Cerebrospinal Fluid (CSF) Analysis

Cerebrospinal fluid (CSF) is a clear, colorless, watery fluid that flows in and around the brain and spinal cord. It controls and coordinates everything we do, including the ability to move, breathe, see think, and more.

Functions of CSF :

1.physical support and protection of central nervous system (CNS) from trauma

2.supplyning nutrient to the CNS and removal of metabolic waste from CNS.

3.Intra-cerebral transport

Neuroendocrine roles i.e. distribution of hormones within the brain

A CSF analysis is a group of tests that use a sample of cerebrospinal fluid to help diagnose diseases of the brain and spinal cord and other conditions that affect the central nervous system.

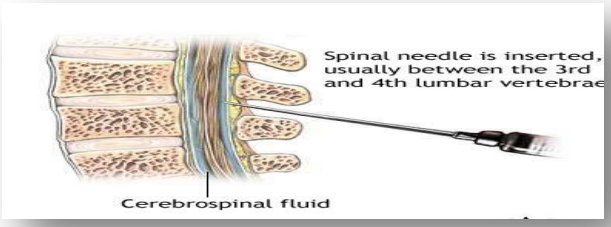
A CSF analysis is used to measure different substances in cerebrospinal fluid. It include tests to diagnose:

- **Infectious diseases of the brain and spinal cord**, including meningitis and encephalitis. CSF tests for infections look at white blood cells, bacteria, and other substances in the cerebrospinal fluid
- **Autoimmune disorders**, such as Guillain-Barré Syndrome and multiple sclerosis (MS). CSF tests for these disorders look for high levels of certain proteins in the cerebrospinal fluid.
- **Bleeding** in the brain
- **Brain tumors**, including from cancers in other parts of the body that may spread to the central nervous system
- **Alzheimer's disease**, which includes memory loss, confusion, and changes in behavior

Procedure

Collection of the sample

The sample should be collected by professional physician using lumber puncture method with complete Aseptic techniques



Collected CSF sample is immediately divided into three tubes

Tube 1. microscopic Investigation.

Tube 2. Microbiology Investigation.

Tube 3. Biochemical Investigation.

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C.S.F Analysis :

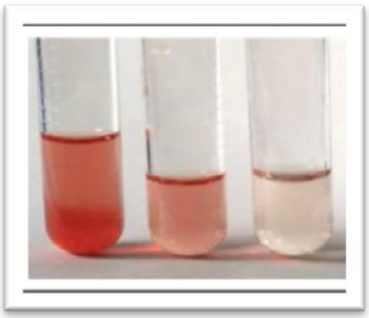
The first step in the CSF analysis is Physical and some of chemical features which includes Color , Appearance, pH and specific gravity

Normal Physical Examination	
Appearance & Color	**Clear ,Colorless**
pH	7.4
Daily Secretion	450-500 ml
Specific Gravity	1.006-1.007

The abnormal appearance of CSF is cloudy ,slightly cloudy or greyish white if there was any infection and the turbidity is due to the presence of the pus .

The abnormal color occurred in different cases for example :

- Pink or Red (bloody) ,these colors may be due to :
  1.Injury of blood vessels during lumber puncture
  2.subarchnoid hemorrhage.



Red(Bloody),pink color CSF

yellow colored CSF

- Yellow colored CSF (Xanthochromia)may be due to:
  1. Patients with 12 hours of subarachnoid hemorrhage
  2. patients with serum bilirubin levels between 10-15 mg/dl .

