Lec. 1 and Lab.

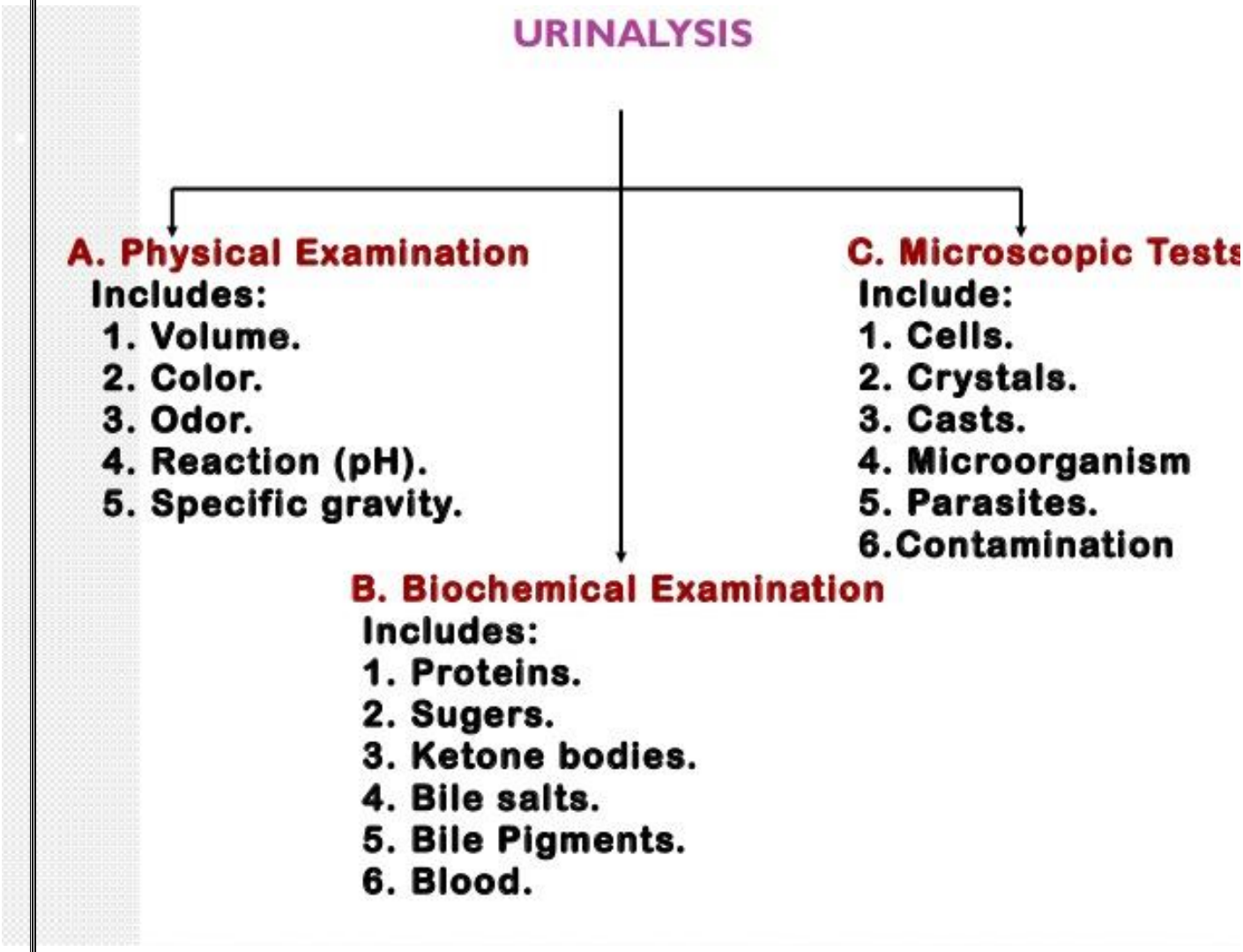
Urine analysis or Urinalysis

A urinalysis is a group of chemical and microscopic tests. They detect the byproducts of normal and abnormal metabolism, cells, cellular fragments, and bacteria in urine.

Urine is the yield filtrate of the urinary system . The physical , chemical, microscopically , microbiological as well as immunological constituents reflects the state of normality or abnormality of the urinary system.

According to the medical demands , urinalysis is classified into two groups of tests:

- Routine urinalysis which is including (General Urine Examination) GUE.
  a. Examination of the physical appearance of urine.
  b. Examination of the chemical composition of urine .
  c. Examination of the microscopic components of urine .



- Specific urinalysis including :
  a. Urine culture .
  b. Microbial sensitivity test .
  c. Immunological tests.

Urinalysis may be performed using a "**clean catch**" sample of urine. To achieve this, the area surrounding the opening of the urethra is cleansed and rinsed well. A small amount of urine should then be allowed to fall into the toilet and the rest should be collected midstream in a clean container and should be performed within **15 minutes** of collecting the urine sample. If that is not possible, the urine should be refrigerated.

A. Physical appearance of urine:

Normal urine is pale yellow to amber in collar with no turbidity (clear to slightly hazy) with ph 4.5- 8.0 . This picture is changed in many abnormalities as follow :

Appearance	Possible cause
Cloudy: urine usually has an unpleasant smell and contain WBC.	Bacterial urinary infection with protein urea.
Red and cloudy due to RBC.	1. Urinary shistosomiasis 2. Bacterial infection 3. Renal haemorrhage (bleeding)
Brown and cloudy due to haemoglobin	1. Black water fever 2. Other conditions that cause intravascular haemolysis (autoimmune haemolytic anemia)
Yellow brown or green brown (bilirubin)	1. Acute viral hepatitis 2. Obstruction jaundice
Yellow orange due to urobilin , oxidized urobilinogen	1. Haemolysis 2. Hepato cellular jaundice
Milky white due to chyle	Bancroft filarial

PH : ph of urine is not very helpful in deciding the urine abnormality . Kidney play an important role in the maintaining acid-base balance , so urine PH is affective by the acidosis or alkalosis of the blood.

NOTES:

↑ protein food → Acidic urine .

↑ vegetable - ↓ COH - ↑ citrus fruits → Alkaline urine.

Specific gravity :

Normal urine is ranging between (1.015- 1.o25) . SG is a measure of the concentration of dissolved solutes (substance in a solution) and it reflect the ability of the kidney to concentrate the urine . it can be measured by refractometry or by chemical analysis .

↑ SG → ↑ Urine con. (1.035) → 1. Diabetes mellitus

2. Large amount of medication

3. Radiologic studies

4. Oligo urea

↓ SG → ↓ Urine con. (1.003) → 1. Diabetes insipidus

2. Renal failure

3. Poly urea

Notes :

If the quantity of urine not enough to determine this test must be write Q.N.S (quantity not sufficient).

B. Chemical and biochemical composition:

Normal chemical composition of urine is as follow :

Ammonia (0.05%) , sulfate (0.18%) , phosphate (0.12%) , chloride (0.6%) , magnesium (0.01%) , calcium(0.015%) , potassium (0.6%) , sodium (0.1%) , creatinine (0.1%) , uric acid (0.03%) and urea (2%).

Normal urine must be not have any biochemical composition accept Urobilinogen the normal range between (0.1-1.0) in normal urine.

Crystals in acidic urine :

a) Amorphous urates
b) Uric acid
c) Calcium oxalate
d) Sodium acid urate

Crystals in alkaline urine :

a) Amorphous phosphates
b) Calcium phosphate
c) Ammonium blur ate
d) Triple phosphates
e) Calcium carbonate

Glucose test :

The normal concentration of glucose in blood is between (80-120 mlg / 100ml blood) . kidney don't allow release the glucose from the blood to urine accept if the concentration increase to 180 mlg / 100 ml in blood this called renal threshold and there are some disease caused release the glucose from urine like diabetes.

There are two types of testing :

1. Using dipstick
2. Benedicts test : procedure is

a) Place 6-8 drops of urine in the test tube.
b) Add 3-5 ml. Of Benedict's solution to the urine.
c) Boil over Bunsen burner for 2 min or put test tube in water bath for 5 min.

d) Place a test tube in rack , allow to cool at room temperature to notes the change the color:
   Clean blue to marked cloudy green———— negative
   Bluish green————————————————+
   Yellowish green————————————————++
   Yellow————————————————————+++
   Orange (coppery)————————————————++++
   (any + = 60 mlg of suger).

Proteins (albumin):

The presence of increase amount of protein in the urine can be an important indicator of renal disease . Albumin is smaller than most other proteins and is typically the first protein that is seen in the urine when kidney dysfunction begins to develop. Albumin level can reflect disease in which the kidney cannot prevent albumin from leaking from the blood in to the urine and being lost.

