Body fluids

Manual

Collected and prepared by

Ibtisam H. AlAswad
Yousif M. EL-Argan
Mohammed M. Laqqan

Medical Technology Department
Islamic University-Gaza

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قسم التحليلات الطبية
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وصف المساق: سوائل الجسم المختلفة من ناحية كيميائية وهماتولوجية، وتقدر نسب المواد فيها، التعرف على القيم الطبيعية، التدرب على كتابة التقارير الطبية الأولية لها، التعرف على الفحوصات السيرولوجية وعلاقة كل منها بالأمراض وتقدير النسب لكل فحص، دراسة تكوين الجسم في جسم الإنسان وطريقة فحصها كيميائياً.

أهداف المساق:
1. التعرف على سوائل الجسم المختلفة.
2. التعرف على طرق الحصول على عينات منها.
3. التعرف على المكونات الطبيعية للبول وكمياتها.
4. التعرف على الطرق المستخدمة في تحديد كفاءة الكلية.
5. التعرف على نسب بعض الحالات المرضية.
6. التعرف على أساليب فحص الكلى

النتائج المتوقعة أن يحصل عليه الطالب:

تحدد الامتحانات لاحقاً.

تاريخ الامتحانات:
امتحان نظري نصف.
امتحان عملي.
امتحان نظري نهائي.
امتحان عملي نهائي.

توزيع الدرجات:
امتحان نظري تصفي 30 درجة.
امتحان عمل نهائى 20 درجة.
امتحان نظري نهائي 40 درجة.
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Urinary system

- The kidneys are retroperitoneal organs (i.e. located behind the peritoneum) situated on the posterior wall of the abdomen on each side of the vertebral column, at about the level of the twelfth rib.

- The left kidney is lightly higher in the abdomen than the right, due to the presence of the liver pushing the right kidney down.

- The kidneys take their blood supply directly from the aorta via the renal arteries; blood is returned to the inferior vena cava via the renal veins.

- Urine (the filtered product containing waste materials and water) excreted from the kidneys passes down the fibromuscular ureters and collects in the bladder. The bladder muscle (the detrusor muscle) is capable of distending to accept urine without increasing the pressure inside; this means that large volumes can be collected (700-1000ml) without high-pressure damage to the renal system occurring.

- When urine is passed, the urethral sphincter at the base of the bladder relaxes, the detrusor contracts, and urine is voided via the urethra.
Function of the urinary system

1. Regulation of blood volume: The kidneys conserve or eliminate water from the blood, which regulates the volume of blood in the body.

2. Regulation of blood pressure: The kidneys regulate blood pressure in 2 ways, by:-
   - Adjusting the volume of blood in the body (by regulating the quantity of water in the blood)
   - Via the action of the enzyme renin. The kidneys secret renin, which activates the angiotensin-aldosterone pathway in which the following take place:

The hormones, renin, angiotensin, and aldosterone work together to regulate blood pressure. A sustained fall in blood pressure causes the kidney to release renin. This is converted to angiotensin in the circulation. Angiotensin then raises blood pressure directly by arteriolar constriction and stimulates adrenal gland to produce aldosterone which promotes sodium and water retention by kidney, such that blood volume and blood pressure increase.

3. Regulation of the pH of the blood: The kidneys excrete H⁺ ions into urine. At the same time, the kidneys also conserve bicarbonate ions (HCO₃⁻), which are an important buffer of H⁺.

4. Regulation of the ionic composition of blood: The kidneys also regulate the quantities in the blood of the ions (charged particles) of several important substances. Important examples of the ions whose quantities in the blood are regulated by the kidneys include sodium ions (Na⁺), potassium ions (K⁺), calcium ions (Ca²⁺), chloride ions (CL⁻), and phosphate ions (HPO₄²⁻).

5. Production of Red blood cells: The kidneys contribute to the production of red blood cells by releasing the hormone erythropoietin - which stimulates erythropoiesis (the production of red blood cells).

6. Synthesis of Vitamin D: The kidneys (as well as the skin and the liver) synthesize calciferol - which is the active form of vitamin D.

7. Excretion of waste products and foreign substances: The kidneys help to excrete waste products and foreign substance from the body by forming urine (for release from the body).

Examples of waste products from metabolic reactions within the body include ammonia (from the breakdown of amino acids), bilirubin (from the breakdown
of hemoglobin), and creatinine (from the breakdown of creatine phosphate in muscle fibers). Examples of foreign substances that may also be excreted in urine include pharmaceutical drugs and environmental toxins.

**Urine:**

Urine, a very complex fluid, is composed of 95% water and 5% solids. It is the end product of the metabolism carried out by billions of cells and results in an average urinary output of 1-1.5 L per day.

Urine consists of thousands of dissolved substances although the three principle constituents are water, urea, and sodium chloride, considerable variations in the concentrations of these substances can occur due to several factors.

Urine may also contain formed elements such as cells, casts, crystals, mucus and bacteria. Almost all substances found in urine are also found in the blood although in different concentration.

**Urine formation**

- **Filtration**

Urine formation begins with the process of filtration, which goes on continually in the renal corpuscles. As blood courses through the glomeruli, much of its fluid, containing both useful chemicals and dissolved waste materials, soaks out of the blood through the membranes (by osmosis and diffusion) where it is filtered and then flows into the Bowman's capsule. This process is called glomerular filtration.

The water, waste products, salt, glucose, and other chemicals that have been filtered out of the blood are known collectively as glomerular filtrate. The glomerular filtrate consists primarily of water, excess salts (primarily Na\(^+\) and K\(^+\)), glucose, and a waste product of the body called urea.

Urea is formed in the body to eliminate the very toxic ammonia products that are formed in the liver from amino acids. Since humans cannot excrete ammonia, it is converted to the less dangerous urea and then filtered out of the blood. Urea is the most abundant of the waste products that must be excreted by the kidneys.

The total rate of glomerular filtration (glomerular filtration rate or GFR) for the whole body (i.e., for all of the nephrons in both kidneys) is normally about 125 ml per minute.
Reabsorption
Reabsorption, by definition, is the movement of substances out of the renal tubules back into the blood capillaries located around the tubules (called the peritubular capillaries). Substances reabsorbed are water, glucose and other nutrients, and sodium (Na+) and other ions. Reabsorption begins in the proximal convoluted tubules and continues in the loop of Henle, distal convoluted tubules, and collecting tubules.

In other words, about 99% of the 180 liters of water that leave the blood each day by glomerular filtration returns to the blood from the proximal tubule through the process of passive reabsorption.

The nutrient glucose (blood sugar) is entirely reabsorbed back into the blood from the proximal tubules. In fact, it is actively transported out of the tubules and into the peritubular capillary blood. None of this valuable nutrient is wasted by being lost in the urine.

Secretion
Secretion is the process by which substances move into the distal and collecting tubules from blood in the capillaries around these tubules. In this respect, secretion is reabsorption in reverse. Whereas reabsorption moves substances out of the tubules and into the blood, secretion moves substances out of the blood and into the tubules where they mix with the water and other wastes and are converted into urine. These substances are secreted through either an active transport mechanism or as a result of diffusion across the membrane. Substances secreted are hydrogen ions (H+), potassium ions (K+), ammonia (NH3), and certain drugs.
In summary, three processes occurring in successive portions of the nephron accomplish the function of urine formation:

1. Filtration of water and dissolved substances out of the blood in the glomeruli and into Bowman's capsule;
2. Reabsorption of water and dissolved substances out of the kidney tubules back into the blood (note that this process prevents substances needed by the body from being lost in the urine);
3. Secretion of hydrogen ions (H\(^+\)), potassium ions (K\(^+\)), ammonia (NH\(_3\)), and certain drugs out of the blood and into the kidney tubules, where they are eventually eliminated in the urine.

❖ **Role of Aldosterone:**
   Low Blood Pressure (Hypotension) → Adrenal gland → Aldosterone hormone

   - Increase Na\(^+\) reabsorption
   - Increase water retention
   - Increase blood pressure

❖ **Role of Antidiuretic Hormone (ADH):**
   ADH regulates water reabsorption in the distal tubule.

 Plasma

Increased Plasma Osmolality (Plasma Concentration)

Posterior pituitary → Feedback mechanism

ADH

Distal tubules → Plasma dilution, decreased plasma Osmolality

   - Increased Water reabsorption
   - Decreased water excretion
Specimen Collection

- The specimen must be collected in a clean dry, disposable container and properly applied screw top lids.
- The container must be properly labeled with the patient name, date, and time of collection. The labels should be applied to the container and not to the lid.
- The specimen must be delivered to the laboratory prompt and tested within 1 hr, specimen must be delivered within 1 hr, should be refrigerated or 2 have an appropriate chemical preservative added. eg. (Toluene, thymol, formalin or boric acid).

Changes in unpreserved urine: (At room temp. for longer than 1 hr.)

1. Transformation of urea to ammonia which increase pH.
   Urea \( \xrightarrow{\text{urease} \ (Bacteria)} \) \( 2\text{NH}_3 + \text{CO}_2 \).

2. Decrease glucose due to glycolysis and bacterial utilization.
3. Decrease ketones because of utilization.
4. Decrease bilirubin from exposure to light.
5. Decrease urobinogen oxidation urobin.
6. Increase bacterial number.
7. Increase turbidity caused by bacteria & amorphous.
8. Disintegration of RBCs casts, particularly in diluted alkaline urine.
9. Increase nitrite due to bacterial reduction of nitrate.
10. Changes in color due to oxidation or reduction of metabolic.

Types of specimens

1. **Random specimen (at any time):** Useful for routine screening but may give false results due to dietary intake or physical activity just prior to the collection of the specimen, hence it’s not useful for quantitative analysis.

2. **First morning specimen:** Valuable, for it’s usually concentrated and more likely to reveal abnormalities and formed elements, it’s also free of dietary influences and changes due to physical activities, its prevents false negative pregnancy test. As well as it’s useful in evaluation of orthostatic proteinuria.

3. **24 hr’s collection:** Used for quantitative determination and for evaluation the kidney function.
4. **Post. Prandial sample:** It taken at specified time after specific meal to know the normal excretion.

5. **Clear catch sample (midstream urine):** Best for bacteriological work, it’s collected by cleaning the genitalia then the patient takes the midstream urine which is suppose to be the most sterile one.

6. **Catheterized urine:** Collected form pediatric or adult that can’t give urine.

7. **Supra - pubic samples:** For bacteriological samples and taken from pediatric mainly.

**Routine Urinalysis**

A routine urine analysis (R+M) Routine + Microscopic includes the examination of physical & chemical characteristics of microscopic studies of some cellular & non-cellular elements.

**Physical examination of Urine (Macroscopic, Gross)**

The first part of a urinalysis is direct visual observation.

1. **Appearance:** (includes color and clarity)

   ◗ **Color**
   
   Normal urine color has a wide range of variation ranging from pale yellow, straw, light yellow, yellow, dark yellow amber due to urochrome pigment (it’s an end product of endogen metabolism), trace of urobilin and uroerythrin.

   **The color is affected by**
   
   1. Concentration of urine
   2. pH
   3. Metabolic activity.
   4. Diet intake (Beet→ red).
   5. Drugs may change urine color.
Abnormalities in color

- **Colorless or pale yellow**
  1. High fluid intake
  2. Reduction in perspiration.
  4. Diabetes Mellitus.
  5. Diabetes Insipidus (Low level of antidiuretic hormone).
  6. Alcohol ingestion
  7. Nervousness.

- **Dark yellow, Amber, orange**
  1. Low fluid intake.
  2. Excessive sweating
  3. Dehydration (burns, fever).
  4. Carrots or vitamin (A)
  5. Pyridium and nitrofurantoin (drugs).

- **Brownish yellow**
  1. Bilirubin → on shaking yellow foam will appear.
  2. Urobilin → on shaking the foam has no color.

- **Yellow – green**
  Bilirubin $\xrightarrow{oxid}$ Biliverdin (greenish)
  Which give a yellow foam & (-) test for bilirubin

- **Blue – Green**
  Pseudomonas Infection

- **Pink – Red**
  Due to the presence of fresh blood or Hb, fresh blood will give smoky color while Hb gives clear reddish urine.
  - **Both may be due to**
    1. Urinary tract infection
    2. Calculi
    3. Trauma
    4. Menstrual contamination.

- **Dark brown**
  Met hemoglobin if bloody sample long staded, Hb will be oxidized.
  Melanin
  \[
  \text{Melanogen} \xrightarrow{\text{light}} \text{Melanin} \xrightarrow{\text{(Brown)}} \text{Malignant Melanoma}
  \]
- **Black Urine**
  Alkaptonuria, a disease of tyrosine metabolism.

- **Clarity (Transparency)**
  Normal urine clear or transparent, any turbidity will indicate.
  1. WBCs (pus).
  2. RBCs
  3. Epithelial cells
  4. Bacteria
  5. Casts
  6. Crystals
  7. Lymph
  8. Semen.

2. **Odor**
   - Fresh normal urine has a faint aromatic odor due to the presence of some volatile acids.
   - In some pathological conditions, certain metabolites may be produced to give a specific odor such as:
     - Fruity odor is due to Diabetic urine acetone.
     - Ammoniac odor → urine standing long time
     - Offensive odor → Bacterial action of pus (UTI).
     - Apple odor → Asparagus
     - Mousy odor → Phenylalanine (phenyl keto urea “PKU” ).

3. **Volume**
   Adult urine volume = 600 – 2500 ml /24hr.
   Children urine volume = 200 – 400ml /24hr. (4ml / kg / hr).

- **Which depends on**
  1. Water in take
  2. External temperature.
  3. Mental and physical state.
  4. Intake of fluid and diuretics (Drugs, alcohol – tea).

- **Abnormalities**
  - Oligouria: marked decrease in urine flow < 400 ml.
  - Polyuria: Marked increase in urine flow > 2500 ml.
  - Anuria: complete stoppage of urine flow.
  - Nocturia: excessive urination during night.
- **Causes of polyuria**
  1. Increased fluid intake (polydipsia ➔ Polyuria).
  2. Increased salt intake and protein diet, which need more water to excrete.
  3. Diuretics intake (certain drugs, drinks).
  4. Intravenous saline or glucose.
  5. Diabetes Mellitus.
  7. Renal disease.
  8. Hypoaldosteronism.

- **Causes of Oligouria**
  1. Water deprivation
  2. Dehydration
    a. Prolonged vomiting.
    b. Diarrhea
    c. Excessive sweating
  3. Renal Ischemia
    a. Heart failure
    b. Hypotension
    c. Transfusion Reaction
  4. Renal Disease
  5. Obstruction by
    a. Calculi.
    b. Tumor.
    c. Prostatic hypertrophy.

- **Causes of Anuria**
  1. Sever Renal Defect and loss of urine formation mechanism.
  2. Due to the presence of stone or tumor.
  3. Post transfusion hemolytic reaction.

4. **Specific Gravity (spg)**
   - Specific gravity (which is directly proportional to urine osmolality which measures solute concentration) measures urine density, or the ability of the kidney to concentrate or dilute the urine over that of plasma.
   - As (spg): Is a measure of number and size of molecules, hence, large molecules such as urea will contribute to reading more than the small molecules, such as Na\(^+\) and K\(^+\) which are actual more important to reflect urine concentration mechanism. Hence, osmolality may express this function with more effectively because it’s the number of particles / kg of substance.
Specific gravity between 1.002 and 1.035 on a random sample should be considered normal if kidney function is normal. Since the spg of the glomerular filtrate in Bowman's space ranges from 1.007 to 1.010, any measurement below this range indicates hydration and any measurement above it indicates relative dehydration.