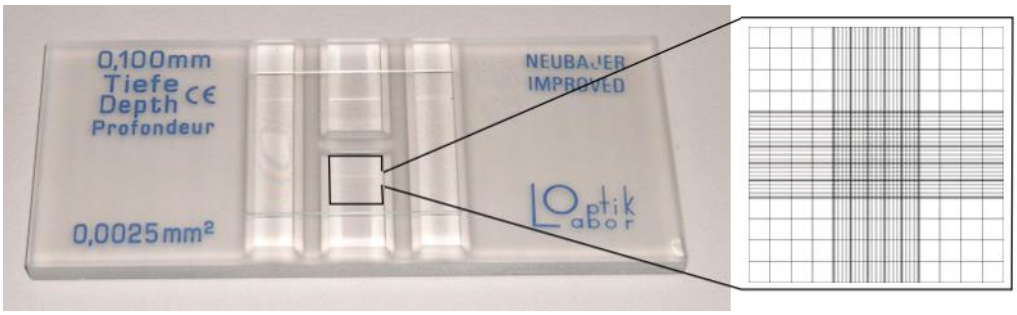
1. Microscopic Investigation

- Wet Preparation includes Total WBC count (uncentrifuged sample)

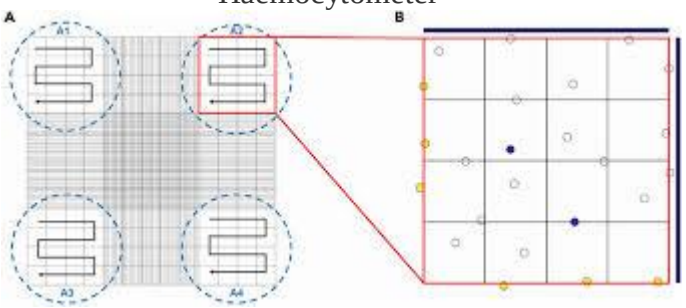
Normal CSF may contain up to 5 WBCs per mm^3 in adults and 20 WBCs per mm^3 in newborns. Eighty-seven percent of patients with bacterial meningitis will have a WBC count higher than 1,000 per mm^3

When the sample is clear , put 0.1 ml of CSF in haemocytometer chamber after fixing cover slid on the chamber ,then read the result under 10 X microscopic lens .

if the sample is cloudy or slightly cloudy you should dilute the sample using diluting Turkish solution (the dilution factor is depending on the turbidity of the sample)



Haemocytometer



Make a 1 in 20 dilution using 0.05 ml of the CSF and 0.95 ml of Türkish solution .Pipette into a small tube then mix well. put 0.1 ml of the sample in the chaber and Leave it on the bench for 3-5 minutes to allow the cells to settle. Place the chamber on the microscope stage. Then count the cells and use the calculation equation as bellow

$$\text{Cells No.} = \frac{\text{No. of cells in 1 large square X dilution factor}}{\text{Sample volume}}$$

- CSF Staining with different stains

The sample should be centrifuged ,then the deposit fixed on slides and stained with

1. Gram's stain to distinguish gram positive and gram negative if there is bacterial infection
   >Crystal violet
   >Iodine
   > Alcohol
   > Safranin

2. Lishman stain to count differential WBC count (Neutrophils, lymphocytes)
3. **Ziehl-Neelsen** stain to distinguish TB bacterial infection

2. Microbiological Investigation
Different media used to isolate and identify bacteria that cause meningitis

CSF analysis in various types of meningitis

CSF analysis	Bacterial	Tuberculosis	Viral	Fungal
Pressure	Increased	Increased	Normal to elevated	Normal to mild increase
Appearance	Turbid	Turbid	Clear	Clear
Glucose	< 40 mg%	Low	Normal to mild less	Low to normal
Proteins	Elevated	Greatly elevated	Normal to mild elevation	Normal to mild increase
RBCs	Elevated	Elevated	Normal	Normal
WBCs	10-2000/ cu mm	Elevated, but < 500	>100/ cu mm	10–50/. cu mm
WBC	Neutrophils	Lymphocytes	Lymphocytes	Lymphocytes

3.Biochemistry Investigation

CSF glucose—normal is about 2/3 the concentration of blood glucose it is about 50-70 mg/dl . Glucose levels may decrease when cells that are not normally present use up (metabolize) the glucose. These may include bacteria, white blood cells, or cells shed by tumors.