There are three manual to detect the protein in the urine :

1. Using dipstick .
2. Using sulfosalicylic acid : put urine in test tube add 3 drops of sulfosalicylic acid (10%) ,report the result as : negative , trase, +, ++, +++ .
3. Heat and Acetic Acid Test (Protein) Principle:
   based on precipitation by heat and coagulation by acids :
   a) Fill test tube with urine (2/3 full) centrifuge.
   b) Heat the upper 2cm of the urine and observe the cloudiness. (Due to phosphates not albumin).
   c) Add 2 to 3 drops of 10% acetic acid . Cloudiness due to phosphates will disappear. Repeat the heating. Persistent cloudiness indicates albumin (Proteinuria) .

Bilirubin test :

Bilirobin is formed in the spleen and bone marrow as a result of the breakdown of hemoglobin . urine bilirobin aids in the diagnosis and monitoring of treatment for hepatitis and liver function. Can be detected by:

1. Foam test :its include put the urine sample to 1/3 of tube test and heated from upper tube until boiling and foam will appear.
   Non color of foam → negative
   Yellow foam → positive
2. Smith test : its include put the smith reagent in the tube test and put urine gradually on the walls of the tube , when the ring will appear so the result is positive or none appear will be negative.

Urobiliogen test (Ehrlich aldehyde):

Bilirobin is transformed through the action of bacterial enzyme in to urobilinogen after it enters the intestines . urobilinogen is one of the most sensitive tests available to determine liver function. Increased urinary urobilinogen occur in prehepatic jaundice (hemolytic anemia), hepatitis, hepatic necrosis and others.

Bile salt test : (Hay's test)

Done by put a little amount of crystal sulfur flower on the surface of urine sample if this crystal will go deep in the tube that’s positive result . while if stay on the surface refer to negative result.

Uric acid test :

Uric acid concentration in urine above and below normal are known as hyperuricosuria and hypouricosuria . such abnormal concentration of uric acid are not medical condition , but are associated with a variety of medical condition. High concentration may be caused arthritis (Gout), Cardiovasecular disease , Diabetes, Metabolic syndrome .

Lec.3 & Lab 3

General stool examination(GSE)

Stool is the waste that discharged from the digestion and absorption of essential food ingredient in the stomach and intestine, additional of the undigested food and unabsorbed secretions of the stomach, liver and intestines like (mucus, pus, salts, bile pigment, fat ….).

GSE is a series of tests done on a stool (feces) sample to help diagnose certain conditions affecting the digestive tract.

Normal stool

The stool of healthy adult are mixed diet is soft, well-formed, semi-solid, alkaline or neutral(PH= 6.9 – 7.2) and color is brown. The odor is aromatic. The feces are normally composed of 25% of feces matter. The stool found in jejunum and ileum are liquid and about 400g / day. But, through pass through cecum and colon, most of water is absorbed. Then converted to contents to a soft, formed mass about 150-120g / day. Mucous scanty, mixed with stool.

Collection of sample

1. Collect in a dry – clean container, must be exam within 1/2 to 1 hours.
2. The sample should be un contaminated with urine or other body secretion.
3. Collect stool before antibiotic therapy.
4. Warm stool are best for detecting ova or parasites, don’t refrigerate specimen.
5. Examine liquid stool and those containing mucus or blood first because they may contain motile amoebae which die quickly.

6. Cover all specimens tightly, place the label on the cup, not on the lid.
7. Never examining stool samples without first putting on gloves.

Physical examination of stool

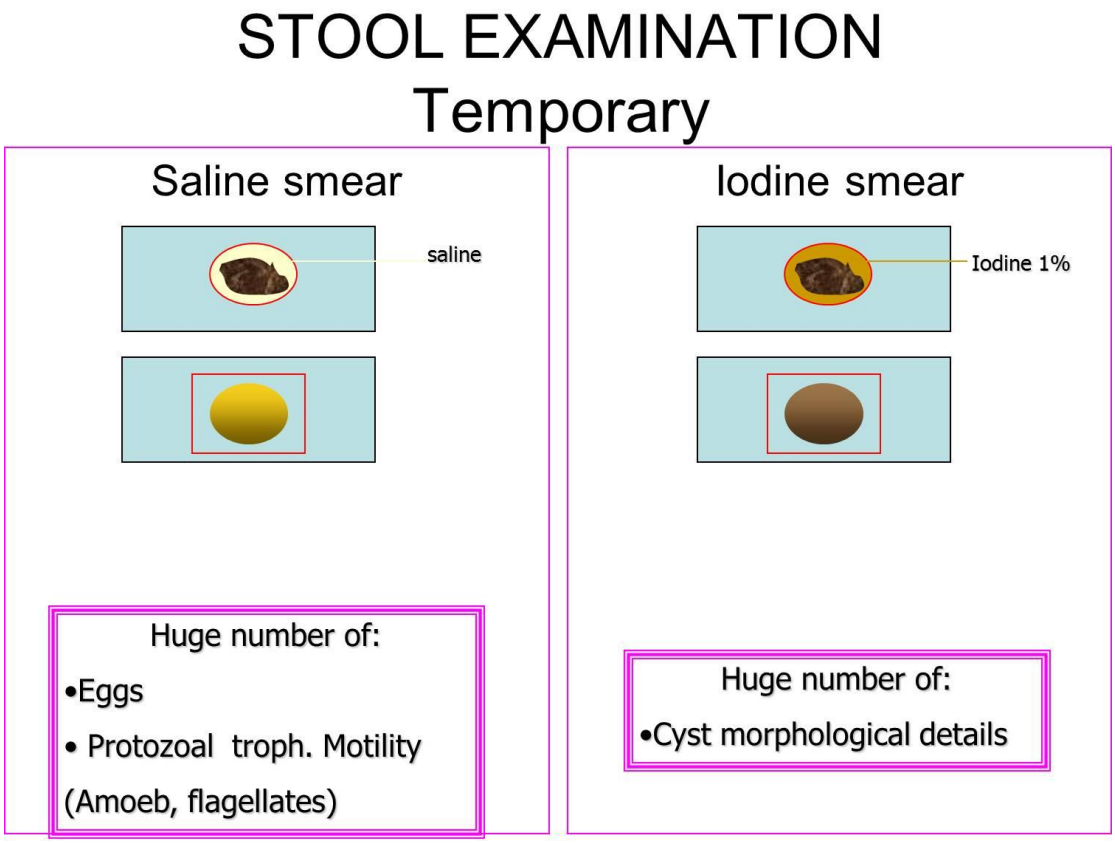
- **Appearance**: the stool appearance solid or semi-solid (soft), liquid or watery, liquid- mucus, soft liquid.
- **Color:**
    1. The normal color of stool is brown due to a bile pigment
    2. Black color is result from upper gastrointestinal tract infection
    3. Pale yellow is refer to pancreatic deficiency
    4. Red or pink is result from lower gastrointestinal tract bleeding
    5. Green color is result from ingestion of spinach or taking oral antimicrobial drugs

Microscopic examination

Preparation of sample: There are more type for preparation, some of them

1. **Wet preparation** (saline solution):
    - Add one drop of normal saline on dry slide
    - Take small amount of stool from center and surface, using wooden stick, and mix it with drops of normal saline
    - Put the cover slide
2. **Iodine preparation**:
   One drop of saline, emulsion is put on glass slide. Add one drop of iodine and place a cover slide over it. It is essential for identical of cyst and trophozoite of protozoa and

helpful for staining inclusion with cysts and troph. Such as nuclei and chromatoid bodies.



3. **Flotation** :

Done by saturate solution of salt or saccharides with high specific gravity. Such as Zinc sulfate flotation is optical for ova and cyst of parasite.

- Put zinc sulfate in test tube and adds the stool
- Mixed the mixture, then add amount of solution flotation to filled test tube (3/4)
- Cover the test tube, and let 1/2 hour to attach ova or cyst on surface of solution. Then exam under microscope.

Large amount of leukocytes:

Usually found in chronic ulcerative colitis, shigellosis, invasive E. coli diarrhea. Less leukocytes is associated with cholera, viral diarrhea and parasite.