- **Low specific gravity**
  1. Diabetes Insipidus
  2. Glomerulonephritis
  3. Sever renal damage (diminish the concentration ability of the kidney)
  4. Excessive water intake.

- **High specific gravity:**
  1. Diabetes mellitus.
  2. Nephrosis
  3. Fever since urine is conc.
  4. Urine preservative substance
  5. X ray contrast media

**Note:**
For every $\mu$g / dl protein spg increased by 0.003.
For every mg / dl sugar spg increased by 0.004.

**Measurement of spg**

1. **Urinometer:** Which is consists of a weighted float a hatched to a scale that has been calibrated in terms of urine spg (1.00 – 1.040) the weighted float displaces a volume of liquid equal to its weight and has been designed to sink to a level of 1.000 in distilled water. The additional mass provided by the dissolved substances in urine causes the float to displace a volume of urine smaller than of distilled water, the level to which the urinometer sinks, is representative of the specimen spg.
Disadvantages of urinometer:
- Inaccurate reading so needs solution of known spg to correct.
- The minimum amount of urine to be measured is about 15 ml.
- If the urine is so turbid it is difficult to read the result.

2. Reagent strip
Which contain polyelectrolyte, when ions increase in urine, more acidic groups are released, the change in pH will take place which change the color of bromothymol blue indicator.

(ions in low spg urine)  (ions in high spg urine)
C – OO – H⁺  C – OO – H⁺
C – OO – H⁺  C – OO – H⁺
C – OO – H⁺  C – OO – H⁺
Less H⁺ release because less urine ions is present  so increased pH  H⁺ replaced by urine ions & released in urine so decreased pH

5. pH
1. One of the important functions of the kidneys is pH regulation, the glomerular filtrate of blood plasma is usually acidified by renal tubules and collecting ducts from a pH of 7.4 to about 6 in the final urine to keep blood pH about 7.4. Hence, urine pH must vary to compensate for diet and products of metabolism, this function takes place in the distal convoluted tubule with the secretion of both H⁺ & NH₃⁺ and reabsorption of bicarbonate ie. In cases of acidosis, urinary pH will be alkaline by stop H⁺ excretion.

2. Normal urine pH is (4.6 – 8.0) as average (6.0).

- Clinical significance of pH
  1. Determine the existence of metabolic acid base disorder
  2. Precipitation of crystals to from stone requires specific pH for each type. Hence, pH control may inhibit the formation of these stones by control diet.
     - High protein will give acidic urine.
     - High vegetable will give alkaline urine
     - In addition to some drugs which control pH.
• Crystals found in alkaline urine: Ca carbonate, Ca phosphate, Mg phosphate, and amorphous phosphate.
• Crystals found in acidic urine: Ca oxalate, Uric acid, Cystine, Xanthine and amorphous urate.

3. May indicate the presence of urinary tract infection caused by urea splitting organisms.

\[
\text{Urea} \xrightarrow{\text{urease}} 2\text{NH}_3 + \text{CO}_2.
\]


5. Determination of unsatisfactory specimens.

\[
\text{Urea} \xrightarrow{\text{urease}} \text{NH}_3 + \text{CO}_2.
\]

Even in abnormal conditions, urine pH mustn’t reach 9, if so or more this will indicate that urine is stand for along time & must be rejected.

• **Test for pH**

  Reagent strips which has an indicator (methyl red – bromothymol blue indicator) or other indicators.

  - Alkaline urine is found in:
    Patient with alkalemia, UTI, diets high with citrus fruits or vegetables.

  - Acidic urine is found in:
    Patient with acidemia, starvation, dehydration, high diets with meat products.

### Chemical properties of urine

1. **Protein**

   A small amount of protein (50 – 150 mg / 24 hrs) appears daily in the normal urine, or 10 mg/dl in any single specimen which is not appear in routine urinalysis procedure. More than 150 mg/day is defined as proteinuria. This amount of protein is form of:

   1. 40% consist of albumin, which may escape from the glomerulus membrane & not reabsorbed.
   2. 40% of (tamn–Horsfall) mucoprotein which is secreted from the renal tubule and other secretions from genitalia.
   3. 20% other traces of non-plasma proteins.
Proteinuria: Is defined as the presence of detectable amount of proteins in urine.

- **Causes of proteinuria**
  1. **Glomerular membrane damage**, which may be:
     a. Primary: due to primary glomerular defect as glomerulonephritis.
     b. Secondary: due to external disease that affects the glomerular function as:
        1- SLE      2- Drug      3- Septicemia
  2. **Prerenal Proteinuria**
     a. Absorption problems
     b. Over flow / over load, increase of LMW protein such as multiple myeloma. Ex. Bence Jones protein.
  3. **Tubular proteinuria:**
     Present of LMW protein, so used immunological method for diagnosis
     Encountered in heavy metal poisoning, Fanconi’s syndrome, Wilson’s syndrome

- **Functional or Nonpathogenic proteinuria due to:**
  1. Fever
  2. Emotional
  3. Cold
  4. Later months of pregnancy
  5. Postural (as long standing & exercises)

- **Tests for protein**
  1. **Dipstick**
     The basis for protein test is the “protein error” of indicators, a term applied to the change in ionization and color of the indicctor, and hence the apparent pH, when an indicator dye is adsorbed to protein the paper spot in the dipstick is impregnated with citrate buffer (PH = 3.0) containing Bromphenol blue, which is most sensitive to albumin but detects globulins and Bence-Jones protein poorly, Bromphenol blue is yellow at pH 3.0 and blue at pH 4.2, at pH (3) the indicator is mostly unionized. If protein is present in the urine into which it is dipped, the ionized fraction binds to the protein, thereby causing more dye to ionize until equilibrium is reached; hence, the impregnated strip has less yellow and bluer color as the protein concentration increases. This reaction is seen visually as a change from yellow to green (a mixture of yellow dye plus blue dye appears green). The color is compared with that of the protein content from (30 – 1000mg /dl).
In rough terms, trace positive results (which represent a slightly hazy appearance in urine) are equivalent to 10 mg/100 ml or about 150 mg/24 hours (the upper limit of normal). 1+ corresponds to about 200-500 mg/24 hours, a 2+ to 0.5-1.5 gm/24 hours, a 3+ to 2-5 gm/24 hours, and a 4+ represents 7 gm/24 hours or greater.
False (+ve) may be due to increased urine pH,
HIN (yellow) → H⁺ + IN⁻ (Blue)

2. Precipitation test

b. Sulfosalicylic acid
Precipitation by heat is a better semi quantitative method, but overall, it is not a highly sensitive test. The sulfosalicylic acid test is a more sensitive precipitation test. It can detect albumin, globulins, and Bence-Jones protein at low concentrations.

Neg. No turbidity
(1+) Turbidly, No granules.
(2+) Turbidity, granulation, flocculation.
(3+) Turbidity, granulation
(4+) Clumps of proteins.

3. Test for bence – Jones protein
Bence Jones protein appears in urine of multiple myeloma patients.
First heat the urine between 40 – 60 °C, precipitation will occur then continue heating till 100 °C so the precipitation will disappear (clear). If you cool the urine till 40 – 60 °C the precipitation will occur again.

- Combined use of dipstick and sulfosalicylic acid
  1. If both are +ve then proteinuria is present
  2. If dipstick 1+ and sulfosalicylic negative then there is probably no pathologic concentration of protein.
  3. If dipstick negative and sulfosalicylic positive then the protein may be Bence Jones protein or one of the heavy chain proteins and should confirmed by immunologic method.
2. **Glucose**

- Under normal conditions, almost all of glucose filtered by the glomerulus is reabsorbed in the proximal convoluted tubule, by an active process to maintain the plasma concentration of glucose. Less than 0.1% of glucose normally filtered by the glomerulus appears in urine (< 130 mg/24 hr).

- If the blood glucose concentration is increased, reabsorption of glucose ceases & glucose appears in urine. Glycosuria (excess sugar in urine) generally means diabetes mellitus.

- **Threshold substances**
  - Substances that are completely absorbed by the tubules when their plasma concentration is normal and not completely absorbed by the tubules if their plasma concentration exceeds normal levels.
  - The threshold of glucose is 180 mg / dl.

- **Glycosuria may be due to**
  1. Reabsorption defect
  2. Increase Blood glucose, in the following cases:
     a. Diabetes mellitus
     b. Alimentary glycosuria (transitory), after meal.
     c. Stress in which elevation of epinephrine leads to increase glycogenolysis, and cortisol increase gluconeogenesis.
     d. Pancreatic disease affect insulin secreting gland.
     e. Decrease reabsorption ability.

- **Tests for sugar** (reagent strip) “Benedicts test”
  1. Cu So → Cu_2O (red ppt).
  2. Glucose → gluconic acid + H_2O_2.

      H_2O_2 + O → toluidine peroxides oxyorthotoludine (blue color).
3. **Blood, hemoglobin & myoglobin**
   Normally there is no blood or Hb in normal voided urine. The presence of these will be refereed to hematuria, hemoglobinuria or myoglobinuria.

- **Causes of hematuria:** (the presence of erythrocytes)
  
a. **Kidney problem such as**
     1. Renal disease.
     2. Renal calculi
     3. Renal tumor.
     4. Trauma.
     5. Effects of toxins that damage the glomeruli.

b. **Lower Urinary tract problem**
   1. Infection
   2. Tumor
   3. Calculi
   4. Trauma

c. **Bleeding disorders and blood disease**
   1. Leukemia.
   2. Hemophilia.
   3. Drugs
   4. Thrombocytopenia.
   5. Sickle cell trait.
   6. Catheterization

**Note:** If hematuria, cast and proteinuria are present then the origin of problem is kidney.

- **Causes of hemoglobinuria**
  The presence of free Hb in urine as a result of intravascular hemolysis due to hemoglobinemia
  1. Hemolytic anemia
  2. Sever burns
  3. Transfusion reaction
  4. Poisoning
  5. Sever physical exercises
  6. Infections with hemolytic bacteria
• **Causes of myoglobinuria**
  The presence of myoglobin, which is heme. Protein of muscles, which facilitate the movement of oxygen within muscles. Hence it will appear in urine in case of:
  • Muscular trauma
  • Convulsions
  • Prolonged coma
  • Progressive muscle disease
  • Alcoholic myoglobinuria

**Tests**

O-toludine Benzidine + $\text{H}_2\text{O}_2 \xrightarrow{\text{peroxides of Hb}} \text{colored pigment (blue / green)} + \text{H}_2\text{O}$.

- RBCs, Hb, and myoglobin will give +ve reaction.
- RBCs will give a spotted reaction pattern & will appear in microscopic test.
- Hb & myoglobin will give diffused reaction pattern; ammonium sulfate will differentiate between them, which precipitate Hb but not myoglobin. In urine sample, both give normal RBCs microscopically (0–2).

4. **Nitrite**
   A positive nitrite test indicates that bacteria may be present in significant numbers in urine. Gram negative rods such as E. coli are more likely to give a positive test.
   - Negative test can not exclude the presence of bacteria.

   ![Nitrate bacteria nitrite](image)

   - **Nitrite present in**
     1. Cystitis
     2. Pyelonephritis
     3. Also we can use the test for:
     - Evaluation of antibiotic therapy
     - Monitoring of patient at high risk for UTI

• **Test: (Greiss – reaction)**

Para-arsenic acid or sulfonamide + Nitrite $\rightarrow$ Diazonium compound.
5. **Bilirubin**  
Bilirubin derived from Hb, is conjugated in the liver and excreted in the bile. Conversion to stereobilinogen (faecal urobilinogen) takes place in the intestinal lumen. Some reabsorbed urobilinogen is excreted in the urine.

**Production of bilirubin**

Red blood cells destruction in spleen and liver

![Diagram of bilirubin production](image_url)
RBCs are destructed in the reticuloendothelial system which gives protoporphyrine from Hb, which transformed in liver to bilirubin, which released into the circulation bounded with albumin; hence it will not be filtered in the kidneys. During circulation it will reach the liver to conjugate with glucoronic acid to from the conjugated form that will be reach the intestine via the bile duct to be reduced by the action of bacteria to give urobilinogen, which has two ways, one is secretion with feces, the rest is absorbed via the intestine and reach blood to be filtered in the kidney & secreted in form of urine urobilinogen

- **Normal urine has a small amount of**
  - Urobilinogen 0 – 4 mg / day
  - Urobilin 10 – 130 mg / day.
  - While no bilirubin is present

- **Conjugated bilirubin** will appear if the normal degradation cycle is obstructed by the bile duct or when the integrity of liver is damaged allowing, leakage of conjugated bilirubin into the circulation such as cholestasis & hepatitis.

- **Bilirubin test**
  1. Reagent strip reaction
     - Diazonium salt + bilirubin → Azodye (Diazonium Compound color)
  2. Tablet contain diazonium salt
  3. Examine the color produced from the conversion of bilirubin to biliverdin.

**Methods**

a. **Oxidation test (Harrison Spot test) = Fouchet test**
   - Filter paper is soaked in saturated BaCl₂, dried, cut in strip.
   - When performing the test, the lower half of the strip is embedded in urine sample & then removed, apply one drop of (FeCl₃ + TCA) (Fouchet reagent) in the line separated the wet & dried half.

   +ve result found as greenish color of the cut off line.

b. **Smith iodine test**

   5ml urine + 2 ml of 0.7 iodine prepared in 95% ethyl alcohol.

   +ve ——— green ring at the junction between the two fluids.
c. **Shake test**: this test neither specific nor sensitive.

   +ve yellow foam

- **Urobilinogen.**
  p-dimethyl aminobenzaldehyde (Ehrlich’s reagent)
  +ve result with urobilinogen (red color).

6. **Ketone bodies**

Ketones are 3 intermediate product of fat metabolism which are

1. Acetone (78%)
2. Aceotacetic acid (20%)
3. Beta-hydroxybutyric acid (2%).

- **Ketonurea occurs in**
  1. Diabetes acidosis
  2. Starvation
  3. Excessive Carbohydrate loss.

**Test**

Sodium nitroprusside react with aceotacetic acid

\[ \text{Acetoacetic acid} \rightarrow \text{Beta-hydroxybutyric acid (2%).} \]

7. **Leukocyte Esterase**

A positive leukocyte esterase test results from the presence of white blood cells either as whole cells or as lysed cells. Pyuria can be detected even if the urine sample contains damaged or lysed WBC’s. A negative leukocyte esterase test means that an infection is unlikely and that, without additional evidence of urinary tract infection, microscopic exam and/or urine culture need not be done to rule out significant bacteriuria.

**Reagent strip.**

Indoxylcarbonic acid Ester \[ \text{leukocyte Esterase} \rightarrow \text{Indoxyl + diazonium salt} \]

(purple color)
Microscopic examination of urine Sediment

In health, urine contains a small number of cells & other formed elements. From the entire length of genitourinary tract, casts and epithelial cells from nephron, epithelial cells from the pelvis, urinary bladder & urethra, mucous thread spermatozoa from prostate, possibly some red or white blood cells and occasional cats. Urinary sediment provides information useful for both prognosis & diagnosis. It constitutes a direct sampling of urinary tract morphology hence; it gives the following information:

1) provides evidence of renal disease as opposed to lower UTI
2) Indicate the type and state of activity of renal lesion or disease.