Glucose must be measured within 20 minutes of the CSF being withdrawn otherwise a falsely low result will be obtained due to glycolysis.

Use the supernatant fluid from centrifuged CSF or uncentrifuged CSF if the sample appears clear.

Glucose can be measured in c.s.f. using a colorimetric technique or a simpler semiquantitative technique using Benedict's reagent.

- **CSF protein**—only a small amount is normally present in CSF because proteins are large molecules and do not cross the barrier between the blood and brain easily. Decreases in CSF protein are not generally considered significant. Increases in protein are most commonly seen with:
  - Meningitis and brain abscess

- Brain or spinal cord tumors

Metabolic Antagonists

Several valuable drugs act as antimetabolites: they **antagonize, or block**, the functioning of metabolic pathways. The chemical reactions of metabolic pathways are catalyzed by enzymes. Enzymes function by binding a molecule referred to as the enzyme substrate. **Antimetabolites are structurally similar to the substrates of key enzymes and compete with the metabolites for the binding site of these enzymes.** However, once bound to the enzyme, the antimetabolites are different enough to block enzyme activity and further progression of the pathway. By preventing metabolism, they are **broad spectrum but bacteriostatic**; their removal reestablishes the metabolic activity.

Antimetabolites					
Sulfonamides	Static	Inhibit folic acid synthesis by competing with *p*-aminobenzoic acid (PABA)	Silver sulfadiazine, sulfamethoxazole, sulfanilamide, sulfasalazine	Broad spectrum	Nausea, vomiting, and diarrhea; hypersensitivity reactions such as rashes, photosensitivity
Trimethoprim	Static	Blocks folic acid synthesis by inhibiting the enzyme tetrahydrofolate reductase	Trimethoprim (in combination with a sulfamethoxazole)	Broad spectrum	Same as sulfonamides but less frequent
Dapsone	Static	Thought to interfere with folic acid synthesis	Dapsone	Narrow—mycobacterial infections, principally leprosy	Back, leg, or stomach pains; discolored fingernails, lips, or skin; breathing difficulties, fever, loss of appetite, skin rash, fatigue
Isoniazid	Cidal if bacteria are actively growing, static if bacteria are dormant	Exact mechanism is unclear but thought to inhibit lipid synthesis (especially mycolic acid); putative enoyl-reductase inhibitor	Isoniazid	Narrow—mycobacterial infections, principally tuberculosis	Nausea, vomiting, liver damage, seizures, "pins and needles" in extremities (peripheral neuropathy)

Sulfonamides or Sulfa Drugs

Sulfonamides, or sulfa drugs, are structurally related to sulfanilamide, an analogue of p-amino benzoic acid, or PABA (figure 9.11).