Mucus in stool:

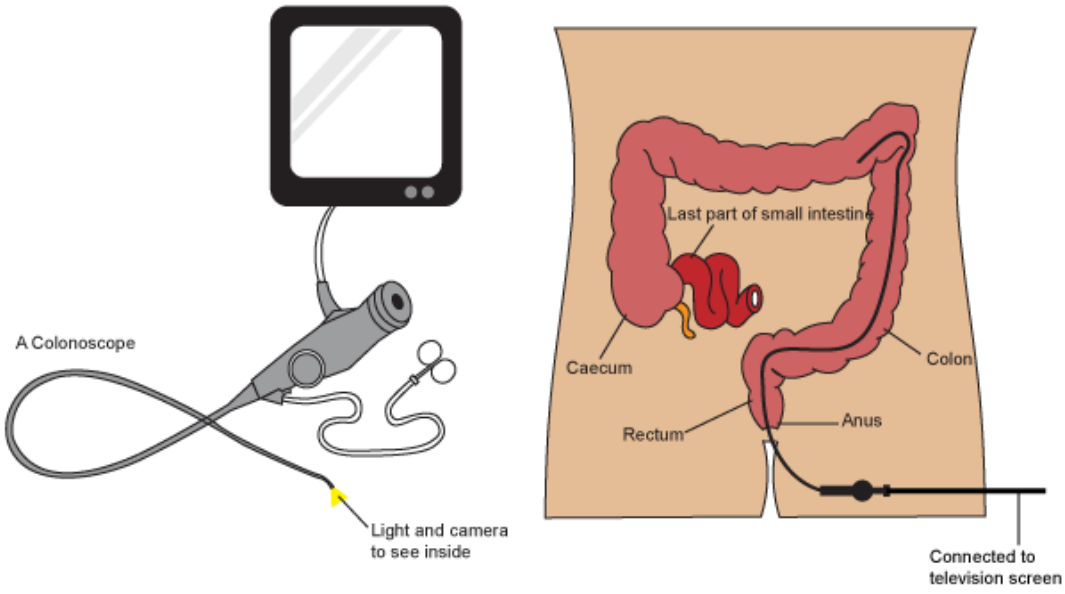
Usually mucus must not be found or absent in stool, mucus seen with inflammatory cases and with ulcerative colitis. Mucus sometimes present with blood or pus cell.

Chemical exam:

1. **Fecal occult blood test(FOBT):**

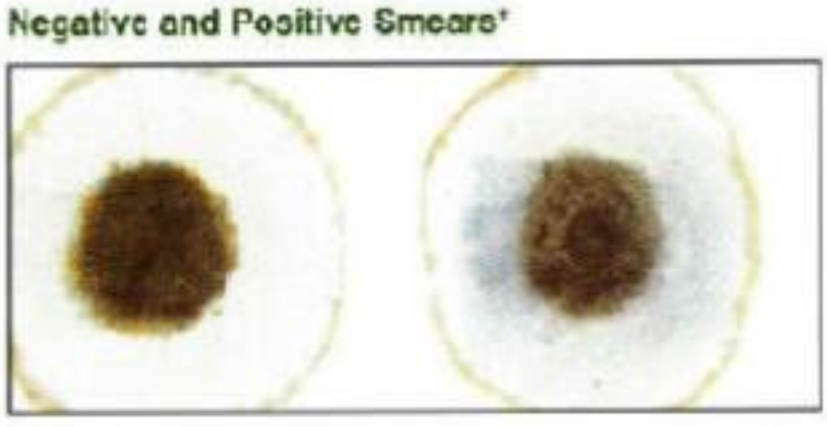
   Presence of blood in stool indicates abnormality. If RBCs are seen in microscope, the bleeding has occurred below the gut region due to a variety of reasons (E. histolytica, bacillary dysentery and ulcerative). But, if the stool black without presence of RBCs this occult of blood is recognized chemically. In this case outer portion of feces avoided and takes the sample from the center.

   Before occult test the patient should not take any medication containing iron like Aspirin or eat red meat that contain peroxidase (false positive)and vitamin C (false negative). Fecal occult blood tests offer an important screening modality for colon cancer, but, because it detects only a minority of cancers and precancerous polyups, by itself it is not sufficient. It needs to be complemented by endoscopic examination of the colon(colonoscopy).



Procedure:

1. **Guaiac test(gFOBT):** principle of test is oxgen liberated from hydrogen peroxide that peroxides of the heam portion in hemoglobin molecule. Its include placing a small sample of stool on chemical solution is put on top of the sample. If the card turn blue, there is occult blood in the stool sample.



2. **Prepare solution of aminopyrine** (0.5g of aminopyrine+ 5 ml ethanol 95%) in test tube
    - Centrifuge the stool + 7 ml D.W for 5 min.
    - Transfer the supernatant into another tube then add 10 drops of acetic acid (10%) and 5 ml of aminopyrine solution and add 10 drops of hydrogen peroxide
    - Result should be read within 5 min. of adding hydrogen peroxide
    - The red color appears, the result is positive to occult blood in feces
3. **Immunologic testing**

2. **Fecal fat test**: is the standard test for diagnosis steatorrhea.

There are three majure causes include:

- Weak of intestine absorption
- Deficiency of pancreatic digestive enzymes
- Deficiency of bile

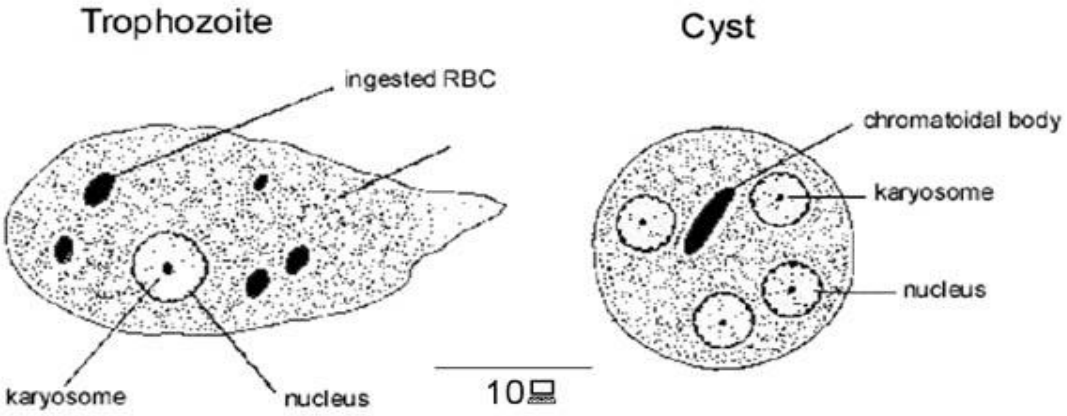
Procedure

1. Put small amount of stool on slide
2. Add 2 drops of ethanol 95% and staining with a Sudan III dye (2 drops), mixed well.
3. Put cover slide and examined under a microscope
4. Visible amounts of fat indicate some degree of fat malabsorption.
    - Fatty acid : appear as needle like crystals that don't stain< 100 fat globules
    - Fatty acid salt: appear as amorphous

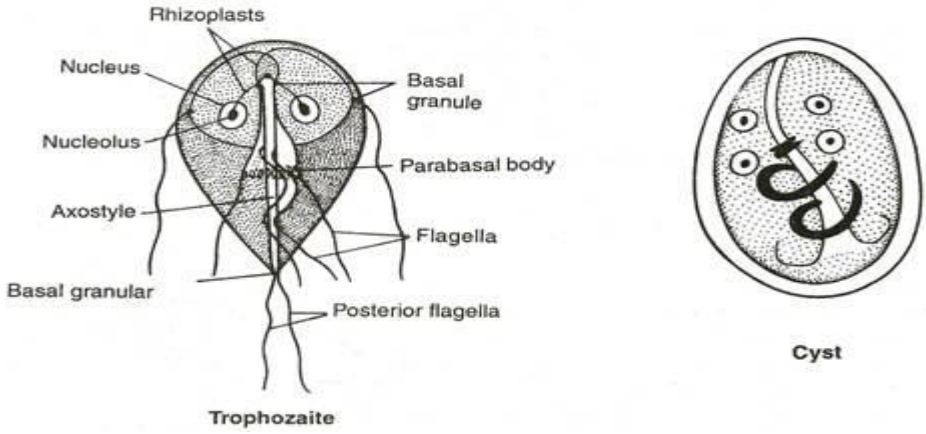
- Triglycerides: appear as large orange or red droplets <50 fat globules

Other parasites:

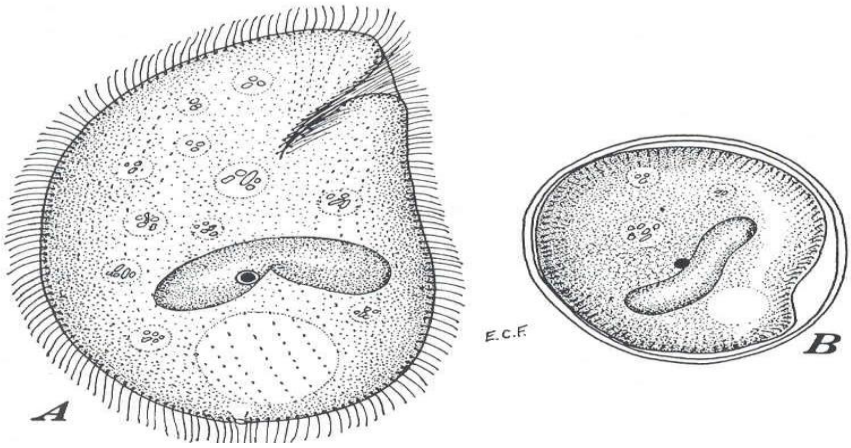
1. **Protozoa**:
    - *Entamoeba histolytica* (dysentery)



    - *Giardia lamblia* (Giardiasis)



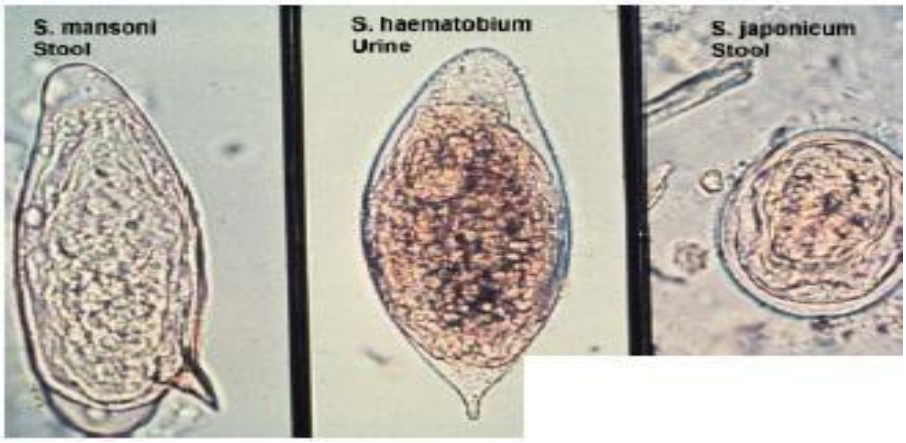
- *Balantidium coli* (Balantidiasis)



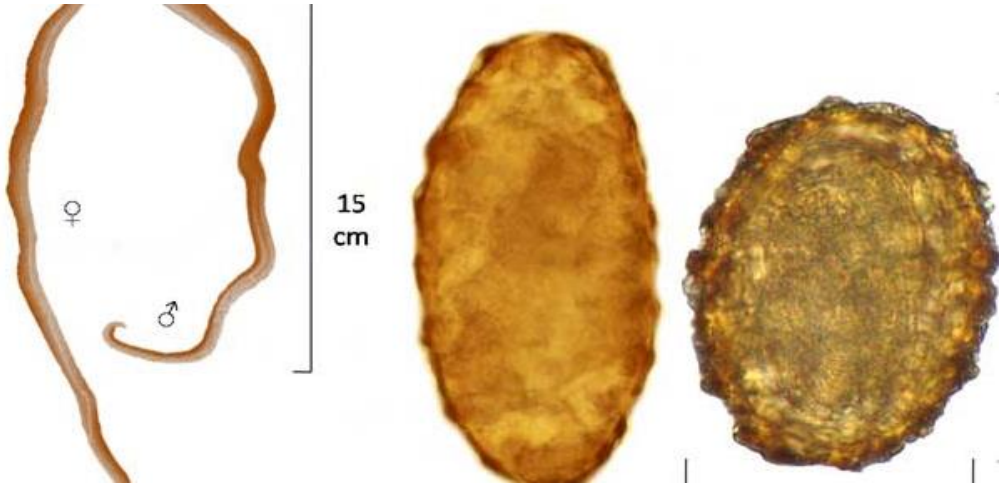
2. Worms

- *Trichuris trichiura*

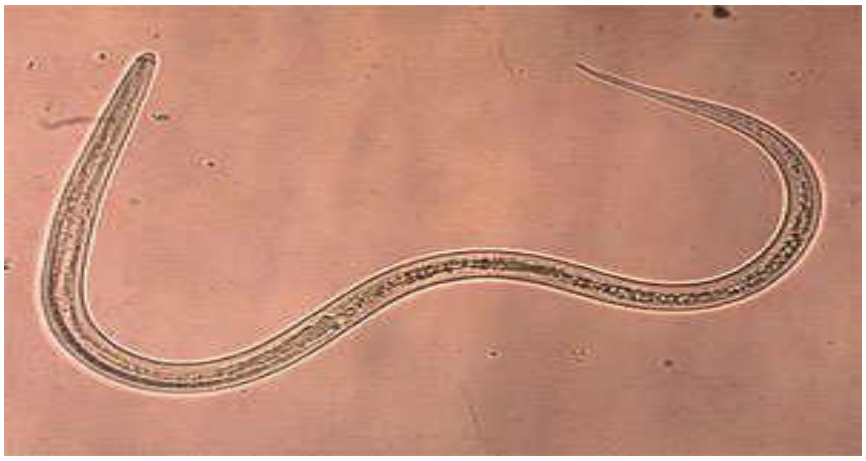
- *Schistosoma* eggs and worm



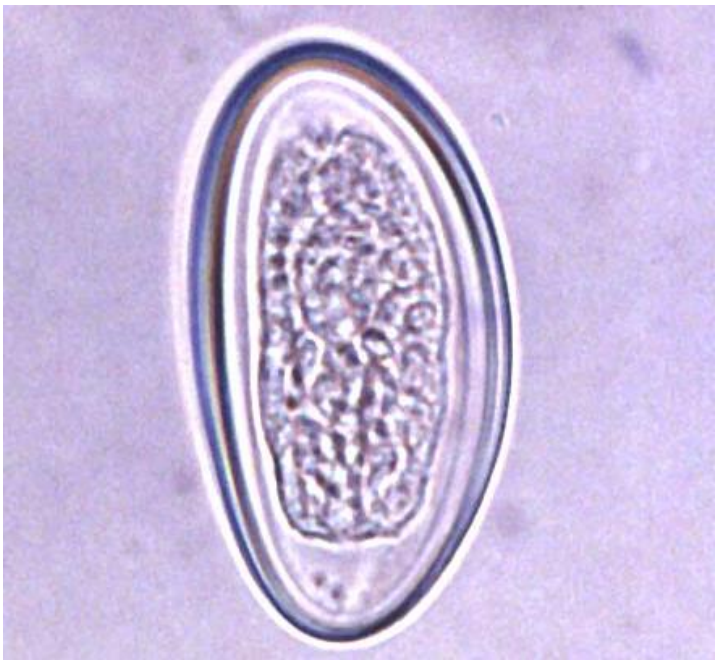
- *Ascaris lumbricoides*



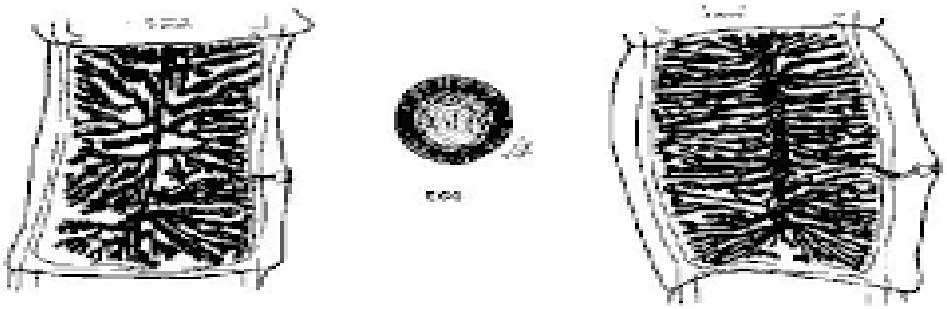
- *Ancylostoma duodenale*



- *Enterobius vermicularis*



- *Taenia*



Lec 2. and Lab 2 **Asst Instructor**

Heba Ameen

Urine Analysis

(Urinanalysis)

C. Microscopic examination of urine

Urine is examined microscopically to refers to all solid materials suspended in the urine specimen. Normal sediment :

1. WBC cells
2. Red cells
3. Casts and crystals
4. Yeast cells
5. T . vaginalis motile trophozoite
6. S. haematobium eggs
7. Bacteria
8. Epithelial cells
9. Spermatozoa

Preparation of sample is as following :

1. Put sample of urine in centrifuged tubes about 3/4 of the tube.
2. Centrifuge at 500- 1000 for 5 min.
3. Pour the supernatant fluid in another tube to be used for biochemical test.
4. Remix the sediment and transfer one drop to a slide and cover with cover slip.
5. Examine the preparation microscopically used 10x and 40x .
6. Examine at the minimum in 10 fields.