Methodology

A sample of well-mixed urine (usually 10-15 ml) is centrifuged in a test tube at relatively low speed (about 2-3,000 rpm) for 5-10 minutes until a moderately cohesive button is produced at the bottom of the tube. The supernatant is decanted and a volume of 0.2 to 0.5 ml is left inside the tube. The sediment is resuspended in the remaining supernatant by flicking the bottom of the tube several times. A drop of resuspended sediment is poured onto a glass slide and cover slipped.

Examination

The sediment is first examined under low power to identify most crystals, casts, squamous cells, and other large objects. The numbers of casts seen are usually reported as number of each type found per low power field (LPF). Example: 5-10 hyaline casts/LPF. Since the number of elements found in each field may vary considerably from one field to another, several fields are averaged. Next, examination is carried out at high power to identify crystals, cells, and bacteria. The various types of cells are usually described as the number of each type found per average high power field (HPF). Example: 1-5 WBC/HPF.
1. **Red Blood Cells 0-2/HPF**

- Hematuria is the presence of abnormal numbers of red cells in urine due to: glomerular damage, tumors which erode the urinary tract anywhere along its length, kidney trauma, urinary tract stones, renal infarcts, acute tubular necrosis, upper and lower urinary tract infections, nephrotoxins, and physical stress. Red cells may also contaminate the urine from the vagina in menstruating women or from trauma produced by bladder catheterization.

- Theoretically, no red cells should be found, but some find their way into the urine even in very healthy individuals. However, if one or more red cells can be found in every high power field, and if contamination can be ruled out, the specimen is probably abnormal.

- RBC's may appear normally shaped, swollen by dilute urine (in fact, only cell ghosts and free hemoglobin may remain), or created by concentrated urine. Both swollen, partly hemolyzed RBC's and created RBC's are sometimes difficult to distinguish from WBC's in the urine.

- In addition, red cell ghosts may simulate yeast. The presence of dysmorphic RBC's in urine suggests a glomerular disease such as a glomerulonephritis. Dysmorphic RBC's have odd shapes as a consequence of being distorted via passage through the abnormal glomerular structure.
• **Ghost cell (erythrocyte cell Membrane)**
  It’s a faint erythrocyte, which is exposed to hemolysis due to the presence of hypotonic alkaline urine, this indicate the presence of Hb in the sample.

• **Dysmorphic cell (shrinking Erythrocytes)**
  May indicate the presence of old RBCs due to:
  - Possible hemorrhage in the upper urinary tract (glomerulus).
  - Or indicate hypertonic urine.

○ **Note:**
  RBCs may be differentiated from yeast by:
  1. Biconcave shape (RBC)
  2. The presence of budding in yeast.

2. **White Blood Cells:** (< 4/HPF)
   - Pyuria refers to the presence of abnormal numbers of leukocytes that may appear with infection in either the upper or lower urinary tract or with acute glomerulonephritis. Usually, the WBC’s are granulocytes. White cells from the vagina, especially in the presence of vaginal and cervical infections, or the external urethral meatus in men and women may contaminate the urine.
   - However, higher numbers may be found in female urine >5 WBC’s

○ **Origin of WBC’s:**
  1. Through glomerular damage
  2. Amoeboid Migration through to the site of infection

○ **Increased WBC’s (Pyuria) present in:**
  1. Inflammation in the genitourinary system due to bacteria (Pyelonephritis – Cystitis – Prostatitis – Urethritis)
  2. Inflammation due to non bacterial agent:(Glomerulonephritis – SLE – Tumor).
Notes:
- WBC’s may be lysed in alkaline hypotonic urine to form (Glitter-Cell) in which granules are moved in Brownian movement.
- WBC’s are usually spherical, dull gray, they may occur singly or in clumps, larger than RBC’s & Less than epithelial cells in size.
- Mostly neutrophil
- In kidney infection, WBC’s tend to be associated with cellular & granular casts, bacteria, epithelial cells & relatively few RBC’s.

2. Epithelial Cells:
- Which may originate from any site of the genitourinary tract.
- Few cells can be found in urine as a result of normal sloughing off old cells.
- A marked increase may indicate inflammation

Type of Epithelial cells:
- Tubular epithelium:
  - The most significant of epithelial cells, because the finding of increased numbers indicates:
    1. Tubular necrosis
    2. Important in renal graft rejection
    3. Tubular damage such as Pyelonephritis, viral infection, and toxic reactions.
  - They are round and slightly larger than white blood cells & distinguished from leukocytes by the presence of a single round eccentrically located nucleus.
  - When lipiduria occurs, these cells contain endogenous fats. When filled with numerous fat droplets, such cells are called oval fat bodies.
b. Transitional cells:
- (Lower tract epithelium) originate from the lining of the renal pelvis, bladder & upper urethra.
- (2-4) time larger than WBC’s, may be rounded, pear shape, or may have tail-like projections
- & May contain 2 nucleoli
- They are seldom pathologically important unless large numbers exhibiting unusual morphology are seen.

c. Squamous cells:
- The most frequently seen and least significant of the epithelium cells, they are derived from the lining of vagina & lower portion of urethra.
- They are large, flat irregularly shaped cells with central nucleus with abundant cytoplasm.

3. Casts:
- Urinary casts are formed only in the distal convoluted tubule (DCT) or the collecting duct (distal nephron). The proximal convoluted tubule (PCT) and loop of Henle are not locations for cast formation. Hyaline casts are composed primarily of a mucoprotein (Tamm-Horsfall protein) secreted by tubule cells. The Tamm-Horsfall protein secretion, forming a hyaline cast in the collecting duct.
- Even with glomerular injury causing increased glomerular permeability to plasma proteins with resulting proteinuria, most matrix or "glue" that cements urinary casts together is Tamm-Horsfall mucoprotein, although albumin and some globulins are also incorporated. An example of
glomerular inflammation with leakage of RBC's to produce a red blood cell cast.

- **Factors** which favor protein cast formation are low flow rate, high salt concentration, and low pH, all of which favor protein denaturation and precipitation, particularly that of the Tamm-Horsfall protein. Protein casts with long, thin tails formed at the junction of Henle's loop and the distal convoluted tubule are called cylindroids. Hyaline casts can be seen even in healthy patients.

- In end-stage kidney disease of any cause, the urinary sediment often becomes very scant because few remaining nephrons produce dilute urine.

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**Type of Casts:**

a. **Hyaline cast:** (0-2) / LPF
   
The most frequently seen cast is the hyaline type, which consist almost entirely of Tamm–Horsfall protein and may appear as a result of strenuous exercise, fever, dehydration and stress and may appear due to pathological conditions as:
   1. Nephritis (pyelonephritis – glomerulonephritis)
   2. Chronic renal disease.

b. **Red blood cell cast:**
   
   Formed of red cells enmeshed in or a Hatched to Tamm – Horsfall protein matrix.
   
The presence of cellular cast is usually indicative of serous disease, although red cells casts have been found in healthy individuals following exercise, color of red cell cast ranging from yellow to brown, & may contain few or packed cells.
They may indicate:
1) Acute glomerulonephritis.
2) Renal infarction.
3) Kidney involvement of sub-acute bacterial endocarditis.
4) SLE

c. **White Blood Cells Cast:**
The presence of WBC’s indicates the presence of infection or inflammation within the nephron
1. Pyelonephritis & glomerulonephritis
2. Renal parenchymal infection

d. **Bacterial Casts:**
Pyelonephritis

e. **Epithelial cell casts:**
The presence of occasional epithelial cells or clumps is not remarkable, but if many epithelial casts are found, the following disease may damage the tubular epithelium.
1. Nephritis
2. Toxins
4. Acute tubular necrosis

f. **Granular Casts:**
Coarsely or finely granular casts are frequently seen, which may be associated with pathological or non-pathological conditions appears to be the lysosomes excreted by renal tubular cells during normal metabolism and increased excretion due to metabolism in stress and exercise.
In disease states, granules may represent disintegration of cellular casts and tubule cell protein aggregates filtered by the glomeruli.
Clinical implications:
1. Acute tubular necrosis
2. Advanced glomerulonephritis
3. Pyelonephritis
4. Lead poisoning

g. **Waxy casts:**
They are refractive with a rigid texture, yellow or gray, or colorless, homogenous appearance, they result from degeneration of granular & hyaline casts & Found in:
1. Tubular inflammation & degradation.
2. Chronic renal failure.
3. Localized nephron obstruction.

h. **Broad waxy casts:**
   Are found in urine considered as the most ominous of all cast types.

i. **Broad casts:**
   All casts forms can occur in the broad from which is formed in the collecting ducts & called renal failure casts.

j. **Fatty casts:**
   Casts contain fat droplets (bodies), refractive formed of oval fat bodies & integrated fats attached to casts matrix to for Fatty casts in lipiduria as (nephrotic syndrome).

4. **Bacteria:**
   Bacteria are common in urine specimens because of the abundant normal microbial flora of the vagina or external urethral meatus and because of their ability to rapidly multiply in urine standing at room temperature. Therefore, microbial organisms found in all but the most scrupulously collected urines should be interpreted in view of clinical symptoms.

   Diagnosis of bacteriuria in a case of suspected urinary tract infection requires culture. A colony count may also be done to see if significant numbers of bacteria are present. Generally, more than 100,000/ml of one organism reflects significant bacteriuria. Multiple organisms reflect contamination. However, the presence of any organism in catheterized or suprapubic tap specimens should be considered significant.

5. **Yeast:**
   Yeast cells may be contaminants or represent a true yeast infection. They are often difficult to distinguish from red cells and amorphous crystals but are distinguished by their tendency to bud. Most often they are Candida, which may colonize bladder, urethra, or vagina.

6. **Mucus:**
   Protein, formed from the epithelium of the genitourinary

7. **Miscellaneous:**
   General "crud" or unidentifiable objects may find their way into a specimen, particularly those that patients bring from home.
   Spermatozoa can sometimes be seen rarely. Trichomonas vaginalis (Contaminated from vaginal secretion), pinworm ova may contaminate the urine. In Egypt, ova from bladder infestations with schistosomiasis may be seen.
Unorganized Sediments

Crystal are frequently found in urine, although they are seldom of clinical significance, identification must be made to ensure that they don’t represent an abnormality. Crystals are formed by the precipitation of urine salts subjected to changes in pH, temperature or concentration, which affect their solubility. Which appear in urine in the form of either true crystal or amorphous material. The most valuable aid in crystal identification is knowledge of urine pH, because this will type the chemicals precipitated hence crystal are categorized in normal of abnormal as well as crystals in acidic or alkaline urine.

1- Normal edogenous crystals:
A. Acidic urine:

- Uric acid plates rhombic, rosettes, wedges & needles. Increase levels are seen in leukemia, gout.

- Amorphous Urate yellow brown granules if present in large amount may give urine pink color.

- Calcium oxalate color less octahedral resembles envelopes. They are associated with high oxalic acid and with chemical toxicity and are seen – in genetically susceptible person following large doses of ascorbic acid.

B. Alkaline Urine
Phosphates are the most common crystals found in alkaline urine.

- Triple phosphate (Colorless prism)
- Amorphous phosphate (granules). If present in large amounts the produce white turbidity in urine.
- Calcium phosphate: (Colorless thin prisms, plates or needless). When found in neutral urine they may be confused with abnormal sulfonamide crystal, however calcium phosphate crystals are soluble in dilute acetic acid and sulfonamide are not.
- Ammonium biurate (Brownish yellow)
- Calcium Carbonate: (Small colorless with dumbbell or spherical shops). They may occur in clumps that resemble amorphous phosphate, but they can distinguish by the formation of gas after the addition of acetic acid.
2- Abnormal endogenous crystals

- Cystine, cholesterol, leucine, tyrosine, bilirubin, sulfonamide, radiographic dye, and medications. Ampicillin.
- Hemosidren, appearing as yellow – brown granules, may also be seen in the nephron.

- **Exogenous crystals**: as starch (gloves) & telcum bounder granules.
Urinary calculi

❖ Introduction
The occurrence of urinary calculi in the developed world is relatively common. Up to 85% of stones are formed from calcium oxalate. Other calculi may be composed of uric acid, struvite or cystine. The most common association is hypercalciuria. The first line investigations of such patients are urine microscopy, renal function tests and imaging of the urinary tract. The emphasis on management should be on prevention with the focus on appropriate diet and increased fluid intake.

Ultrasound is very useful for the identification of urinary calculi in cats and dogs. Unlike radiography, all types of calculi are visible on ultrasound and it provides a rapid and non-invasive method of diagnosis. The aim of this article is to demonstrate the characteristics of this condition which allows differentiation from other conditions of the urinary bladder.

❖ How bladder stones are formed
All bladder stones, whether they are struvite or any other type, are formed by minerals which first precipitate out in the urine as individual microscopic crystals. Over time, these crystals unite and small grains of sand are formed. Once these first grains are present, additional precipitation forms on their surface and the tiny specks are gradually built into stones that sometimes reach 3" to 4" in diameter. As a simple example, this is the same process that occurs when you put sugar into a hot cup of coffee. At first it all dissolves, but as the liquid cools its carrying capacity decreases and the sugar returns to its granular form. There is no such temperature change in the bladder but the dissolved minerals still precipitate out in the form of microscopic crystals. If this happened only rarely, no harm would be done as they would be flushed out of the body with the urine. In certain animals, however, large quantities of minerals are rapidly formed and clinical urolithiasis develops.
What causes bladder stones?
The process is really quite simple, but what causes it to occur only in certain dogs, cats or humans? As we understand it today, the factors that bring it about are genetic predisposition, bacterial infections, diet, and urine pH. Any one of these could be solely responsible but it is usually a combination of any or all of them.

- **Genetics:** The genetically controlled physiology of some animals causes them to produce within their bodies the higher levels of the substances that are precursors of the crystals. They are then excreted or formed in the urine.

- **Bacterial Infections:** Bacterial infections of the bladder (referred to as cystitis) play a large role in struvite stone formation for two reasons: (1) they tend to make the urine more alkaline (with pH higher than 7.0) and (2) by-products of their metabolism actually initiate chemical reactions that cause the magnesium ammonium phosphate crystals to form. Most bacterial infections of the bladder tend to raise the pH of the urine. This is important in this specific condition as struvite crystals are more PH to remain in solution if the liquid is acidic (with a pH lower the 7.0). That is, they would continue to be dissolved in the liquid and no crystals would form. Additionally, many of the bacteria that cause a cystitis also produce an enzyme (a compound that causes chemical reactions to occur) called urease. This enzyme starts the process by reacting with urea molecules found in the urine to form ammonium and carbon dioxide. The ammonia is slowly converted to ammonium ions while the carbon dioxide unites with other compounds, freeing up phosphates. Then, through a chain of chemical reactions that seem to feed on each other and at the same time raise the pH of the urine into the alkaline range even more, the magnesium that is normally present within the urine unites with the ammonium and phosphate to form magnesium ammonium an phosphates (struvite). If the crystals are formed rapidly and in large quantities, they will unite together to form stones. However, if only small amounts are formed over a longer period of time they would simply be flushed out in the urine without producing any problems.