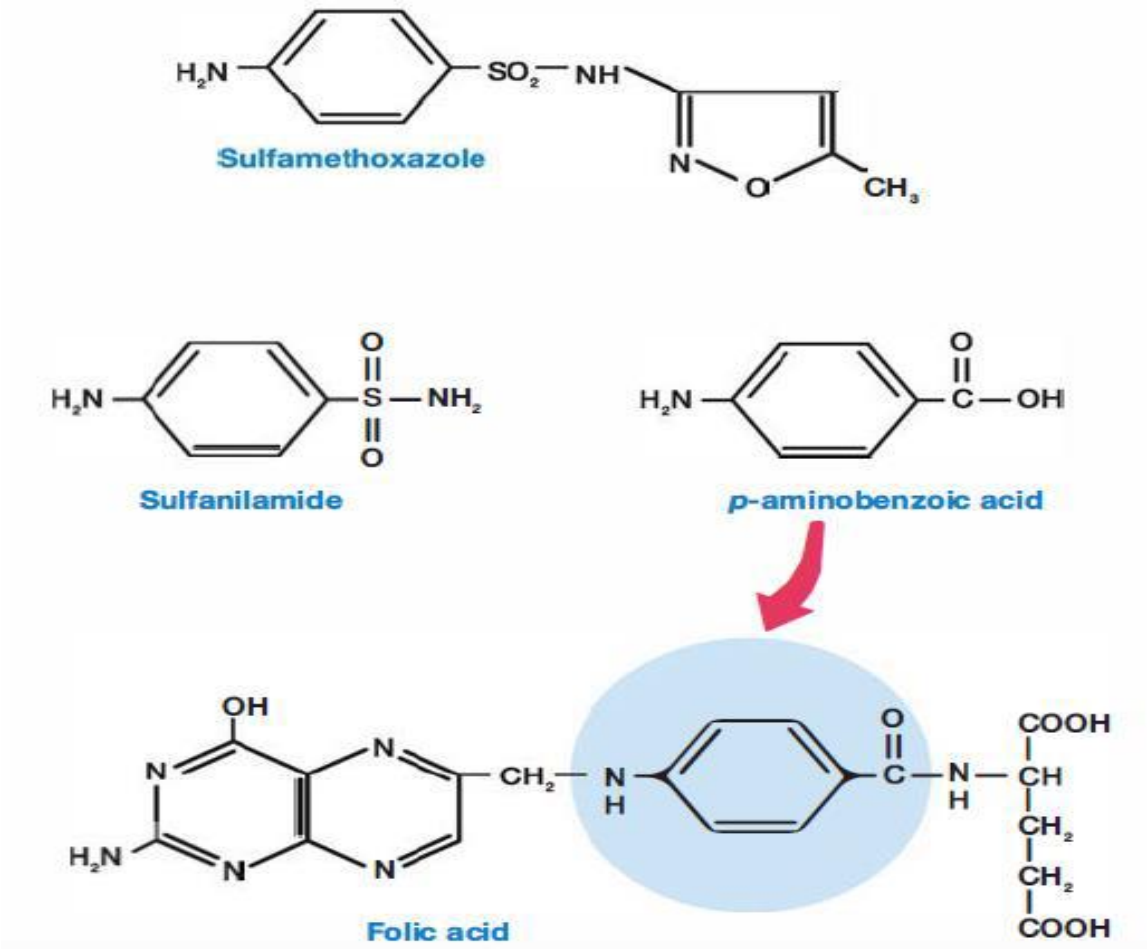


Figure 9.11 Sulfa Drugs. Both sulfanilamide and sulfamethoxazole compete with *p*-aminobenzoic acid to block folic acid synthesis.

PABA is an important component (cofactor) of many enzymes and is needed for folic acid (folate) synthesis. Folic acid is a precursor of **purines and pyrimidines, the bases used in the construction of DNA, RNA, and other important cell constituents (e.g., ATP**). When sulfanilamide or another sulfonamide enters a bacterial cell, it competes with PABA for the active site of an enzyme involved in folic acid synthesis, causing a decline in folate concentration (see figure 10.18).