1. White blood cells (pus cells):

The number of WBCs in urine sediment is normally low (or very few). When the number is high, it indicates an infection or inflammation somewhere in the urinary tract and called (pyuria). pyuria is significant in most UTI caused by *E. coli, Proteus, Klesiella , Staph, Pseudomonas* and many others. WBCs can also be a contaminant, such as those from vaginal secretions. WBCs in pyuria mostly PMNs which are round, 10-15 µm in diameter that contain granules and often found in clump.

Interpretation of the results

2-3 → trace

7-10 → +

15- 20 → ++

25- 30 → +++

Upper 30 → ++++ (full fields)

2. Red blood cells (RBCs):

Normally no blood is found in urine except in certain physiological conditions like during menstruation period or by hemorrhoids. The presence of RBCs in urine indicator to damage in the kidney or tumor and can be indicator for damage in other organs and called hemoglobinuria (hematuria) . RBC will appear like circular disk and smaller from the WBCs , interpretation of the results of RBCs is the same in WBC.

3. Epithelial Cells:

Normally found in women but, abnormal in male. A few epithelial cells from the bladder or from the external urethra can be found in the urine sediment. Cells from the kidney (kidney cells) are less common. Its large size and fusiform and mononuclear cells but sometimes have two nuclei . In urinary tract conditions such as infections and inflammation or in physical conditions such as pregnant

women and vaginal contamination of the specimen, more epithelial cells are present.

4. Casts:

Casts are cylindrical particles sometimes found in urine that are formed from abnormal protein secreted by kidney cells. They are formed in the long, thin, hollow tubes of the kidneys because they are formed in the kidney tubules . mostly , they can be seen in centrifuged urine with 10x . The presence of casts in urine indicator for renal disorder and there are different types of casts can be seen in urine cause different kidney diseases,

Types of casts:

a. **Hyaline casts**: they are colorless and empty. A few may be seen in the urine of healthy persons after stress mucosal effort but if its increase that indicates damage of glomerular filter.
b. **Waxy Casts:** they are thicker and denser with twisted shape, referred to as severe chronic renal disease (renal failure) casts.
c. **RBC Casts:** RBCs may be found in a cast ,orange color either as the result of leakage of RBCs through the glomerular membrane or by bleeding into the tubules at any point along the nephron.
d. **WBC Casts**: are generally composed of neutrophils, found in case of inflammation of the kidney tubules (UTIs).
e. **Granular casts**: are generally the result of degeneration of cells in cellular casts or serum proteins and other substances and associated with renal damage.
f. **Other Casts:** like fatty casts, bacterial casts, crystal casts and epithelial casts.

5. Yeast cells :

Can be seen this cells in the urine of person suffering from diabetic , immunosuppression and can be found in urine of women with vaginal candidiasis (*candida albicans*). Some time will confuse between the shape of RBC and yeast cells in microscope so will be used acetic acid ; if the cells are RBC , they will haemolysis by the acid, but yeast stay not affected. Also can recognized the yeast by its shape (smaller and have budding).

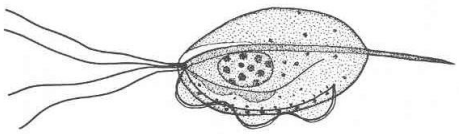
6. Parasite:

a. Trichomonas vaginalis trophoziote:

Trichomonas vaginalis is one of the causative agents of vaginitis in women and some time seen in the men's urine. Can be detected by his shape or characteristic motility.

b. ***Schistosoma haematobium*** **eggs** :

can be excreted in urine after about 4-6 weeks of *Sch*. Infection and can be detected by its shape and size (large, bear terminal spine).



7. Spermatozoa :

Found in the urine of men or women after sexual connecting.

8. Crystals:

Urine contains many dissolved substances (solutes) – waste chemicals that the body needs to eliminate. These solutes can form crystals, solid forms of a particular

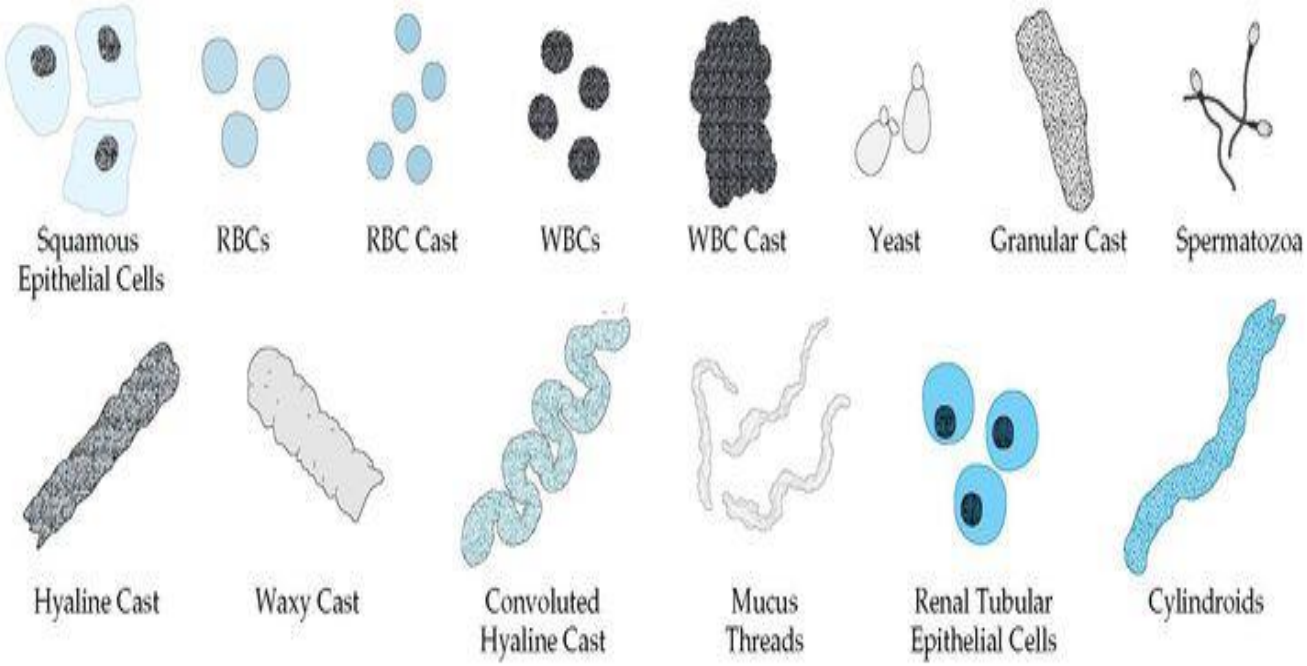
substance. Crystals may be associated with formation of urinary tract stones. The PH of urine is an important to identify the crystals.

Types of the crystals:

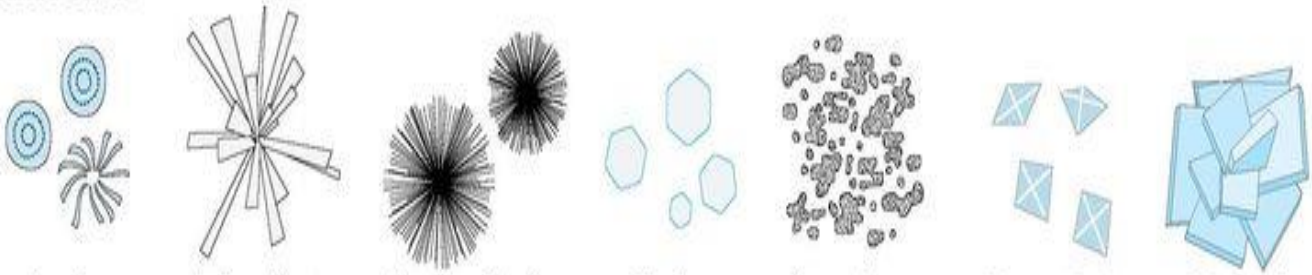
a. **Tyrosine crystals**: which are yellow or dark color and look like needles massed together , found in severe liver disease.
b. **Triple phosphate crystals:** found in the alkaline urine (PH>7), with no clinical significant (normal).
c. **Calcium oxalate crystals** : which may indicate urinary calculi in acidic urine (PH <7) and indicate to form the stone, its shape like envelope .
d. **Uric acid crystals**: yellow or pink – brown indicates to urinary calculi.
e. **Amorphase urate**: found in the acidic urine like sand granule
f. **Amorphase phosphate**: found in alkaline urine
g. **Calcium carbonate:** found in alkaline urine and it's like dumbbell
h. **Other types** : such as calcium phosphate , cholesterol cystine and sodium carbonate crystals.