- **Diet:** Diet also plays a role in struvite formation. The urea that we mentioned above is formed when protein within the bladder is broken down by bacteria. The body's breakdown of large dietary proteins into smaller molecules also produces urea. Diets with excessively high levels of proteins simply provide the system with more urea to work with in the formation of ammonium and carbon dioxide. In truth, this may be the only
factor in animals fed all-meat diets. Commercially prepared dog foods, even the highest protein varieties, would not be a factor in most animals.

- **Renal tubular acidosis:** This is an acidification defect of the renal tubular cells resulting in high urinary pH with increased risk of calcium phosphate precipitation. The disorder is very prevalent in female patients (often with an early onset), who often suffer from multiple stone formation.

❖ **Types of urinary calculi:**
There are several different types of bladder stones, depending on their chemical make-up. In this article we will deal with the more common struvite stones that are composed of magnesium ammonium phosphate. Others are made of calcium oxalate, calcium apatite, cystine, or ammonium urate. Each form has its own different peculiarities as to which breed is most often affected and what factors affect the formation. However, by understanding the struvite ones we will learn a lot about urolithiasis in general.

- **Hypercalciuria**
Criteria for this condition are controversial and the calcium secretion in urine is influenced by factors such as sex, age and diet. There are four types of hypercalciuria:
  - Absorption hypercalciuria is associated with increased intestinal absorption of calcium, probably due to increased synthesis of vitamin D3.
  - In renal hypercalciuria, renal tubular calcium reabsorption is defective resulting in a higher urinary output.
  - The dietary type is caused by high ingestion of food ingredients rich in calcium, especially milk. Sodium, sugar and animal protein can also increase the urinary calcium output. The potential for hypercalciuria to develop should be borne in mind when prescribing calcium supplements.
  - Resorption hypercalciuria is due to increased skeletal calcium resorption as seen in patients with primary hyperparathyroidism.

- **Uric acid disorders**
Many of these patients have an unusually severe form of stone disease. The patients may have increased serum uric acid, and/or increased secretion of uric acid in the urine and/or uric acid as a component stone. One subgroup is idiopathic with a constant low urinary pH that may provoke uric acid precipitation. Patients with ileostomy, Crohn's disease and ulcerative colitis are prone to uric acid stone formation due to a loss of alkali and fluid. **Hyperoxaluria** (urinary oxalate > 40 mg/day [> 440 µmol/day]) can be primary or caused by excess ingestion of oxalate-containing foods (e.g., rhubarb, spinach, cocoa, nuts, pepper, tea) or by excess oxalate absorption due to various enteric
diseases (eg, bacterial overgrowth syndromes, chronic pancreatic or biliary disease) or ileojejunal surgery. The clinical history and amount of oxalate in the urine will help determine the cause.

The clinical approach

Examination
The physical examination should include inspection for gouty tophi and palpation of the abdomen including the renal angles, and lymph node areas. The blood pressure should be measured, and if indicated, rectal and/or pelvic examination performed.

Investigations
In taking the blood samples it is important to relieve the pressure of the tourniquet while still taking the blood (otherwise there may be falsely elevated values).

Table. Investigations for recurrent urinary calculi
• Blood sample to measure serum levels of:
  • Calcium.
  • Phosphate.
  • Uric acid.
  • Alkaline phosphates.
  • sodium potassium chloride.
  • Magnesium.
  • Creatinine.
Microscopy of urine

Urine examination is recommended on a freshly voided morning sample using dip stick test and microscopic examination and culture. Microscopy may reveal the following crystals:
- oxalate (envelope)
- calcium phosphate (amorphous)
- triple phosphate (coffin lid)
- uric acid (needle shaped) and cystine (hexagonal), as well as any other formed elements. As urine cools to room temperature, crystal deposition renders this of doubtful significance.

- Twenty-four hour urinary sampling
Although this has been a standard method of determining urinary calcium and uric acid its value has been called into question since the daily lives of both these substances can fluctuate quite widely in the same individual. At least two consecutive 24 hour samples should be taken in patients having formed a high number of stones and/or failing to respond satisfactorily to treatment. The main value of this test is in estimating the total volume of urine, which in these patients should not be below two liters per day.

- Stone analysis
This is regarded as essential, because the content of the stone may determine the subsequent evaluation of the patient. The presence of uric acid, cystine or struvite will have therapeutic consequences. Chemical qualitative analysis is generally adequate.

- X-ray/imaging techniques
If a calculus is suspected, intravenous pyelography (urogram) should be included. It may also reveal structural abnormalities.
For follow up and for patients with suspected obstruction, a plain film of the urinary tract is considered sufficient.
For patients experiencing a colic episode, arranging X-rays is not urgent. Even with severe obstruction, significant renal damage will not occur for several weeks.
Diagnostic ultrasound is an alternative to the urogram. Combined with a plain X-ray, this method may be preferred for assessing patients with recurrent stones and has a considerably lower radiation dose.
Notes: The triple phosphate doesn’t form stone alone, because it is ppt in alkaline media and this media will be change directly by eating.

1-Powder+ Uric acid indicator(U.A).
2-Incubation for 5 min. in RM.
3-Color pink will be appear if the U.A form it.

Another Test
1-Powder + 6%HNO₃+heat to boil then leave it to cooled ppt .then filtrated it. The filtrate make the following on it:
- Filtrate + FeCL₃ +ammonia: If white ppt appeared so there is oxalate
- Filtrate + Reagent(phosphor): Blue Color will be appear if the there is phosphor.
- Filtrate + H₂SO₄: after a good mixed filter it and add HCL to anew filter then KMO₄ and heat slowly if the golden color appeared the calculi is Ca if no the color change the calculi is Mg.
Cerebrospinal fluid

- Cerebrospinal fluid (CSF) is a clear, colorless liquid that fills the ventricles (cavities) of the brain and the spinal cord, surrounds them as well, and acts as a lubricant and a mechanical barrier against shock.
- The nervous system of the vertebrate embryo consists of a hollow tube with a canal running through its whole length. As the organism develops, the canal becomes narrow in the spinal cord, while widening in the brain and creating the ventricles. CSF is formed primarily in the ventricles, which serve as a network of interconnected holds. The fluid flows down through the brain-stem canal, and leaves the central nervous system by being absorbed into surrounding tissue spaces.
- CSF has a slightly alkaline chemical composition, similar to blood. It contains no red blood cells, and low amounts of protein and lipids in comparison to blood. It is about 99 percent water. There are about 100 to 150 milliliters of cerebrospinal fluid in the normal adult body.

Formation

CSF is a secretion product mainly of the choroids plexus (ultra filtration of blood in the choroids plexus) on the ventricles but also of the ependymal lining of the ventricles of the brain and possibly of the cerebral subarachnoid space. It assumes its final composition as a result of material exchange between with the blood and adjacent brain tissue. Secretion predominates in the ventricles and absorption in the subarachnoid space (arachnoid villi); a flow of CSF is produced from the ventricles into the subarachnoid space. CSF circulates through the Foramen of Monro from the two lateral ventricles to the third ventricle, down the Aqueduct of Sylvius to the fourth ventricle and into the subarachnoid space via the posterior Foramen of Magendie and the lateral Foramina of Luschka. Some of the CSF travels down the central canal of the spinal cord.
• 0.3 ml/min.
• 20 ml/hr.
• 500 ml/day.

Functions of the CSF
1. The CNS: brain and spinal cord are leave floating by the cerebrospinal fluid medium in which they are suspended. This provides the nervous system with support and protection against rapid movements and trauma. It is appears to act as a cushion.
2. The CSF is believed to be nutritive for both neurons and glial cells.
3. The CSF provides a medium for removing waste products of cellular metabolism form the nervous system. In this capacity, it functions like a lymphatic system.
4. The CSF plays a role in maintaining the constancy of the ionic composition of the local microenvironment of the cells of the nervous system. The extracellular space of the brain freely communicates with the CSF compartment and therefore the composition of the two fluid compartments is similar.
5. The presence of a number of biologically active principles (releasing factors, hormones, neurotransmitters, metabolites) within the CSF suggests that it may function as a transport system.
6. Since the CSF and brain extracellular space are in continuity analysis of the composition of the CSF provides diagnostic information about the normal and pathological state of the nervous system function.

Sample collection
Lumbar puncture (spinal tap) is the most common means of collecting a specimen of CSF. The patient is positioned on his side with his knees curled up to his abdomen and his chin tucked in to his chest (occasionally this procedure is performed with the person sitting bent forward). The skin is cleaned, and a local anesthetic is injected over the lower spine. The spinal needle is inserted, usually between the 3rd and 4th lumbar vertebrae. Once the needle is properly positioned in the subarachnoid space, pressures can be measured (normal pressure between 80-190 mm) and fluid can be collected for testing (10-20 ml of CSF is collected). After the sample is collected, the needle is removed, the area is cleaned, and a bandage is applied. The patient will be asked to remain flat, or nearly flat, for 6 to 8 hours after the test. Alternative methods of obtaining CSF are rarely used, but may be indicated if there is a problem such as lumbar deformity or infection, which would make lumbar puncture impossible or unreliable.
Remark
1. The specimen must be collected under sterile conditions, sealed immediately to prevent leakage or contamination, and sent to the laboratory without delay.
2. The specimen should be labeled with the patient’s name, age, date, room number, and suspected disease. The laboratory staff should be alerted so that they can prepare to examine the specimen immediately.
3. Blood sample should be collected 30 min. before lumbar puncture for glucose, protein and immunoglobulin determination.
4. Specimens are usually collected in three sterile tubes, labeled 1, 2, and 3 in order in which they are drawn, tube 1 for chemistry and serology, tube 2 for microbiology, while tube 3 is used for cell count and differential (2-4 ml in each tube).
5. The attending physician should be notified as soon as results are obtained so that appropriate treatment can be started.
6. CSF specimens for additional chemical and serological tests should be frozen, hematology tubes are refrigerated, and microbiology tubes remain at room temperature.

❖ Purpose of CSF Analysis
The purpose of a CSF analysis is to diagnose medical disorders that affect the central nervous system. Some of these conditions include:
1. Viral and bacterial infections, such as meningitis and encephalitis.
2. Tumors or cancers of the nervous system.
3. Bleeding (hemorrhage) around the brain and spinal cord.
4. Multiple sclerosis: a disease that affects the myelin coating of the nerve fibers of the brain and spinal cord.
5. Syphilis, a sexually transmitted disease.
Macroscopic examination

Total volume
- In adult, CSF volume is 90-150 ml.
- In neonates, CSF volume is 10-60 ml.

Distribution
- 20 ml in the ventricles.
- 60 ml in the subarachnoid space.
- 70 ml in the spinal canal.

Appearance
Normal CSF is crystal clear and the consistency of water. The major terminology used to describe CSF appearance includes crystal clear, cloudy or turbid, milky, xanthochromic, and bloody.

Cloudy, turbid or milky: May be caused by WBCs (over 200 cells/µl), RBCs (over 400 cells/µl), microorganisms (bacteria, fungi, amebas), contrast media, aspiration of epidural fat during lumbar puncture, or radiographic contrast media.

Xanthochromic: Xanthochromia is a term used to describe CSF supernatant that is pink, orange or yellow. It may be caused by the following:
1. Oxyhemoglobin: from lysed RBCs present in CSF before lumbar puncture, or traumatic tap with lysis of RBCs after lumbar puncture.
2. Bilirubin from lysed RBCs in CSF, or increased direct bilirubin with normal blood-brain barrier, or premature infants an immature blood-CSF barrier plus elevated total Bilirubin.
3. CSF protein levels over 150 mg/dl or traumatic tap with sufficient plasma concentration to produce protein concentration over 150 mg/dl.
4. Contamination of CSF by Merthiolate used to disinfect the skin.
5. Carotenoids in CSF due to systemic hypercharotenemia
6. Melanin in CSF due to meningeal melanosarcoma.

Bloody: grossly bloody CSF can be an indication of subarachnoid hemorrhage, but it also may be due to the puncture of a blood vessel during the spinal tap procedure. We can differentiate between both by:
1. Uneven distribution of blood: Traumatic tap often shows significant clearing of blood between the first and third tubes.
2. Centrifugation: Traumatic tap often shows significant clear supernatant after centrifugation.
3. Clot formation: Fluid collected from traumatic tap may form clots.
due to the introduction of plasma fibrinogen into the specimen.
4. The presence of erythrophagia in blood film of subarachnoid hemorrhage.
5. Xanthochromic supernatant: intracranial hemorrhage is associated with small Xanthochromia caused by release of Hb from hemolysed RBCs. Care should be taken, however to consider this examination in conjunction with those previously discussed, because a very recent hemorrhage would produce a clear supernatant, and introduction of serum protein from a traumatic tap could also cause the fluid to appear xanthochromic.

❖ **Specific gravity:** 1.006 – 1.008

❖ **pH**: Alkaline

❖ **Spontaneous clotting**
Clotting occurs when there is an excess of fibrinogen in the specimen, usually associated with a very high protein concentration. This finding occurs classically in association with tuberculous meningitis or with tumors in CNS.

<table>
<thead>
<tr>
<th>Microscopic examination</th>
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</thead>
</table>

❖ **CSF cell count**
- The cell count that is routinely performed on CSF specimen is the WBC's count.
- *NOTE*: Cell counts should be done within 30 minutes after withdrawal of the specimen to avoid cell disintegration. Specimen that can't be analyzed immediately should be refrigerated.
- Normal adult CSF contains 0 to 5 WBC's /µl. the number is higher in children and as many as 30 WBC's /µl can be consider normal in newborns.

❖ **Materials**

**Diluting Fluid**
Crystal Violet, 0.2 gm
Glacial Acetic Acid, 10 ml
Distilled Water, 100 ml
Procedure
- (Clear specimen is counted undiluted while the dilution is for the turbid one)
  1. Mix specimen thoroughly by gentle inversion.
  2. Draw the diluting fluid to the 1.0 mark of a white cell diluting pipette.
  3. Draw the well-mixed sample of CSF to the 11.0 mark of the pipette.
  4. Mix well, about 1 minute.
  5. Discard approximately 1/3 of the fluid and charge the haemocytometer.
  6. Allow to sit undisturbed for a few minutes in order for cells to settle.
  7. Count all the cells in the entire ruled area (9 mm²) under low power for the total WBC count.
  8. Calculation: as in WBC’s total count.