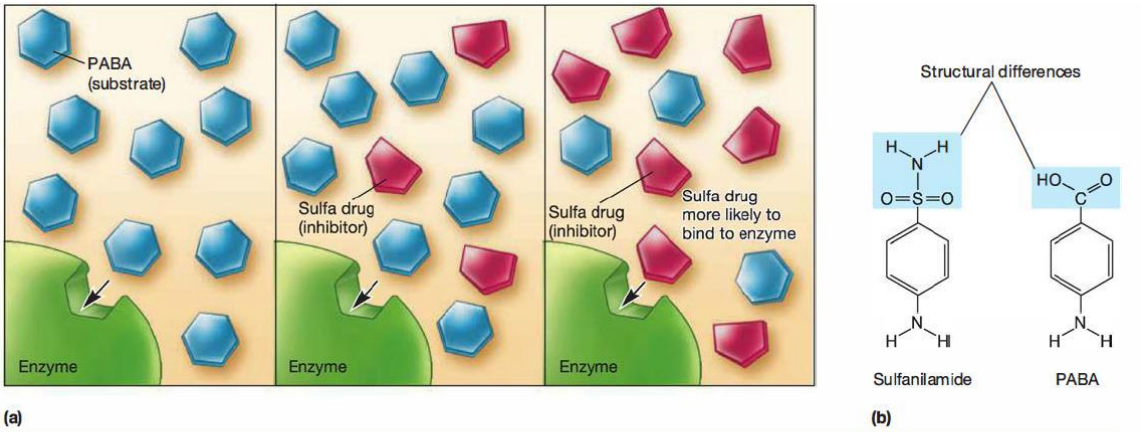


Figure 10.18 Competitive Inhibition of Enzyme Activity. (a) A competitive inhibitor is usually similar in shape to the normal substrate of the enzyme and therefore can bind the active site of the enzyme. This prevents the substrate from binding, and the reaction is blocked. (b) Structure of sulfanilamide, a structural analogue of PABA. PABA is the substrate of an enzyme involved in folic acid biosynthesis. When sulfanilamide binds the enzyme, activity of the enzyme is inhibited and synthesis of folic acid is stopped.

The resulting inhibition of purine and pyrimidine synthesis leads to ending of protein synthesis and DNA replication. Sulfonamides are selectively toxic for many bacteria and protozoa because these microbes manufacture their own folate and cannot effectively take up this cofactor, whereas humans do not synthesize folate; instead, we must obtain it in our diet. Sulfonamides thus have a high therapeutic index. However, the increasing resistance of many bacteria to sulfa drugs limits their effectiveness.

Trimethoprim

Trimethoprim is a **synthetic antibiotic** that also interferes with the production of folic acid. It does so by binding to dihydrofolatereductase (DHFR), the enzyme responsible for converting dihydrofolic acid to tetrahydrofolic acid, competing against the dihydrofolic acid substrate (figure 9.12). Trimethoprim is **abroad-spectrum antibiotic** often used to treat respiratory and middle ear infections, urinary tract infections, and traveler's diarrhea.

It is often combined with sulfa drugs to increase effectiveness of treatment by blocking two key steps in the folic acid pathway. The inhibition of two successive steps in a single biochemical pathway means that less of each drug is needed in combination than when used alone. This is termed a synergistic drug interaction.

Protein Synthesis Inhibitors

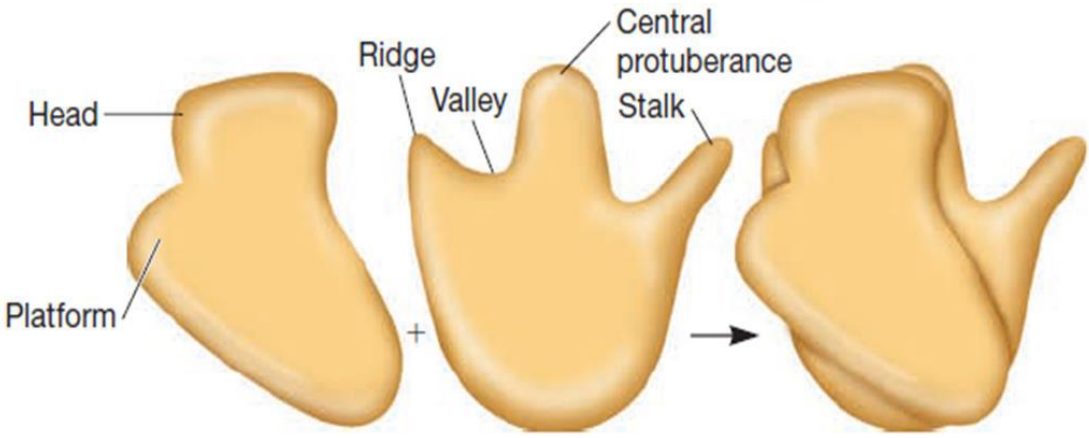
Many antibiotics inhibit protein synthesis by binding with the bacterial ribosome and other components of protein synthesis. Because these drugs distinguish between bacterial and eukaryotic ribosomes, their therapeutic indication is fairly high but not as high as that of cell wall synthesis inhibitors. Several different steps in protein synthesis can be affected by drugs in this group.