9. **Bacteria**: usually seen as rods or cocci or streptococci in very few number which is normal but when number will increase in urine called bacteriuria and culture is recommended. Before culture must be staining bacteria by gram stain to identify the shape of bacteria.

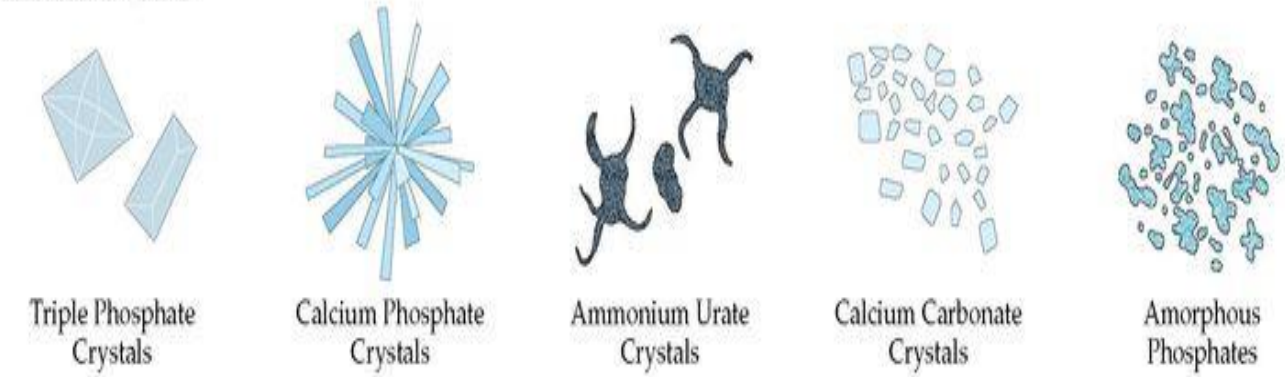
NORMAL URINE



ACID URINE



ALKALINE URINE



Culture urine

Urine sample must be :

1. Collated in sterile container
2. Collection type is mid stream (clean-catch)
3. Collecting before 1/4 hour from culturing to avoid grow and increase in number of bacteria
4. The patient not taken any antibiotic at least before three days from the culture

Culture media :

1. Blood agar
2. Mackonkey agar
3. Cystine lactose electrolyte deficient agar CLED (green to yellow).

NOTES:

- **Type and number of bacteria in culture should be taken in consideration.**
- **Culture results of less than 10^4 (10.000) bacteria / 1ml of urine is not significant, whereas 10^5 or more bac. / 1ml of urine is a significant bacteriuria.**
- **When the results of urine culture shows mixed types of bac. , the results are not significant and must repeated after 2 days**
- **In some cases the culture give negative result but the routine test detect bac. in urine , the reason may be type of bacteria like TB .**
- **Most important pathogens can be recovered in urine culture is :**

  1. E. coli

2. Proteus sp.
3. Pseudomonas sp.
4. Klebsiella sp.
5. Staph. sp
6. Enterococcus sp.

Lec. 4 & Lab. 4

Seminal fluid examination

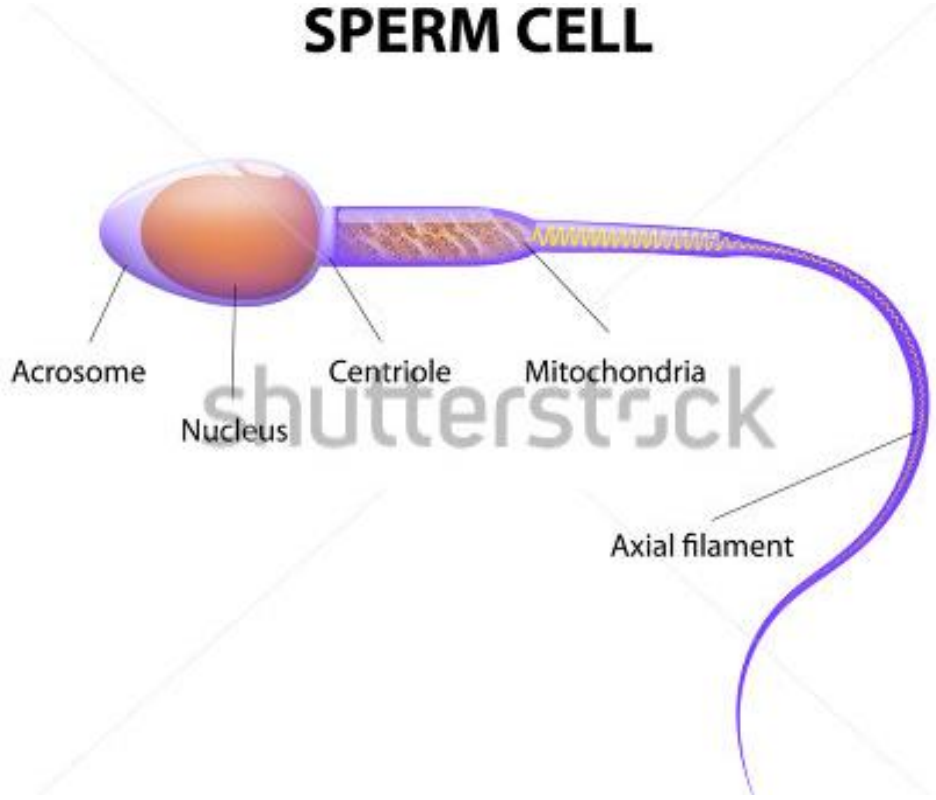
Semen: is a thick , cloudy, white substance that contains sperm and fluid. The fluid provided nutrients for sperm. Semen contains citric acid, free amino acid, fructose, enzymes, prostaglandin, potassium and zinc.

Parts of sperm:

Head: contains genetic material of the father in its nucleus.

Tail: responsible for motility

Mid- piece of sperm contain of mitochondria, which provide energy for sperm motion.



Semen analysis: is one of the first tests done to help find out if a man has a problem (infertility). The case of male

infertility may be due to deficiency and quantity of spermatozoa.

Collection of samples:

1. The seminal fluid must be collected in clean, dry and wide container
2. Specimen must be fresh, and analysis within 30 min. of collection and don’t refrigerated sample
3. Ask of the patient to avoid any sexual activity for 3-5 days before semen analysis
4. Avoid drinking alcohol few days
5. Put the semen in the incubator at 37C till it's being liquid, if specimen collects at home, keep it out of direct sunlight. Carry the semen container near of the body, to keep it in at body temperature
6. Any delay in exam may be effect on test result (PH, temperature, insufficient nutrients)

Microscopic examination

1. **Volume**: normal range (1.5-5)ml. The sample measured by small cylinder (1-6ml)
2. **Color** : the normal is milky, but yellow color refer superlative infection
3. **Viscosity** (liquefaction): freshly semen is viscous and take less than 20 min. for the sample to change from a viscous into liquid. Any long of liquefaction (more than 30 min at 37C) may indicate an infection. Very viscous may be inhibited of motility and activity of sperms.

   Not : liquefaction can be measured by using pipette:

   - Normal semen sample leave the pipette as small drops

- Abnormal semen samples the drops form mucus thread. Watery semen indicated a low sperm concentration or the absence of sperm.

4. **Reaction(PH):** in normal condition is alkalinity. PH is alkaline range of 7-8. It is test by put drop of semen on litmus paper. Abnormal PH may be indicated on inflammatory disorders of the prostate and seminal vesicles.

Microscopic examination

Sperm motility: is the ability of sperm to move. Its normal found low count of non motile sperms in semen sample

Procedure of sperm motility:

1. Put 20-25 µl of semen sample on dry clean slide, put cover slide over it.
2. Examine several fields on low power then on high power, see motility, pus, RBCs, Epithelial cells and morphology of sperms
3. If the motility is very slow, put the semen sample in incubator at 37C because which may be restore motility for 10-15%

There are 4 types of sperm movement :

1. **Active**: sperm with progressive motility. These are the strongest and swim fast in straight line
2. **Slow** (non-linear motility): these also move forward but tend to travel in a curved or slowly movement
3. **Sluggish** : sperm move their tails, but don't move forward (local motility only)
4. **Dead** (non-motility): sperm don't move at all

Azoospermia :it's one of semen problems and mean the absence of sperms in the semen sample even after centrifugation of sample

Sperms morphology: the normal is 100% of sperms have normal shape, semen that contain 20% abnormal sperms is stile fertile

Teratozoospermia: is found many of sperm have abnormal shape above 20%.

