Pyuria (pus in urine) dehydration, stress, fever,	Fewleukocytes can normally be found.
2	Epithelial cells	Male: 2 to 3 per HPF Female: 2 to 5 per HPF	> 5 epithelial cells per HPF	tubular damage	Normally few epithelial cells found.
3	Casts	Absent	Present	The casts may be present in glomerular damage, renal inflammation and renal infection. These are seen in even the	

			Hyaline casts	mildest kind of renal disease.	Occasional-Hyaline casts,may be present due to 1) Physical exercise. 2) Physiologic dehydration
			Red cell casts	Glomerular diseases,	
			White cell casts	Renal infection	
4.	Crystals Acidic crystals	Uric acid Calcium sulfate	When) present in fresh urine in high proportion	Presence of renal calculi.	
		Calcium oxalate	(When) present in highconcentration	May indicate presence of renal drugs and foods (spinach) calculi, vitamin ‘ C’, berries,tomatoes	
			Present in high concentration	May indicate presence of renal calculi	
		Ammonium Magnesium phosphate (Triple phosphate)	Present		
	Bacteria	Absent		May present in acidic urine containing sugar	After storage at room temperature bacterial growth may take place in urine.
	Yeast cells		Present	*Trichomonasvaginalis*(from vagina) *Trichomonashominis*(from rectum)	
	Parasites	Absent	Present		

NORMAL URINE



ACID URINE



ALKALINE URINE