NOTES:
- RBCs will be lysed with this method. To perform RBCs count use either undiluted CSF or if many RBC’s present, saline diluent.
- Correction for contamination:
  WBC’s (added) = (WBC’s (blood) x RBC’s CSF) / RBC's (blood)

Differential cell count:
The differential count should be performed on stained smear and not from the cells in the counting chamber, 100 cells should be counted, classified, and reported in term of %

<table>
<thead>
<tr>
<th>Differential</th>
<th>Adult</th>
<th>Neonates</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lymphocyte</td>
<td>34 ± 62 %</td>
<td>18 ± 20 %</td>
</tr>
<tr>
<td>Neut.</td>
<td>2 ± 5 %</td>
<td>3 ± 5 %</td>
</tr>
<tr>
<td>Mono.</td>
<td>20 ± 36 %</td>
<td>22 ± 72 %</td>
</tr>
<tr>
<td>Eosinophil</td>
<td>Rare</td>
<td>Rare</td>
</tr>
</tbody>
</table>

Causes for increased Neutrophils in CSF
1. Meningitis:
   a. Bacterial meningitis.
   b. Early viral meningoencephalitis.
   c. Early tuberculous meningitis.
   d. Early mycotic meningitis.
   e. Amebic encephalomyelitis.
2. Other infections:
   a. Cerebral abscess.
   b. Subdural empyema.
3. Following seizures.
4. Following CNS hemorrhage:
   a. Subarachnoid hemorrhage.
   b. Intracerebral hemorrhage.
5. Following CNS infarct.
6. Reaction to repeated lumbar puncture.
7. Injection of foreign materials in subarachnoid space (e.g., contrast media).
8. Metastatic tumor in contact with CSF.

**Causes for increased Lymphocytes in CSF**
1. Meningitis:
   a. Viral meningitis.
   b. Syphilitic meningoencephalitis.
   c. Tuberculous meningitis.
   d. Fungal meningitis.
   e. Bacterial meningitis due to *listeria monocytogenes*.
   f. Parasitic infestations of the CNS e.g., toxoplasmosis.
2. Degenerative disorders.
   a. Multiple sclerosis.
3. Other inflammatory conditions.

**Causes for increased Eosinophil in CSF**
1. Parasitic infestations.
2. Fungal infection.
3. Rickettsial.
4. Other (intracranial shunt, foreign material).

**Increased number of Monocytes** usually occurs as part of a "mixed reaction"

- With Neutrophil, lymphocytes, and plasma cells: In Tuberculous meningitis, Fungal meningitis, chronic bacterial meningitis
- Without Neutrophil: In viral meningoencephalitis and Syphilitic meningoencephalitis
**Biochemical estimation**

- CSF is formed by filtration of the plasma, one would expect to find the same chemicals in the CSF as are found in the plasma.
- This is essentially true; however, because the filtration process is selective and the chemical composition is also adjusted by the blood-brain barrier, normal values of CSF chemicals are not the same as the plasma values.
- Abnormal values are the result of alteration in the permeability of the blood-brain barrier or increased production or metabolism by the neural cells in response to a pathologic condition, and they seldom have the same diagnostic significance as plasma abnormalities.

❖ **Proteins:**
- The most frequently performed chemical test on CSF is the protein determination. Normal CSF contains a very small amount of protein.
- Normal CSF protein concentration (mg/dl) is less than 1% of serum protein concentration (g/dl) and usually listed as 15 to 45 mg/dl with slightly higher values found in infants and elderly people.
- Clinical significance of elevated protein: Marked elevated CSF protein is found in the following pathologic conditions, *Froin's syndrome* (complete spinal block), *Cerebral Tumors* *Meningitis*.
- A rise in CSF protein is seen in various diseases as a result of three primary mechanisms:
  1. Decreased clearance of normal protein from the fluid and degeneration of neural tissue.
  2. Increased local synthesis of immunoglobulin.
  3. Increased capillary permeability due to the blood-brain barrier damage.

❖ **The IgG-albumin index**
The IgG-albumin index can be used to distinguish diseases affecting permeability (meningitis, cerebral infarctions, tumors of the brain or spin) from diseases resulting in increased immunoglobulin (usually IgG) synthesis (multiple sclerosis) and some inflammatory diseases (idiopathic polyneuropathies). A normal range for this index has been proposed to be 0.34-0.58. In diseases associated with increased IgG production, the ratio is elevated, whereas in diseases affecting CSF permeability, the ratio is decreased because of increased CSF albumin concentration. Of course some disorders can affect both CSF IgG concentration and blood permeability.
The IgG-albumin index = (CSF IgG/Serum IgG) / (CSF albumin/Serum albumin)

- **Glucose**
  - Glucose enters the CSF by active transport across the blood-brain barrier. The CSF glucose concentration is slightly lower than that in plasma and usually between 60 – 70 % of plasma glucose concentration.
  - The blood glucose sample is needed for comparison. Ideally the blood glucose sample should be drawn at least 30 min. before the lumbar puncture to allow time for equilibrium between the blood and the fluid.

  - **Clinical significance:**
    - Low CSF glucose values can be of considerable diagnostic value in determining the causative agents in meningitis.
    - The finding of markedly decreased CSF glucose accompanied by:
      1. An increased WBC's count and a large percentage of Neutrophil is most indicative of bacterial meningitis.
      2. WBC's count and a large percentage of Lymphocytes is most indicative of tubercular meningitis.

- **Remark:** Decreased glucose values are thought to be caused primarily by alterations in the mechanism of glucose transport across the blood-brain barrier and by increased utilization of glucose by the brain cells. Consumption of glucose by the microorganisms and leukocytes that are present in the fluid could not account for such decreased values as it would not be possible to explain the variations in glucose concentrations seen in different types of meningitis.

- **CSF Lactate**
  - Measurement of lactate concentrations in cerebrospinal fluid (CSF) may be useful as part of the investigation of inborn errors of metabolism in which lactic acidosis occurs. This includes disorders of gluconeogenesis, pyruvate dehydrogenase complex, the Krebs cycle and the mitochondrial electron transport chain. Measurement of lactate in CSF has also been advocated for investigating children with unexplained neurological disease.
### Chemicals in CSF

<table>
<thead>
<tr>
<th>Component</th>
<th>Normal Range</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glucose</td>
<td>60 – 70% of plasma glucose (45-75 mg/dl)</td>
</tr>
<tr>
<td>Protein</td>
<td>15 – 45 mg/dl</td>
</tr>
<tr>
<td>Creatinine</td>
<td>0.6 – 1.2 mg/dl</td>
</tr>
<tr>
<td>BUN</td>
<td>6 – 15 mg/dl</td>
</tr>
<tr>
<td>Ca++</td>
<td>4 – 6 mg/dl</td>
</tr>
<tr>
<td>Chloride</td>
<td>710 – 750 mg/dl</td>
</tr>
<tr>
<td>Lactic acid</td>
<td>10 – 22 mg/dl</td>
</tr>
<tr>
<td>Bilirubin</td>
<td>Nil</td>
</tr>
<tr>
<td>RBCs count</td>
<td>0 – 10 cells/µl</td>
</tr>
<tr>
<td>WBCs count</td>
<td>0 – 5 cells/µl</td>
</tr>
</tbody>
</table>

### Microbiological examination

- **Stain & Culture:**
  If infection is suspected, the centrifuged CSF sediment is stained for _bacteria_
  - Acid-fast for TB, or immunofluorescence staining, for Cryptococcus sp (India ink).
  - Larger amounts of fluid (10 mL) improve the chances of detecting the pathogen, especially acid-fast bacilli and certain fungi in stains and culture.
  - CSF should be cultured aerobically and anaerobically and for acid-fast bacilli and fungi.
  - Latex particle agglutination and coagglutination tests are used to rapidly identify bacteria, especially when stains and cultures are negative (e.g., in partially treated meningitis).

- **Serological examination:**
  Except for enteroviruses, viruses are seldom isolated from the CSF. Viral antibody panels are commercially available. Venereal Disease Research Laboratories (VDRL) testing and cryptococcal antigen testing are often routinely performed.
# Summary

<table>
<thead>
<tr>
<th>Cause</th>
<th>Appearance</th>
<th>Neutrophil</th>
<th>Lymphocyte</th>
<th>Protein</th>
<th>Glucose</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>bacterial meningitis</strong></td>
<td>Yellowish, turbid</td>
<td>Markedly increase</td>
<td>Slightly increase or Normal</td>
<td>Slightly increase or Normal</td>
<td>Decrease</td>
</tr>
<tr>
<td><strong>Viral meningitis</strong></td>
<td>Clear fluid</td>
<td>Slightly increase or Normal</td>
<td>Markedly increase</td>
<td>Markedly increase</td>
<td>Normal</td>
</tr>
<tr>
<td><strong>Tuberculous meningitis</strong></td>
<td>Yellowish and viscous</td>
<td>Slightly increase or Normal</td>
<td>Markedly increase</td>
<td>Slightly increase or Normal</td>
<td>Decrease</td>
</tr>
<tr>
<td><strong>Fungal meningitis</strong></td>
<td>Yellowish and viscous</td>
<td>Slightly increase or Normal</td>
<td>Markedly increase</td>
<td>Slightly increase or Normal</td>
<td>Normal or decrease</td>
</tr>
</tbody>
</table>
Synovial Fluid analysis

- **Alternative names:** Joint fluid analysis; Joint fluid aspiration

- **Definition**
  Synovial fluid is viscous colorless liquid that found in the joint cavities.

- **Formation of synovial fluid**
  It is formed as an ultrafiltrate of the plasma across the synovial membrane into which a mucopolysaccharide containing hyaluronic acid and small amount of protein (low molecular weight proteins) is secreted by the cells of synovial membrane. Except for high molecular weight proteins (more than 12 kilo Dalton in size), the plasma filtration is non-selective; therefore, normal synovial fluid has essentially the same chemical composition as the plasma.

- **Function of synovial fluid**
  1. Lubricate the joint space; as a lubricant to the surfaces of the frequently moving joints.
  2. Supplies nutrients to particular cartilage; as nutrition must be provided by moving synovial fluid in and out of the cartilage, it may be clear that joint movement is essential to cartilage nutrition and maintenance.
  3. Shock absorber; our cartilage, immersed in the synovial fluid, protects our bones from the tremendous impact they would receive when we walk, run, jump, etc. This fluid also has remarkable properties as a shock absorbing, or hydraulic fluid.

- **Why the test is performed**
  1. To identify joints.
  2. To diagnose certain types of arthritis and inflammatory joint diseases, and relieve pain and distention from fluid the cause of swelling in the accumulation in the joint.

- **Sample collection (Arthrocentesis)**
  - The Fluid is collected by a physician by means of needle aspiration of the knee called arthrocentesis.
  - Arthrocentesis (removal of SF from a joint) done when patient presents with effusion
  - The normal amount of fluid contained in the knee cavity is less than 3.5 ml and increased in joint disorders.
- **Fluid from syringe is divided into 3 tubes:**
  1. 2-5 ml in EDTA tube for cell count, crystal exam. (Liquid EDTA or Na heparin may be used while powdered EDTA and lithium heparin should be avoided as anticoagulant).
  2. 5-10 ml in plain sterile tube for microbiology (culture and stain).
  3. Plane tube for Chem. and Immunologic tests.

---

### Gross (macroscopic) examination

- **Total volume:**
  The total volume collected recorded on each time.

- **Viscosity:**
  - Normal synovial fluid is highly viscous due to the polymerization of the hyaluronic acid, which is essential for the proper lubrication of joints.
  - Joint fluid is aspirated into a pipette and then released. If the falling drop of joint is drawn out into a 4 to 6 cm long or longer tenacious band the viscosity is normal. If the drop falls like water, the viscosity is low.

- **Decrease synovial fluid viscosity is usually associated with:**
  - i) Depolymerization of hyaluronate complex which present in the following conditions:
    1. Rheumatoid arthritis.
    2. septic arthritis.
    3. gout.
  - ii) Dilution of hyaluronate complex or even decrease production due to rabid effusion which take place in trauma.

- **The mucin clot formation test**
  - **Principle:**
    Mucin (a hyaluronic acid–protein complex) is precipitated by acetic acid. the morphology of the precipitated is a reflection of the hyaluronic acid content and quality of the joint fluid.
  - **Reagent:**
    Acetic acid, 7N: Mix 408 ml glacial acetic acid and 1 L distilled water.
o **Procedure:**
Add 1 ml joint fluid to 4 ml distilled water in a test tube. Then add 0.14 ml of 7N Acetic acid and stir briskly with a glass rod. Examine immediately and after 2 hr.

o **Interpretation:**
If a hyaluronic acid is normal, a tight ropy mass forms in a clear solution, indicating “good” mucin. “Fair” mucin is indicated by a softer in a turbid solution precipitated that shreds into the solution. “Poor” precipitated consists of shreds

❖ **Clot formation:**
Because of lack of fibrinogen and other clotting factors, normal joint fluid not clots. Inflammatory processes allow the plasma clotting factors to escape into the joint fluid, which then clot.

❖ **Color and Transparency**
1. **Color:** *Normal SF is pale yellow*
   o **Abnormal colors:**
     • Dark red or dark brown (bloody) {hemarthrosis} may due to:
       1. fracture through the joint surface
       2. tumor involving the joint
       3. traumatic arthritis
       4. hemophilic arthritis
       5. may be present in septic or rheumatoid arthritis.
   o Traumatic tap may be identified by uneven distribution of blood which may appear as streak in the syringe
     • Deeper yellow or Green tinge:
       1. Bacterial infection.
       2. Chronic rheumatoid arthritis.
     • Xanthochromia which is difficult to be evaluated owing to the yellow color of synovial fluid, hence any color is usually due to hemarthrosis.
     • Milky:
       1. gouty arthritis
       2. tuberculous arthritis
       3. chronic rheumatoid arthritis
       4. SLE
       5. calcium hydroxyapatite crystals
2. Transparency: Normal = crystal clear
   - Turbid: leukocytosis (more than 200 cell/µl).
   - Cloudy Milky, fatty: cholesterol crystals.
   - Fibrin.
   - Degenerative synovial cells which gives free floating tissue aggregates.

Microscopic Examination

- **Cell count and differential**
  - The white cell counting technique is used, but isotonic saline solution (ex. 0.3 saline, 0.1 HCL, 1% saponin in saline) is substituted the usual acetic acid since the latter precipitates the hyaluronic acid–protein complex and that lead to WBCs clumping due to mucin clot.
  - The cell count should be completed without delay to prevent spontaneous clumping of leukocytes.
  - Degeneration of leukocytes begin one hour after sample collection
  - If the fluid is highly viscous it must be incubate at 37°C with hyaluronidase enzyme 0.05% for 5 min.
  - Normal value: 200WBCs/µl.
  - RBCs usually present in very low numbers but may be present because of trauma of aspiration.

- **Differential cells count:**
  Mononuclear cells including Monocytes, lymphocytes, macrophage and synovial tissue cells are the primary cells in the normal synovial fluid
  Polymorphnuclear cells 0-25% (mean=6%)
  Lymphocytes 0-78% (mean=25%)
  Monocytes 0-71% (mean=48%)
  Macrophages 0-26% (mean=10%)

- **Types of cells seen:**
  - Lymphocytes, Monocytes, Macrophages
  - Synovial tissue cells
  - PMN, LE cells
  - Reiter cells: vacuolated macrophage containing a neutrophil
  - Ragocytes: PMN containing RA factor inclusions.
  - Fat droplets.
  - Bacteria.
Increased No. of neutrophil indicate septic inflammation > 80%
1. Bacterial arthritis
2. Gouty arthritis
3. Rheumatoid arthritis

Increased No. of lymphocytes indicate non septic inflammation
1. Viral infection
2. Rheumatoid arthritis in which lymphocytes may be found.

Increased No. of eosinophile > 2 %
1. Metastatic carcinoma in the synovium.
2. Acute rheumatic fever.
3. Rheumatoid arthritis.