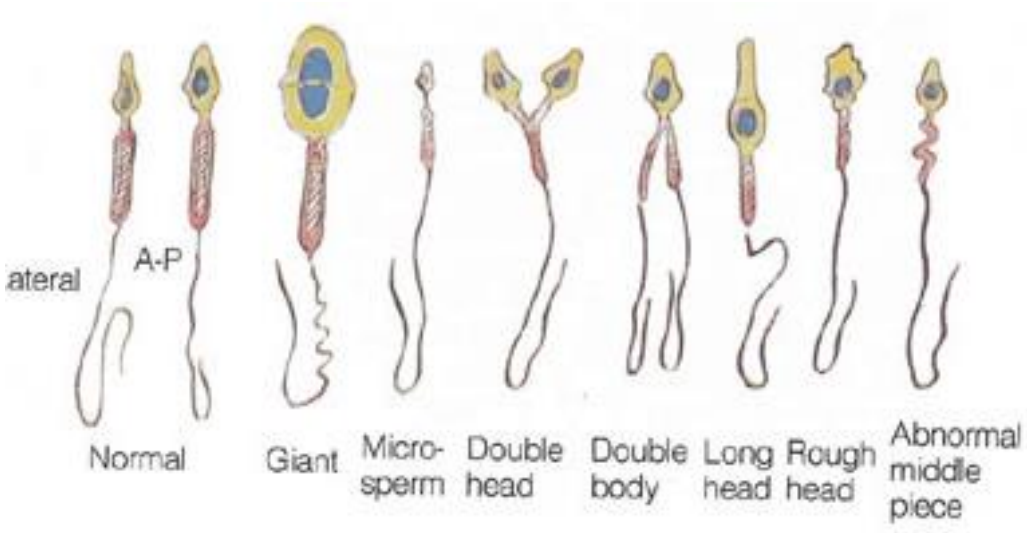
Sperms can be abnormal in shape:

Head :

1. Two heads
2. Giant head
3. Tiny head
4. Rather head like oval
5. Rough head
6. Tapering head
7. Amorphous head
8. Small head
9. Abnormal mid piece

Tails :

1. Two tail
2. Short tail
3. Bent tail
4. Colloid tail
5. Double body



6. Sperm clumping : under microscope seen the sperms sticking together head to head, mid piece to mid piece and tail to tail. Clumping of sperms prevent sperm motility to swimming towards the egg. Semen that contain clumps may be indicate the presence of antibodies to sperm

   Not :some abnormal sperm are usually found in every normal semen sample

White blood cells (pus):

You must be differentiated between sperm precursor cell (which normally found in the semen) and pus cell.

Count of total sperm : the sperm count done to see of found enough number of sperm. The normal count of semen sample is range (20-120million / ml). the sample is considered low sperms, if it has less than 20 million sperm per ml. this case called **Oligozoospermia.**

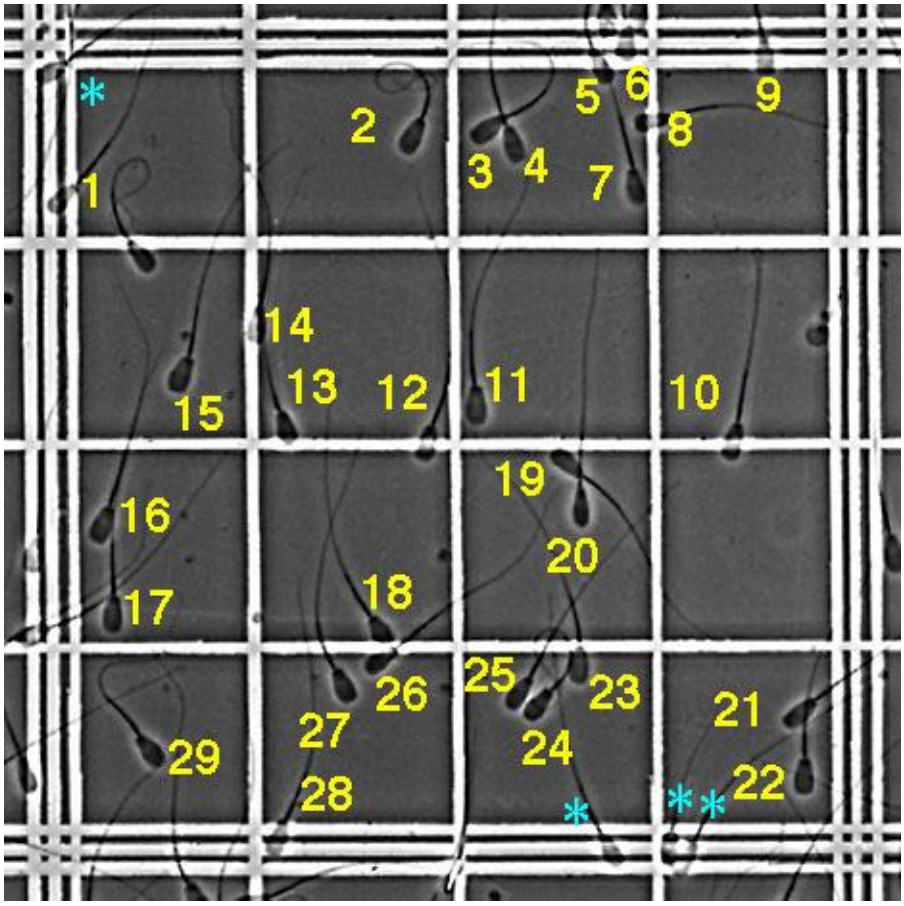
Cause of low sperm count:

1. Smoking cigarettes
2. Drugs reduce count to 50%

3. Drinking alcohol
4. Environmental toxin like radiation
5. Testicular injury
6. Nutrient deficiency
7. Problems with sperm production(genetic or hormonal)

Procedure of sperm count : first, seminal fluid must be diluted by preparing diluting fluid as the following:

1. Prepare diluting solution by (mix 5gm of sodium bicarbonate + 1 ml formalin + 100ml D.W)
2. After liquefaction take 400µl of diluted solution in test tube, then add 20µl of semen sample
3. Wait 5-10 of min.
4. Take 20µl of diluted semen sample and place it over haemacytometer (chamber)
5. After charging the chumber with diluted sample, allow the semen to settle down for 2min.
6. By microscope on high power, count the sperm in one large square (medium square) then multiplied the number of sperm in one large (e.g350) by 200.000, the result is 70.000.000 miliion/ml.



Abnormalities of semen sample:

- Aspermia : absence of semen
- Azoospermia: absence of semen
- Hypospermia: low semen volume
- Oligozoospermia: low sperm count
- Asthenozoospermia: poor sperm motility
- Teratozoospermia: more morphology defect

Lec. 5 & Lab. 5

Cerebral spinal fluid analysis (CSF)

CSF: it is a clear, colorless fluid, that occupies cavity of brain and spinal cord. It is produced in the brain (approximately 50-70). A 600-700 ml of CSF is produce daily.

Functions:

1. CSF protect the nervous system from injury.
2. Its supplies nutrients to the tissues of the central nervous system and removes waste by returning them to the blood.

Compositions:

The CSF contain approximately 15-45 mg/ dl protein and glucose con. in CSF of 60-80% of con. of blood (50-80% mg/dl in children and adult, but higher in infant), WBCs (1-6 µl lymphocytes and no neutrophils), urea, creatnine that filtration from plasma and K^{+}, Ca^{++}, Mg^{++}, Cl^{-} and Na^{+} ions.