Protein Synthesis Inhibition					
Aminoglycosides	Cidal	Bind to small ribosomal subunit (30S) and interfere with protein synthesis by directly inhibiting synthesis and causing misreading of mRNA	Neomycin, kanamycin, gentamicin Streptomycin	Broad (Gram-negative, mycobacteria) Narrow (aerobic Gram-negative)	Ototoxic, renal damage, loss of balance, nausea, allergic reactions
Tetracyclines	Static	Same as aminoglycosides	Oxytetracycline, chlortetracycline	Broad (including rickettsia and chlamydia)	Gastrointestinal upset, teeth discoloration, renal and hepatic injury
Macrolides	Static	Bind to 23S rRNA of large ribosomal subunit (50S) to inhibit peptide chain elongation during protein synthesis	Erythromycin, clindamycin	Broad (aerobic and anaerobic Gram-positive, some Gram-negative)	Gastrointestinal upset, hepatic injury, anemia, allergic reactions
Chloramphenicol	Static	Same as macrolides	Chloramphenicol	Broad (Gram-positive and -negative, rickettsia and chlamydia)	Depressed bone marrow function, allergic reactions

Procaryotic ribosomes are smaller than the ribosomes of eucaryotic cells. Procaryotic ribosomes are called 70S ribosomes (80S in eucaryotes), are constructed of a 50S and a 30S subunit. The S in 70S and similar values stands for Svedberg unit. This is the unit of the sedimentation coefficient, **a measure of the sedimentation velocity in a centrifuge; the faster a particle travels when centrifuged, the greater its Svedberg value or sedimentation coefficient.** The sedimentation coefficient is a function of a particle's

molecular **weight, volume,and shape**. Heavier and more compact particles normally have larger Svedberg numbers and sediment faster.

70s ribosomes consists of two subunits small one (30s) compose of (16s rRNA) with 21 (by weight) proteins and the large one (50s) contains proteins (34 by weight) and two molecules of rRNA (23s rRNA, 5s rRNA) is similar to Archaea that contain (23s rRNA, 5s rRNA , and 5.8 s rRNA) and with protein weight 68 is more similar to eukaryotic ribosomes. many antibiotics inhabit Proteins synthesis such as **Gentamycin, Streptomycin bind to 30s, but Chloramphenicol, Erythromycin are bind to 50s subunit** because there is a difference between the ribosomes of prokaryotic and eukaryotic these antibiotics will **only affect bacterial cells without affecting the host cells.**



(a) 30S subunit 16S rRNA and 21 polypeptides (0.9×10^6 daltons)

(b) 50S subunit 5S rRNA, 23S rRNA and 34 polypeptides (1.8×10^6 daltons)

(c) 70S ribosome (2.8×10^6 daltons)

Transcription and translation

Most of the genes found in bacterial genomes encode proteins. However, DNA does not serve directly as the template for protein synthesis. Rather, the genetic information in the gene is **transcribed** to give rise to **a messenger RNA** (mRNA), which is **translated** into a **protein.** For this to occur, protein-coding genes must contain signals that indicate where transcription should start and stop, and signals in the resulting mRNA that indicate where translation should start and stop. during transcription only one strand of a

gene directs mRNA synthesis. This strand is called the template strand, and the complementary DNA strand is known as the **coding strand** because it is the same nucleotide sequence as the mRNA, except in DNA bases. Messenger RNA is synthesized from the 5' to the 3' end in a manner similar to 30