Wet preparation:
No crystals
No rheumatoid arthritis cells
No cartilage fibers
No bacteria

Crystals that may be found:
- Crystals indicate the presence of crystal induced arthritis.
- Test must be performed at the collection time because crystals are affected by pH, temperature, as refrigeration of sample will increase MSU.

Two types of crystals may be found
- Endogenous crystals:
  1. Monosodium urate (uric acid) MSU, needle –like seen in gout
  2. Calcium pyrophosphate dehydrate (CPPD), seen in pseudogout they are needles, plates or rod-like rhomboid
  3. Cholesterol, flat rectangular notched plate
  4. Apatite =small needles
  5. Calcium oxalate envelop shape

- Exogenous crystals:
  1. Gloves powder
  2. Corticosteroids
Chemical examination

Because SF is chemically an ultrafiltrate of plasma, chemistry tests values are approximately the same as serum values

❖ Glucose:
  o Normal value: 0-10% lower than glucose plasma or serum level, because equilibration is slow, hence test must performed after at least 6 hrs of fasting
  o Because the joint fluid glucose equilibrates with blood glucose, whenever the Joint fluid glucose is assayed the blood glucose level should also be determined.
  o In inflammatory joint disease ex: rheumatoid arthritis, the synovial glucose level is about 60% of that in plasma and in septic arthritis it drops to 40% of the plasma concentration.

❖ Total protein: 1.07-2.13g/dl (nearly 1/3-1/2 that of plasma)
  o The normal proteins of plasma also enter synovial fluid by passive diffusion.
  o In contrast to small molecules, however, protein concentrations remain substantially less in synovial fluid than in plasma. In aspirates from normal knees, the total protein was only 1.3 g/dl, a value roughly 20% of that in normal plasma.
  o Protein concentration greater than 3.0g/dl may be due to increased permeability and immunoglobulin synthesis in the following cases:
    1. Rheumatoid arthritis.
    2. Gout.
    3. Septic arthritis.

❖ Uric acid
  o Crystals of uric acid usually accumulate in the synovial fluid of hyperuricemic patients during the intercurrent periods between attacks of gout.
  o They usually result from either, overproduction of uric acid or under excretion by the kidney.
  o Serum uric acid levels are generally not helpful in acute attacks and may be normal. However, when levels are chronically greater than 10 mg/dl, the chance of an acute attack is >90
- **Alkaline phosphatase**
  - Increased in most cases of arthritis

- **Microbiologic exam**
  - Gram stain and culture

- **Seroological exam**

  *RF, ANA, and LE cells*

  - **RF**
    Rheumatoid factor (RF) is found in synovial fluid of about 60% of RA patients, usually at a titer equal to or slightly lower than that of serum. It is presence, however, is non-specific, and measurement are generally not helpful for diagnosis or prognosis.
Seminal fluid analysis

A semen analysis measures the amount of semen a man produces and determines the number and quality of sperm in the semen sample.

A semen analysis is usually one of the first tests done to help determine whether a man has a problem fathering a child (infertility). A problem with the semen or sperm affects more than one-third of the couples who are unable to have children (infertile).

Purpose of the test:
There are basically four indications for the examination of seminal fluid:
1. The investigation of fertility: male infertility is primarily responsible in 30%-50% of infertile marriages.
2. To determine the effectiveness of vasectomy.
3. To determine the suitability of semen for artificial insemination.
4. Medico legal: testes to detect semen are frequently requested in alleged rape or in association with other sexual crimes of violence.

Fluid Fractions

1. Bulbourethral & Urethral glands (2-5%) are very small mucus secreting glands.
2. Prostate: (produce about 13-33% of the fluid volume of semen) Prostate glands secretion is a milky, alkaline fluid that plays a role in activating sperm, the secretion contains acid phosphatase and proteolytic enzymes that act on the fluid from the seminal vesicles, resulting in the coagulation and liquefaction of the semen.

Coagulation and liquefaction
Coagulation and subsequent liquefaction are believed to be three stage processes:
1. Coagulation results from the actions of a prostatic clotting enzyme on a fibrinogen-like precursor formed by the seminal vesicles.
2. Liquefaction is initiated by enzymes of prostatic origin.
3. The protein fragments are degraded further to free amino acids and ammonia by the action of several poorly characterized proteolytic enzymes, including an amino peptidase and pepsin. Clearly, a semen analysis should not be performed immediately following sample production. The sample should be mixed well in the original container by swirling for several seconds prior to removing the lid. Do not invert the container.
3. **Seminal vesicles** (produce about 46-80% of the fluid volume of semen) Viscous, yellowish secretion is rich in fructose, vitamin C, prostaglandin, and other substances, which nourish and activate the sperm passing through the tract. This component has high flavin content, which is largely responsible for the fluorescence of semen.

4. **Testis & Epididymis**: (5%) Spermatozoa are produced in the testis under the influence of testosterone, and then the epididymis (is the first part of the duct system) provides a temporary storage site for the immature sperm that enter it from testis. This fraction still in the inactive form until ejaculation due to the high content of carnitine, glycercylphosphorylcholine and diminished oxygen supply.

- The sperm within the semen are the cells that actually fertilize the egg and are therefore the most important to assess. However, the sperm account for only 1-2% of the seminal fluid volume. Problems with the surrounding fluid may also interfere with the movement and function of the sperm. Therefore, both the sperm and the fluid must be tested.

**Specimen collection:**
- Specimen should be collected into pre warmed (37°C), sterile, non-toxic, wide-mouth container, after a couple has abstained from sexual activity for 2-3 days.
- Verbal and written instructions should be given to the patient to ensure appropriate collection & delivery of semen sample to the laboratory. Ideally the sample should be collected in a room set aside for this purpose at the clinic laboratory in order to reduce ejaculation-analysis interval but this is not always possible.
- The patient should be advised to urinate and then wash and dry his hands and genitals thoroughly prior to ejaculation to avoid bacterial contamination. It is important to note that contamination of the semen sample with either soap or water may adversely affect sperm quality.

**Methods of collection:**
1. Masturbation (the method of choice for all seminal fluid tests)
2. The use of condom: it is not recommended for fertility testing because the condoms may contain spermicidal agents (used to determine the effectiveness of vasectomy).
3. By coitus interrupts (withdrawal method): the sample may be mistimed and part of the ejaculate may thus be lost.
• The sample should be clearly labeled with:
  1. the patient's name
  2. ID or clinic number (if available)
  3. Date and time of sample collection.

• The following should be recorded on the laboratory analysis form:
  1. The period of abstinence (in days).
  2. If sample collection was complete or incomplete.
  3. The time interval from collection to analysis.

The sample should be transported upright, at body temperature if possible, and
should be delivered to laboratory as soon as possible after collection and
certainly within one hour of ejaculation. If the sample is cold on receipt, this
should be noted in laboratory records. Patients should be advised not to expose
the sample to extremes of temperature.

Examination of seminal fluid

When evaluating semen specimens in cases of infertility, the following
parameters are routinely measured: volume, viscosity, pH, sperm count
(concentration), motility, and morphology.

❖ Initial evaluation (macroscopic examination) of sample
- After ejaculation, the seminal secretions form a coagulum, which gradually
  liquefies 10-20 min. In most cases, the semen sample should become fully
  liquefied within 60 minutes of production.
- Once liquefaction is complete then the physical appearance of the sample
  should be recorded in the laboratory records. If liquefaction does not occur
  then this abnormality should be noted.

❖ Viscosity of the ejaculate:
Estimate the viscosity of the semen by aspirating the semen into the measuring
pipette and allowing the semen to drop by gravity and will not appear clumped.
Observe the length of the thread. With excessively viscous samples, thorough
mixing can be difficult and accurate estimation of sperm concentration and
motility may be impossible

❖ Volume:
- This is a measurement of the volume of the ejaculate, Normal is (2-5
  milliliters). Using either a graduated cylinder with a conical base or a
disposable wide- mouthed pipette (accurate to 0.1ml) measure the ejaculate
volume to the nearest 0.1ml.
- Excessively small or large volumes are important in the transport of semen
  within the female reproductive tract and should be noted.
• The volume may be low if a man is anxious when producing a specimen, if all of the specimen is not caught in the collection container, or if there are hormonal abnormalities or ductal blockages.

❖ **Color of seminal fluid:**
Semen is normally a gray-yellow opalescent fluid. Its opacity is due to the most part, to its high protein content but is of course also produced by the many millions of spermatozoa as well as the cellular debris that is normally suspended within it.

❖ **PH:**
The normal pH of semen is slightly alkaline (7.2- 8.0) but increases with time.

### Microscopic examination

1- **Concentration (sometimes referred to as the "count")**
• This is a measurement of how many million sperm there are in each milliliter of fluid.
• There are various techniques for obtaining this number - some prove to be more accurate than others are.
• Average sperm concentration is more than 60 million per milliliter (60-150 million/ml).
• Counts of less than 20 million per milliliter (<20 million/ml) are considered sub-fertile.

❖ Several terms are used to describe both sperm concentration and sperm count:
• **Azoospermia** describe a total absence of spermatozoa in semen. (After centrifuge sperm count is zero/HPF).
• **Oligozoospermia** refers to a reduced number of spermatozoa in semen and is usually used to describe a sperm concentration of less than 20 million/ml. Sperm count 5-10 sperm/HPF.
• **Severe oligospermia**, sperm count 1-2 sperm/HPF.
• **Polyzoospermia** denotes an increased number of spermatozoa in semen and is usually refers to a sperm concentration in excess of 350 million/ml.
Methods of measuring sperm concentration

A) By using hemacytometer
- The sperm count is performed in the same manner as blood and CSF counts; that is by diluting the specimen and counting the spermatozoa in a neubauer chamber.
- Sperm can be counted by make dilution 1:20 in WBC pipette or by automatic pipette (which is more accurate) with a solution containing sodium bicarbonate (5g) and formalin (1ml) (immobilize & preserve the spermatozoa), tap water (100 ml) will suffice as a diluent.
- The sperm should then be counted - do not count headless or "pin-heads" sperm and do not count tailless heads.
- Traditionally, the sperm concentration is expressed in millions per milliliter (x10^6/ml) of semen and the total sperm/ejaculate is reported in millions (x10^6) per ejaculate.

❖ Calculations
1. Using a 1:20 dilution and two large WBC’s squares counted
   The sperm concentration/ml = No of sperms counted x 100,000
2. Using a 1:20 dilution and five small RBC’s squares counted
   The sperm concentration /ml = No of sperms counted x 1,000,000

B) Direct smear
The application of a drop of well-mixed semen to a clean glass slide under a lightly applied glass coverslip will allow visualization of the sperm in a specimen of semen.

2- Motility (sometimes referred to as the "mobility")
This describes the percentage of sperm, which are moving. 50% or more of the sperm should be moving. In order to achieve fertilization, a sperm must not only be able to move but be capable of movement that results in forward progression is often also known as progressive activity.
There are four classifications of motility

1. Rapid progressive motility - the sperm are moving swiftly across the field usually in a straight line
2. Slow or sluggish progressive motility - the sperm may be less linear in their progression
3. Non-progressive motility - sperm are also described as twitching or shaking
4. Immotility - sperm do not move at all.

- Eosin stain is used to differentiate live (unstained) and dead (stained) spermatozoa.

Other cells in semen
- The presence of other cell types in human semen other than spermatozoa are common and include:
  1. Leukocytes normally (1-4/HPF), increase number (leukocytospermia) indicates reproductive tract infection
  2. Epithelial cells normally (1-2/HPF)
  3. Spermatocytes (Immature germ cells) 1-2/HPF.
  4. Erythrocytes (1-2/HPF). Increased number may indicate a reproductive tract infection or damage to a small capillary during sample production.
  5. Bacteria and protozoa such as Trichomonas vaginalis are uncommon in human semen but their presence is indicative of possible male reproductive tract infection and should be reported to the referring doctor for further evaluation.
3- Agglutination

- The presence of agglutination should be recorded as this may indicate immunological infertility. Assess the spermatozoa in 10 random fields - estimate the average percentage of spermatozoa clumped together to the nearest 5%.
- Only count motile sperm attached to other motile sperm - do not assess immotile sperm stuck together or motile sperm adhering to mucus threads, other cells or debris, this is non-specific aggregation.

4- Morphology

- This describes the shape of the sperm. The sperm are examined under a microscope and must meet specific sets of criteria for several sperm characteristics in order to be considered normal. Most commercial laboratories will report WHO morphology (i.e. use World Health Organization criterion). 30% of the sperm should be normal by these criteria.
- Generally accepted that a high incidence of morphologically abnormal spermatozoa in a semen sample is associated with reduced fertility.
- Human sperm can be visualized using bright-field microscopy on fixed, stained specimens.
- Examples of fixed stained preparations (Papanicolaou stain, Vital staining with eosin/nigrosin, giemsa stain).
- Normal spermatozoa should have an oval shaped head (4-5.5µm long and 2.5-3.5µm wide).
- The midpiece should be cylindrical (3-5µm long and 1.0µm wide).
- The tail should also be cylindrical (45-50µm long and 0.5µm wide) with a narrower terminal segment (4-6µm long).
- There should be no head, midpiece or tail defects, and no cytoplasmic droplet more than one-third the size of a normal sperm head.
Defects to be scored

a) Head shape/size defects - such as large, small, tapering, pinhead form, amorphous, vacuolated, multiple heads or any combination of these.

b) Neck and midpiece defects - such as non-inserted or bent tail, distended, irregular / bent midpiece, thin midpiece (no mitochondrial sheath), absent tail (free or loose heads) or any combination of these.

c) Tail defects - such as short, multiple, hairpin, broken, irregular width, coiled tails, tails with terminal droplets or any combination of these.

d) Cytoplasmic droplets - greater than one-third the size of a normal sperm head.

Each spermatozoon is scored as either normal or abnormal with each of the defects being tallied separately. If a majority of the cells have a particular morphological defect this should also be noted.

In stained preparations 100-200 sperm should be scored using a x100 oil-immersion bright field objective.
Useful biochemical Test

**Fructose:**
- Fructose is the primary energy source for sperm. It is required for spermatozoa survival in an anaerobic environment and it stimulates sperm motility.
- Spermatozoa, which are subjected to centrifugation and thus separated from the seminal plasma, will not survive anaerobically unless seminal plasma or carbohydrates source is added back to separated spermatozoa. Seminal plasma fructose is produced by the seminal vesicles. Fructose production is stimulated by testosterone. Since the seminal vesicles do not have a large storage capacity, collection of several ejaculates within a few days will yield decreased fructose values. It takes about two days for fructose levels in the seminal vesicle to be replenished.
- Fructose measurements are useful diagnostically in men with low-volume ejaculates. The absence of fructose can indicate the congenital absence or infections that affect the seminal will also result in absent or reduced fructose concentration.

**Reagent:**
- ZnSO$_4$ (5%)
- Ba(OH)$_2$ (4.76%)
- Resorcinol (1%)
- Conc. HCL
- Water bath (90°C), automatic pipette, tubes.