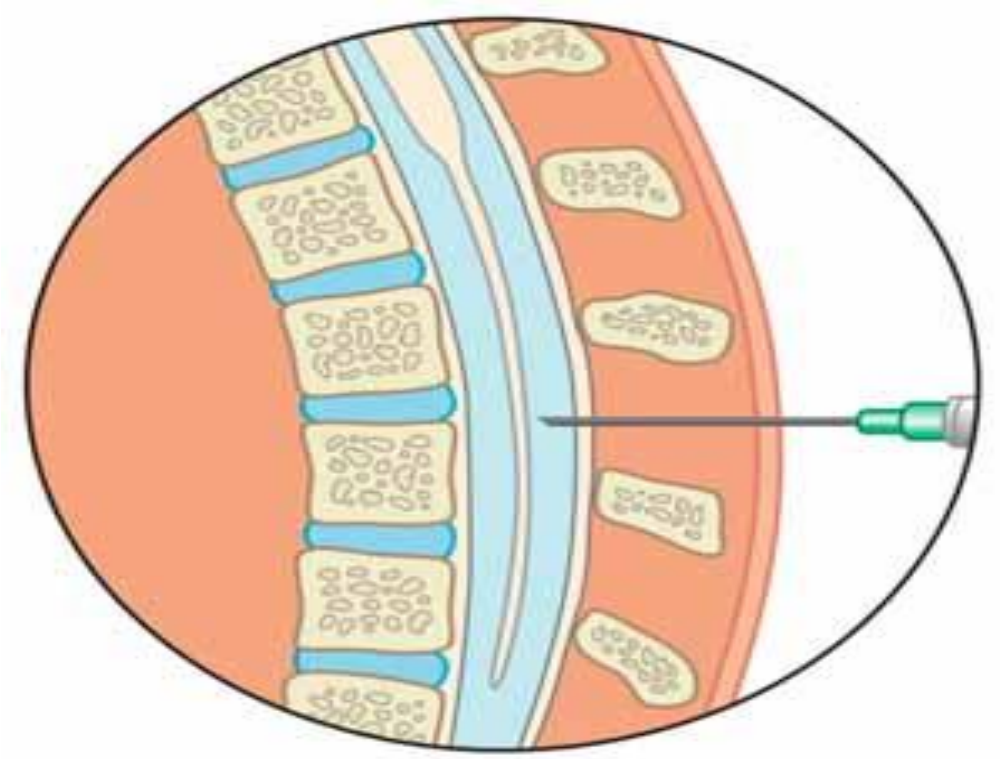
CSF analysis: includes tests in clinical biochemistry, hematology, immunology and microbiology.

Important of CSF analysis : to diagnose the following

1. Meningitis (inflammatory of membrane that surrounding brain and spinal cord)
2. Bleeding in the central nervous system
3. Tumors
4. Parasite and bacterial infection in the central nervous system.

Collection of CSF (consideration and procedure)

1. CSF is collected by technique called lumber puncture or spinal tap at the junction of L3/L4 or L4/L5 vertebrae



2. The patient sites in a bending position
3. Clean with antiseptic solution the entire puncture site with surrounding area
4. Collecting specimen consisting of up to 20 ml CSF within 1-2 min. take up to 4 tubes, about 2-3 ml in each tube
   Tube 1 : used for chemistry test
   Tube 2 : used for microbiology test
   Tube 3: used for hematology test
   Tube 4 : used for special test like protein electrophoresis, immunologic studies and syphilis test
5. Put small sterile dressing to the puncture site
6. CSF sample should never be place in the refrigerator (if viral studies are done on small part of sample should be frozen)
7. CSF must be taken to the lab immediately, within 1 hr. and maximum 2 hr. because cell and trypanosome are rapidly lysis in CSF samples, glucose is also destroyed, unless preserved with fluoride oxalate

8. It is preferred to collect sample before snit microbial therapy is started

Macroscopically examination of CSF

1. **Color** :
    - The normal CSF is colorless
    - The red or pink color, allowed the tube until RBC to settle or centrifuge for 5 min. then examined the supernatant fluid:
        a. If the supernatant fluid is clear. The blood is there because accidental injury to blood vessel
        b. If the supernatant fluid is red, the blood is there because a subarachnoid hemorrhage
    - Yellow color (xanthochromia):
        a. Due to subarachnoid hemorrhage. It is mainly resulted from oxyhemoglobin which appear in 4 to 6 hr. after 24hrs. xanthochromia is increased because of conversion hemoglobin to bilirubin
        b. Sever jaundice
        c. Large amount of pus cells
        d. False positive by iodine contamination
2. **Appearance:**
   normal CSF is clear, specific gravity 1006-1009. Abnormal CSF may appear cloudy, smoky or bloody.
3. **Clot formation** :
   within 10 min. of collecting seen clots. Small clotting in CSF is abnormal and indicates T.B. meningitis. Large clotting indicates pus inflammation in meanings and increase protein and fibrinogen.

Microbiology test

The sample of CSF will be cultured on different media and then centrifuged the sample to examine under microscope

- ✓ **Culture of CSF** : by loop and burner take part of CSF and stretching on different media, incubated at 37C.

  Types of agars:

  - Blood agar to detect the growth of *Strep* and *Staph*
  - Chocolate agar to detect the growth of *Niesseria meningitides* and *Hemophilus influenza*
  - Mac connkey agar to detect the growth of E . coli and other Enterobacteria
  - Lowenstein Jensen agar to detect the growth of *Mycobacterium tuberculosis*
  - Sabourouds agar to detect the growth of fungi

- ✓ **Microscopic examination :**

- **Wet preparation** : after centrifugation for 20 min. with half of centrifuge speed to seen blood cell and motile trypanosomes in African area that indicates on central nervous system infection
- **Gram stain smears** : to seen *strep pneumonia* and *Hemophilus influenza* and *Niesseria meningitides*
- **Ziehl- neelsen stain smears**: if *tuberculosis* is suspected
- **Inndia ink** : to seen *Candida albicans* or other fungi

Biochemical test

1. **Glucose** : a blood glucose sample must be obtained at least 60 min. before lumber puncture for comparison. For determination of glucose concentration in the CSF, all methods that are used for determination of blood glucose concentration can be applied:

There are two methods :

a. Benedicts test
b. Enzymatic method (using kit with spectrophotometer)

Not: glucose con. ↑ → diabetic

glucose con. ↓ → hypoglycemia (can cause coma)

2. **Protein** : normally low in CSF . source of CSF protein :
    - Filtrate from plasma (small M.W protein)
    - Local synthesis (immunoglobulin)

There are two methods to measured P. in CSF

1. **Turbidimetric method (total P.)**
   Principle : salfosalic acid denaturated the P. (albumin and immunoglobulin)that present in CSF. The turbidity formed is matches with standard P. tube
    a. Pipette 3 ml of salfosalic acid in to test tube. Add 1 ml of CSF and mix. Leave the tube for 5 min.
    b. Compare the cloudiness of the test sample against the P. standard

Increase P. in meningitis, subarachnoid hemorrhage and African trypanosomiasis and the presence of pus

2. **Pandy method (measured immunoglobulin only)**
   Procedure :
    - Measured one ml of pandy reagent (phenol solution) in to a small test tube
    - Plase the tube in front of a piece of black
    - By using a dropping pipette, slowly add three drops of CSF, examine the solution after the addition of each drop
    - Read the result immediately
    - Results :

Positive → white cloud formed
Negative → no white cloud formed

Normal composition of CSF

Appearance :	Clear ,Colorless
Lymphocytes :	1 - 5 /H.P.F.
pH :	7.4
Total Volume :	100 - 150 ml
Daily Secretion :	450 - 500 µl
Specific Gravity :	1.006 - 1.007
Protein :	15 - 45 mg/dl
Glucose :	50 - 80 mg /dl
Chloride :	115 - 130 mmol /L
Calcium :	1.0 - 1.40 mmol/L
Phosphorus :	0.4 - 0.7 mmol/L
Magnesium :	1.2 - 1.5 mmol/L
Potassium :	2.6 - 3.0 mmol/L

Hematology test

1. **WBC count (chamber method):** cell count should not be performed on specimens counting a clot because the clot would to invalidate result. WBC count should be without centrifugation

   Procedure :

   - Cover the counting chamber with cover slide
   - Put 20 µl of CSF and 400 µl of Turk solution or saline (WBC dilute) in small test tube
   - Gently mixed and filled the chamber with fluid
   - Leave the counting chamber on the bench for 5 min. put the chamber on the microscope stage
   - Count the cell in low power and report the result

Not:

- ✓ if undiluted CSF is used, examine the cells using high power that cells are leukocytes not RBC.
- ✓ Acetic acid cannot be used as a diluents for WBCs count because of the precipitation of protein

2. Differential leukocyte count:

Procedure:

1. Centrifuge of CSF for 10 min. transport the supernatant fluid in to another tube
2. Mix the deposit by taping the end of the tube. Spread on the clean slide and leave to dry
3. Fix with methanol and stain with write stain. Examined under microscope

   Not: heat fixation affected on the morphology of the cells