**Procedure**
- In three tubes B, T, ST we put

<table>
<thead>
<tr>
<th></th>
<th>B</th>
<th>St</th>
<th>T</th>
</tr>
</thead>
<tbody>
<tr>
<td>H$_2$O</td>
<td>3ml</td>
<td>0 ml</td>
<td>2.9ml</td>
</tr>
<tr>
<td>Sample</td>
<td>--------</td>
<td>--------</td>
<td>0.1ml</td>
</tr>
<tr>
<td>St</td>
<td>--------</td>
<td>3 ml</td>
<td>------</td>
</tr>
<tr>
<td>ZnSO$_4$</td>
<td>0.5ml</td>
<td>0.5ml</td>
<td>0.5ml</td>
</tr>
<tr>
<td>Ba(OH)$_2$</td>
<td>0.5ml</td>
<td>0.5ml</td>
<td>0.5ml</td>
</tr>
</tbody>
</table>

- Then mix, we will show white ppt, leave it 5 min.
- Centrifuge at high speed for 5 min.
- After that we take from the three tubes 2 ml of supernatant of each, as the table under we do:
- Mix, put it in water path at least time 10 min. we will show pink color.
- Cool the tubes under tap water, and then read it on 490 nm wave length of spectrophotometer.

## Calculation:

Conc. St = 350mg / dl

\[
\begin{align*}
\text{Abs. } T &= 0.502 \\
\text{Abs. } St &= 0.575 \\
\text{Conc. } T &= \left( \frac{\text{Abs. } T}{\text{Abs. } St} \right) \times \text{Cons. } St \\
&= \frac{0.502}{0.575} \times 350 \\
&= 305.565 \text{mg/dl}
\end{align*}
\]

### Biochemical changes that may be seen in the semen with a Variety of different causes of infertility

<table>
<thead>
<tr>
<th>Genital tract lesion causing infertility</th>
<th>Sperm concentration</th>
<th>pH of semen</th>
<th>Seminal volume</th>
<th>Seminal fructose</th>
<th>Seminal acid phosphatase</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bilateral epididymal obstruction</td>
<td>Azoospermia</td>
<td>Normal</td>
<td>Normal</td>
<td>Normal</td>
<td>Normal</td>
</tr>
<tr>
<td>Congenital absence of vasa deferentia</td>
<td>Azoospermia</td>
<td>Normal or reduced</td>
<td>Reduced</td>
<td>Absent</td>
<td>Raised</td>
</tr>
<tr>
<td>Ejaculatory duct obstructions</td>
<td>Azoospermia</td>
<td>Normal or reduced</td>
<td>Reduced</td>
<td>Absent</td>
<td>Raised</td>
</tr>
<tr>
<td>Polyzoospermia</td>
<td>Raised &gt;350x10^6</td>
<td>Normal</td>
<td>Normal</td>
<td>Reduced</td>
<td>Normal</td>
</tr>
</tbody>
</table>
Serological Analysis

- **Anti-Sperm Antibodies**
  Antibodies to sperm can be present in the serum of some females as well as males, the seminal fluid and the cervical mucosa are statistically associated with an increased risk of infertility. It is known that antibodies directed toward various sperm antigens can result in reduced fertility in men.

- **Pyospermia and the microbiology of semen**
  Increased number of WBC’s in semen is known as pyospermia and in occasions, is a cause of infertility in men, and so must be examined microbiologically.
  The first step in the microbiological examination is to make a Gram stain and then make semen culture.
Serous Fluids

- **Introduction**
  - Serous fluids are the fluids contained within the closed cavities of the body. These cavities are lined by an adjacent membrane, which forms a double layer of mesothelial cells, called the serous membrane.
  - The cavities are the pleural (around the lungs), pericardial (around the heart), and peritoneal (around the abdominal and pelvic organs) cavities.
  - A small amount of serous fluid fills the space between the two layers and serves to lubricate the surfaces of these membranes as they move against each other.
  - The fluids are ultrafiltrate of plasma, which are continuously formed and reabsorbed at a constant rate, leaving only a very small volume within the cavities. An increased volume of any of these fluids is referred to as an effusion. Effusions may be either transudates or exudates.
  - Exudates are usually effusions, which result from conditions that directly affect the membranes lining the serous cavity.

- **Formation**
  
  Serous fluids are formed as ultrafiltrate of plasma, with no additional material contributed by the membrane cells. The small amount of protein is removed by the lymphatic system. Production and reabsorption are subject to hydrostatic & colloidal (oncotic) pressures from the capillaries serving the cavities. Under normal conditions, colloidal pressure from serum proteins is the same in the capillaries on both sides of the membrane. Therefore, the greater hydrostatic in the systemic capillaries on the parietal side favors fluid production through the parietal membrane and reabsorption through the visceral membrane.
Pleural fluid

In human anatomy, the pleural cavity is a body cavity containing the lungs; the lungs are surrounded by two serous membranes, the pleurae. The outer pleura (parietal pleura) covers and is attached to the chest wall. The inner pleura (visceral pleura) covers and is attached to the lung and other structures, i.e. blood vessels, bronchi and nerves. Between the two is a thin space known as the pleural space, which normally contains a small amount of pleural fluid, when there is an excess fluid accumulation in the pleural cavity, this is called pleural effusion, which may be transudates, exudates or fluid from extra pleural origin such as:

1. Ruptured esophagus which is characterized by increase fluid amylase and decrease of PH.
2. Pancreatitis which is characterized by increase amylase.

- **Transudates**
  Effusion that forms because of systemic disorder that disrupts the balance in the regulation of fluid filtration and reabsorption such as:
  1. The changes in the hydrostatic pressure (increasing) created by a mechanical process such as congestive heart failure (CHF) or by pulmonary embolism.
  2. Decrease the plasma oncotic pressure such as nephrotic syndrome or hepatic cirrhosis

- **Exudates**
  Effusions that are produced by conditions that directly involve the membranes of the particular cavity (from an inflammatory process which including infections and malignancies) that leads to:
  1. Increased capillary permeability.
  2. Decreased lymphatic resorption.
Laboratory differentiation of Transudates & Exudates

<table>
<thead>
<tr>
<th>Criteria</th>
<th>Transudates</th>
<th>Exudates</th>
</tr>
</thead>
<tbody>
<tr>
<td>Appearance</td>
<td>Clear</td>
<td>cloudy</td>
</tr>
<tr>
<td>specific gravity</td>
<td>&lt; 1.016</td>
<td>&gt; 1.016</td>
</tr>
<tr>
<td>Total protein</td>
<td>&lt; 3.0 g/dl</td>
<td>&gt; 3.0 g/dl</td>
</tr>
<tr>
<td>Fluid : serum protein ratio</td>
<td>&lt; 0.5</td>
<td>&gt; 0.5</td>
</tr>
<tr>
<td>Lactate dehydrogenase LDH</td>
<td>&lt; 200 IU</td>
<td>&gt; 200 IU</td>
</tr>
<tr>
<td>Fluid : serum LDH ratio</td>
<td>&lt; 0.6</td>
<td>&gt; 0.6</td>
</tr>
<tr>
<td>Cell count</td>
<td>&lt; 1000/µl</td>
<td>&gt; 1000/µl</td>
</tr>
<tr>
<td>Spontaneous clotting</td>
<td>No</td>
<td>possible</td>
</tr>
</tbody>
</table>

Gross Examination

- **Volume:** 1-15 ml
- **Color and Appearance:**
  - Transudates, Clear, Pale Yellow
  - Exudates, cloudy, opaque appearance indicates more cell components.
  - Bloody fluid: Malignancy, pulmonary infarct, and trauma. We can differentiate between old bleeding and traumatic tap as seen with other fluids.
  - Milky
    1. Chylous due to: Damaged or obstruction of thoracic duct.
    2. Pseudochylous due to: break down of cellular lipids in chronic effusion.
  - White fluid: Chylothorax, cholesterol effusion, or empyema.
  - Black fluid: Aspergillus niger (fungi) infection
  - Purulent fluid: Indicates infection
  - Turbid and greenish yellow : Rheumatoid effusion
**Microscopic examination**

- **RBC’s**
  - Little value
- **WBC’s**
  - Total lower than 1000/µl
- **LE cells**
- **Macrophages**
- **Mesothelial cells**

![Cell count:](performed in counting chamber)

- **Total RBC count**
  - RBCs (5000-6000) are needed to give red appearance to pleural fluid
  - RBCs > 100,000 is grossly hemorrhagic and suggests malignancy, pulmonary infarct, or trauma but occasionally seen in congestive heart failure alone.
  - Hemothorax suggests trauma, bleeding from a vessel, bleeding disorder, or malignancy.

- **Total WBC count**
  - Transudates are usually > 1000/µl
  - WBC’s > 10,000/µl indicates inflammation, most commonly with pneumonia, pulmonary infarct, Pancreatitis.
  - WBC’s > 50,000/µl is typical only in Para pneumonic effusions, usually empyema
  - In malignancy & tuberculosis are usually < 5000/µl.

### WBC’s differential

- **Mononuclear cells** predominate in transudates and early effusions and chronic exudates.
- **PMNs** predominate in early inflammatory effusion neutrophil:
  - 90% in the following
  1. Acute inflammation due to pneumonia
  2. Pulmonary infection
  3. Pancreatitis

After several days, mesothelial cells, macrophage, lymphocytes may be predominating.
• **Lymphocyte** (80-90%) increased in the following cases:
  1. Tuberculosis
  2. pneumonia
  3. True Chylous
  4. S.L.E
  5. Uremic effusion
  6. Subacute inflammation

• **Eosinophilia**: Eosinophilie in pleural fluid ( > 10% of total WBC) is diagnostically significant
  1. Pneumothorax.
  2. Post pneumonia effusion.
  4. Pulmonary infection.
  5. Congestive heart failure.
  6. S.L.E.

• **LE cells**: occasionally LE cells make the diagnosis of SLE. There is an increased number of macrophages and mesothelial cells.

## Biochemical examination

- **Protein and LDH**
  - To differentiate transudates from exudates.
  - Protein electrophoresis shows an elevation of albumin and absence of fibrinogen in comparison to that of plasma.

- **Glucose**
  Same as serum value in transudates. Usually normal but if it lowers than 60 mg/dl may be found in:
  1. Rheumatoid arthritis.
  2. Empyema
  3. Malignancy
  4. TB
  5. Esophageal rupture.
  6. SLE

- **Amylase**
  1. Increase in acute pancreatitis (may reach 2 times plasma amylase)
  2. Perforated peptic ulcer.
**Lipids**
- Triglycerides
- Lipoproteins
- Cholesterol.

<table>
<thead>
<tr>
<th>Serology</th>
</tr>
</thead>
</table>
| - Used to differentiate effusions of immunologic and malignant origin from those of non inflammatory and non malignant origin. The tests includes:
  - Tumor Marker: CEA (60-70% of lung cancer) 40-50% of other malignancies.
  - RF, complement, ANF, immunoglobulin
|   |
| - Increased levels of immunoglobulins and CEA or decreased complement is indicative of inflammatory and neoplastic reaction |

<table>
<thead>
<tr>
<th>Microbiology</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gram stain, acid-fast stain, cultures.</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Pericardial fluid</th>
</tr>
</thead>
<tbody>
<tr>
<td>- The pericardial space enclosing the heart normally contains about 25 to 50 mL of a clear, straw colored ultrafiltrate of plasma, called pericardial fluid.</td>
</tr>
<tr>
<td>- When an abnormal accumulation of pericardial fluid occurs, it fills up the space around the heart and can mechanically inhibit the normal action of the heart.</td>
</tr>
<tr>
<td>- In this case, immediate aspiration of the excess fluid is indicated.</td>
</tr>
</tbody>
</table>
Pericardial effusion

- Pericardial effusion is usually caused by:
  1- Infection: Which may be bacterial, tuberculosis, fungal or viral.
  2- Neoplasm: Which may be due to metastatic carcinoma or lymphoma.
  3- Myocardial infarction.
  4- Hemorrhage due to trauma.
  5- SLE.

- Sample collection called pericardiocentesis.

**Gross appearance**

- **Volume:** 10-50ml
- **Appearance:** clear pale yellow.
  - Bloody due to T.B., or other wide variety of diseases
  - Milky (chylous and pseudochylous).

**Microscopic examination**

- **RBCs:** Little value
- **WBCs:** Total
- **LE cells**

**Biochemical examination**

- Protein (little value in differential diagnosis.
- Glucose
- Lipids
  - Triglycerides
  - Lipoproteins
  - Cholesterol

**Serology**

- ANA

**Microbiology**

- Gram stain, acid fast stain and cultures.
Peritoneal fluid (Ascitic)

Peritoneal effusion:

- is a common complication in many diseases which may be:

  o Transudate due to:
    1. Congestive heart failure
    2. Constrictive pericarditis
    3. Hypoproteinemia
    4. Nephrotic syndrome
    5. Liver cirrhosis

  o Exudate due to:
    1. Peritoneal malignancy.
    2. Tuberculous peritonitis.
    3. Pancreatic ascites.
    4. Billie peritonitis.
    5. Trauma.

Gross appearance

- **Volume:** lower than 50 ml.
- **Appearance:** clear pale yellow.
- **Turbidity**
  1. Appendicitis
  2. Pancreatitis
  3. Strangulated intestine
  4. Ruptured bovel
  5. Bacterial peritonitis
- **Milky**
  1. Chylous
  2. Pseudochylous.
• **Greenish**
  1. Perforated duodenal ulcer
  2. Perforated intestine
  3. Chlocystitis
  4. Perforated gall bladder
  5. Acute pancreatitis

### Microscopic examination

- RBCs: Little value
- WBCs: Total
- LE cells

### Biochemical examination:

- Protein
- Glucose
- Lipids
- Cholesterol
- Amylase
- ALP
- LDH
- Tumor Marker: CEA
- Ammonia if it is increased due to:
### Differentiation between peritoneal fluid exudates and transudate

<table>
<thead>
<tr>
<th>Criteria</th>
<th>Transudate</th>
<th>Exudate</th>
</tr>
</thead>
<tbody>
<tr>
<td>Protein</td>
<td>&lt;3.0 (30% higher)</td>
<td>&gt;3.0</td>
</tr>
<tr>
<td>Fluid: Serum Protein Ratio</td>
<td></td>
<td>&gt;0.5 suggests malignancy</td>
</tr>
<tr>
<td>LDH</td>
<td></td>
<td>Elevated in malignancy</td>
</tr>
<tr>
<td>Fluid: Serum LDH Ratio</td>
<td></td>
<td>&gt;0.6 suggests malignancy</td>
</tr>
<tr>
<td>CEA</td>
<td></td>
<td>&gt;10 suggests malignancy</td>
</tr>
<tr>
<td>Fluid: Serum CEA Ratio</td>
<td></td>
<td>&gt;2 suggests malignancy</td>
</tr>
<tr>
<td>Glucose</td>
<td></td>
<td>&gt;60</td>
</tr>
<tr>
<td>Fluid: Serum Glucose Ratio</td>
<td>&gt;1</td>
<td>&lt;1</td>
</tr>
<tr>
<td>Amylase</td>
<td></td>
<td>Increase in pancreatic ascites</td>
</tr>
<tr>
<td>Lipase</td>
<td></td>
<td>Increase in pancreatic ascites</td>
</tr>
<tr>
<td>pH</td>
<td></td>
<td>May be decrease in infectious</td>
</tr>
<tr>
<td>Triglycerides</td>
<td></td>
<td>Increase in chylous ascites</td>
</tr>
</tbody>
</table>

### Serology

ANA

### Microbiology

Gram stain, acid fast stain, culture

THE END
A sweat test measures the amount of salt chemicals (sodium and chloride) in sweat. Sodium and chloride are part of your body’s electrolyte balance, and combine to form the salt found in sweat. They help regulate the fluid balance in your tissues. It is done to help diagnose cystic fibrosis.

Normally, sweat on the skin surface contains very little sodium and chloride. People with cystic fibrosis (CF) have 2 to 5 times the normal amount of sodium and chloride in their sweat.

Cystic fibrosis

Cystic fibrosis (CF), mucoviscoidosis, or mucoviscidosis, is a life-threatening hereditary disease which is inherited as autosomal recessive and is characterized by:

1. Increased viscosity of mucous secretions, including those of (pancreas, intestinal glands, tracheal, peritracheal, bronchial).
   - Thick mucus production, as well as a less competent immune system, cause mucus to build up and clog some of the organs in the body, particularly in the lungs and pancreas. When mucus clogs the lungs, it can make breathing very difficult. The thick mucus also causes bacteria (or germs) to get stuck in the airways, which causes inflammation (or swelling) and infections that leads to lung damage.
   - Mucus also can block the digestive tract and pancreas. The mucus stops digestive enzymes from getting to the intestines. The body needs these enzymes to break down food, which provides important nutrients to help us grow and stay healthy.

2. Increased concentration of electrolytes especially Na and CL, in secretion of other gland notably (sweat glands, Parotid salivary glands, lachrymal glands).
Symptoms of cystic fibrosis (CF)

1. Thick, viscous mucus secretions in the lungs
2. Repeated infections: The accumulation of sticky, thick mucus in the lungs creates a favorable environment for infectious microorganisms to inhabit and flourish.
3. Stools, pale or clay colored, foul smelling, or stools that float
4. Meconium ileus is a typical finding in newborn babies with CF
5. Recurrent pneumonia
6. Chronic cough, possibly with blood streaking
7. Wheezing
8. Bronchitis
9. Chronic sinusitis
10. Asthma
11. Weight loss, failure to thrive in infants, abdominal swelling
12. Excessive salt in sweat, dehydration
13. Failure of newborn to pass stool
14. Abdominal pain, flatulence
15. Fatigue
16. Changes in color and amount of sputum (material coughed up from the lungs)

Sweat analysis

Two methods of sweat analysis are most frequently used: chloride concentration and conductivity measurement. The sweat chloride analysis is recommended as the diagnostic test for CF. Sweat conductivity may be used to screen for CF.

Sample collection

- Earlier methods to stimulate the production of sweat including the use of humid high temperature room, and encasing the pts. Body in plastic.
- Current methods: A tiny amount of a sweat-stimulating liquid is applied to a small patch of skin on the arm or leg. An electrode is then placed over the site and a weak electrical current stimulates the area. This is a painless procedure that may create a tingling or warm sensation. After several minutes, the area is cleaned and sweat is collected for about thirty minutes, either into a plastic coil of tubing or onto a piece of gauze or filter paper. The sweat obtained is then analyzed.
Performance of the test

- done on a baby's right arm or thigh. On an older child or adult, the test is usually done on the inside of the right forearm. Sweat may be collected and analyzed from two different sites.

- The skin is washed and dried, and then two small gauze pads are placed on the skin. One pad is soaked with a medicine that makes the skin sweat, called pilocarpine. The other pad is soaked with salt water such as NaNO₃.

- Other pads called electrodes are placed over the gauze pads. The electrodes are hooked up to an instrument that produces a mild electric current, which pushes the medicine into the skin. Another testing method collects the sweat into a coil (macroduct technique).

- After 5 to 10 minutes, the gauze pads and electrodes are removed, and the skin is cleaned with water and then dried. The skin will look red in the area under the pad that contained the medicine.

- A dry gauze pad, paper collection pad, or special tubing is taped to the red patch of skin. This pad is covered with plastic or wax to prevent fluid loss (evaporation).

- The new pad will soak up the sweat for up to 30 minutes, then it is removed and placed in a sealed bottle. It is then weighed to measure how much sweat the skin produced, and it is checked to find out how much salt chemical (sodium and/or chloride) the sweat contains.

- After the collection pad is removed, the skin is washed and dried again. The test site may look red and continue to sweat for several hours after the test.

- The sweat test usually takes 45 minutes to 1 hour.

- Then we measure CL by automated or manual titration method and Na by flame photometry or ion exchange electrode.

Results

A sweat test measures the amount of salt chemicals (sodium and chloride) in sweat. Generally, chloride (sweat chloride) is measured.

Sweat chloride

- Normal Less than 40 mill moles per liter (mmol/L).
- Borderline 40–60 mmol/L.
- Abnormal More than 60 mmol/L.
**Abnormal (high) values**: Usually mean a person has cystic fibrosis. Some people with cystic fibrosis have borderline or even normal sweat chloride levels
Sputum

❖ Definition
Sputum is a secretion that is produced in the lungs and the bronchi (tubes that carry the air to the lung). This mucus-like secretion may become infected, blood stained, or contains abnormal cells that may lead to diagnosis.

❖ Composition
Tracheo bronchial secretions are an inconstant mixture of plasma, water, electrolytes and mucin. In addition to contamination from upper and lower respiratory tract with cellular exfoliation, nasal and salivary gland secretions and normal bacterial flora of the oral cavity.

❖ Sources
- Submucous glands: present between the surface epithelium and cartilaginous plates
- Surface epithelium: three types of secretory cells can be distinguished:
  1. Serous cells
  2. Celara
  3. Goblet cell: thick mucin-type secretion that is diluted by a more serous mixture of acid glycoproteins, sialoproteins and sulfoproteins are secreted by submucous gland

❖ Viscoelastic character of sputum
- The physical properties of sputum reveal secretions to be viscoelastic that is some of the properties of liquid and some of solid.
- The consistency is dependent mainly on the molecular structure of the glycoproteins and on the degree of hydration.
- Sialic acid is the most important single component of sputum viscosity.

❖ Sample collection:
- Sputum is what comes up with deep coughing. Increasing the amount of fluids in the night before the test may help to get the sample.
- First morning specimen is best, most tracheobronchea secretions are not ejected from the mouth but are swallowed during sleep
- Patent cooperation and understanding, instruct patient to rinse mouth with water prior to each collection.
- Cough up from deep down in the chest, the specimen should be collected in a sterile container and then send immediately to lab and not be allowed to stand for long time
- Specimen expectorated sputum, not saliva or nasal aspirates three consecutive mornings is advisable.
Macroscopic Examination

Consistency and appearance: viscoelastic, clear and watery, usually specific diseases have characteristic consistencies and appearance.

- **Color**
  - Color is determined by the material contained and color can indicate the pathologic process
  1. **Yellow color:**
     - Pus and epithelium cells
     - Pneumonia
  2. **Green:**
     - Pseudomonas
     - Sputum left standing more than 24 hrs tend to become greenish through the break down of neutrophils and the release of neuroperoxidase enzyme.
  3. **Rust red:**
     - Decomposed Hb
       1. Pneumococcal pneumonia
       2. Pulmonary gangrene
  4. **Bright red:**
     - Recent hemorrhage
       1. Acute cardiac failure
       2. Pulmonary infection
       3. TB
       4. Ruptured blood vessels.

- **Miscellaneous finding:** (cheesy masses, bronchial cast, broncholiths, parasites).
- **Odor:** No odor

- **Sputum Exam for Mycobacterium**
  - Acid fast organisms
  - Sputum gram stain
  - Sputum Culture

THE END
Amniotic Fluid

Definition
- A clear, slightly yellowish liquid that surrounds the unborn baby (fetus) during pregnancy; it is contained in the amniotic sac.
- The fetus floats in the amniotic fluid. During pregnancy the amniotic fluid increases in volume as the fetus grows. Approximately 1000 ml of amniotic fluid surround the baby at full term (40 weeks gestation).

Function
1. Protects from outside injury by cushioning sudden blows or movements
2. Allows for freedom of fetal movement and permits musculoskeletal development
3. Maintains a relatively constant temperature for the environment surrounding the fetus
4. Protects the fetus from heat loss
5. Is a source of oral fluid to the fetus
6. Allows for symmetrical growth and development of the fetus
7. It contains bacteriostatic properties which can protect the intrauterine environment from infection.
8. It can serve as a short term fluid and nutrient supply.
9. It is needed for proper development of gastrointestinal, musculoskeletal and pulmonary systems.

Formation
Amniotic fluid is composed of a mixture of different substances that reach or are removed from the amniotic cavity by various routes:
1. Fetal urine appears in the amniotic cavity as soon as the metanephros develops and increases gradually.
2. Pulmonary secretions increase gradually.
3. Oro-nasal secretions may contribute a small and indeterminate amount.
4. The fetal skin.
5. Intramembranous secretions.
6. Transmembranous passage.
**Volume**
- Volume of amniotic fluid is varied according to the period of pregnancy.
  - 10 weeks: 30 ml.
  - 20 weeks: 350 ml.
  - 37 weeks: 100-1500 ml.
  - Third trimester: 400 ml

- An excessive amount of amniotic fluid is called polyhydramnios. This condition often accompanies multiple pregnancy (twins or triplets), or some congenital defect such as hydrocephalus.

- An abnormally small amount of amniotic fluid is known as oligohydramnios. This condition can cause deformities in the fetus. And may be due to congenital anomalies such as renal agenesis or bladder outlet obstruction.

**Amniocentesis**
- Removal of a sample of the fluid is called amniocentesis.
- Amniotic fluid test or AFT: is a test to analyze the liquid (amniotic fluid) that surrounds an unborn baby (fetus). It can be done after about the 14th week of pregnancy (14th - 22nd), when there is enough amniotic fluid for testing. Amniocentesis may be done to:
  1. To predict the severity of hemolytic disease of the newborn in RH erythroblastosis fetalis.
  2. To assess intrauterine fetal maturity before cesarean section to assure the delivery of an infant with a good chance of survival.
  3. To detect fetal sex in pregnant women heterozygous for X-linked recessive disorders such as hemophilia and muscular dystrophy.
  4. To discover genetic fetal disorders in genetic high-risk patients, with chromosomal translocation (Down’s syndrome) or congenital metabolic disorders.
  5. To assess pulmonary maturity.
  6. To determine fetal trouble, Rh isosensitization, diabetes mellitus, preeclampsia, and eclampsia.
  7. Determine if the amniotic fluid is infected.
  8. Early in the pregnancy to determine if the fetus has certain types of birth defects.
2nd trimester amniocentesis

- For genetic and developmental defects, amniocentesis is usually performed at about 16 weeks gestation, although it can be done safely at any time after 16 weeks gestation.
- Amniotic fluid contains cells that have been shed by the developing fetus. These cells can be tested for more than 100 types of defects that are associated with inherited (genetic) diseases (such as Down syndrome or cystic fibrosis) or development defects (such as spina bifida). Testing for these diseases is most commonly done between the 14th and 18th week of pregnancy (2nd Trimester), when the pregnancy can most easily be ended if the fetus is severely disabled.

- However, amniocentesis cannot detect many common birth defects such as cleft lip and palate, heart problems, and some types of mental retardation.

3rd Trimester amniocentesis

- Amniocentesis can also detect the sex of the fetus. In (3rd Trimester) we make theis to alloimmune hemalytic diseases and respiratory destress.

- Ultrasound is used to guide the procedure. The doctor withdraws a small amount of amniotic fluid by inserting a needle into your abdomen through the uterus and into the amniotic sac. The fluid is sent to a lab and tested for specific substances called phospholipids. This will help the doctor predict whether the baby's lungs are sufficiently developed for delivery.

Specimen:

- **Container:** Amber plastic transport tube with amber stopper (If amber tubes are unavailable, cover standard transport tube completely, top and bottom, with aluminum foil). Identify specimen with patient name directly on the container and on the outside of the aluminum foil. Secure with tape. Amber plastic transfer tube and amber stopper.

- **Storage Instructions:** Freeze within 4 hours to transport to laboratory; stable refrigerated up to 1 week. Protect from light. Avoid repeated freezing and thawing of the specimen, which may cause the sample to precipitate, resulting in a lower than expected value.
- If cell culture is requested the specimen should be kept at 37°C.
Physical Examination

❖ **Color**

**Normal:** Colorless or pale straw.

**Abnormal:**
1. Yellow-orange is indicative of blood incompatibility and the presence of bile pigment released from red blood cell hemolysis.
2. Dark yellow aspirate indicates probable fetal involvement.
3. Brown due to severe hemolysis
4. Green due to contamination with meconium
5. Yellow–brown opaque fluid may indicate intrauterine death, although not necessarily from erythroblastosis.

❖ **Turbidity:** Turbid due to presence of variant cell types.

### Amniotic Fluid Supernatant analysis (2\textsuperscript{nd} trimester)

1. **Alpha-fetoprotein (AFP)**
   - This test can help determine principle protein in weeks and if there is an opening in the fetal skin. The most common place is the spine. This would be a neural tube defect (NTD), such as spina bifida or anencephaly.
   - The normal value in non-pregnant is 20mg/dl and in fetal serum 300mg/dl, in this (NTD) the protein will be increased.
   - The correction to it is: MOM= average pt. duplicate result \ medium of normal reference. The normal between 0.4 to 2.0.
    2. ALT
    3. GGT
    4. ACHE: Pseudo choline esterase serum (if it is increased there is (NTD).

❖ **Assessment of fetal maturity**
   - If the fetus is mature enough to breathe on its own once born, then immediate delivery may be in the best interests of both the mother and fetus.
   - If test results are inconclusive (do not indicate fetal lung maturity), further testing may be done, or medication may be
provided to speed up fetal lung maturity. Delivery may be delayed for a day to a week.

- If there is any complication like (BM, HDN, and Premature lobar) we will make cesarean.

**TESTS**

Used to Attempt to prevent respiratory distress syndrome (RDS) from low surfactant in early delivery, by evaluation of fetal pulmonary maturation. Amniotic fluid test for fetal maturity; indicator to determine optimal time for obstetrical intervention in cases of possible fetal distress: maternal diabetes, toxemia, hemolytic disease of the newborn (erythroblastosis fetalis), post maturity.

1. Creatinine: normal value: 1.5-2.0mg/dl or greater indicates fetal maturity.
2. Optical Density.
3. Cytogenic studies.
4. Lipids (Lecithin / Sphingolipid)

**Limitations** False-negative results (L/S <2.0 but no lung disease) occur in 5% of cases; false-positive results occur in 0.6% of cases. Half of the false-positive results occur in diabetics. Use a cutoff 3.5 for diabetic mothers.

**Chemical Components Of Amniotic Fluid :**

- **Ca** 4m\(^{\text{eq}}\)dl
- **Cl** 12mEq/l
- **K** 4.9mEq/l
- **Na** 32mEq/l
- **Glucose** 30 mg/dl
- **Creatinine** 1.8mg/dl
- **Uric Acid** 31mg/dl
- **Albumin** 1.42 g/dl
- **Alpha globin** 0.19 g/dl
- **Alpha 2 globin** 0.16 g/dl
- **Beta globin** 0.49 g/dl
- **Gama globin** 0.32 g/dl.

**The End**