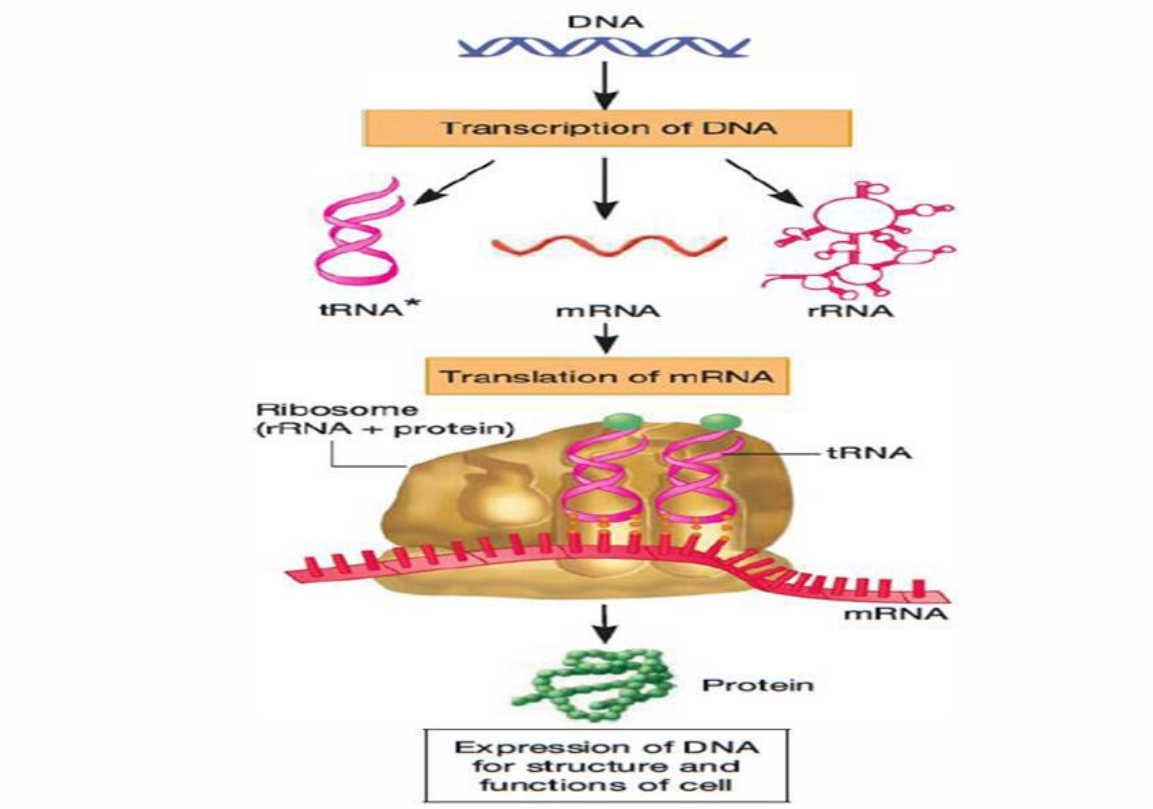
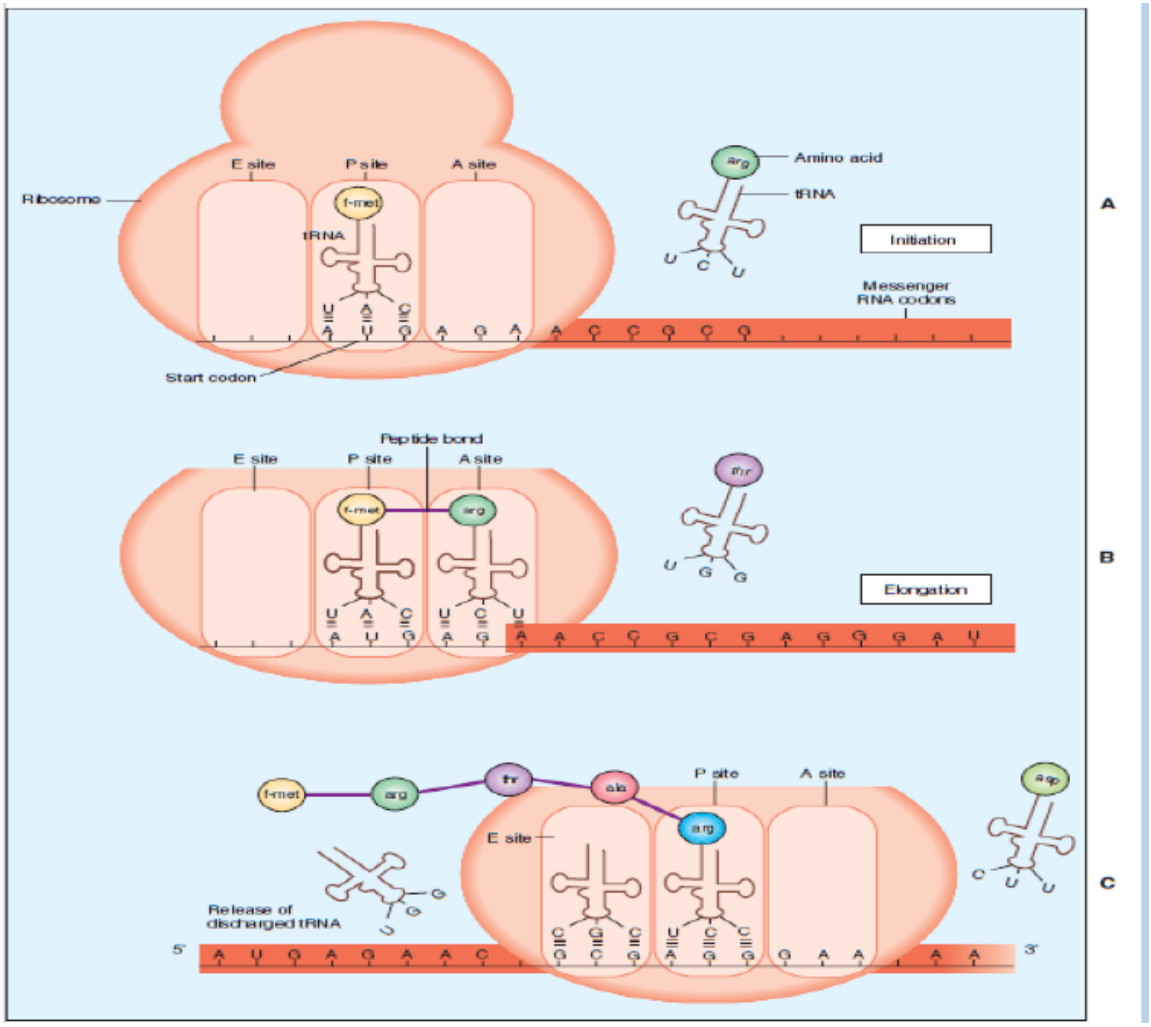


Figure 13.19 Transcription Yields Three Major Types of RNA Molecules. Messenger RNA (mRNA) molecules arise from transcription of protein-coding genes. They are translated into protein with the aid of the other two major types of RNA: transfer RNA (tRNA) molecules carry amino acids to the ribosome during translation; ribosomal RNA (rRNA) molecules have several functions, including catalyzing peptide bond formation.

The ribosome has three sites for binding tRNAs: (1) the peptidyl or donor site (P site), (2) the aminoacyl or acceptor site (A site), and (3) the exit site (E site). At the beginning of an elongation cycle, the P site is filled with either fMet-tRNA or a tRNA bearing a growing polypeptide chain (peptidyl-tRNA), and the A and E sites are empty. Messenger RNA is bound to the ribosome in such a way that the proper codon interacts with the P site tRNA (e.g., an AUG codon for fMet-tRNA). The next codon is located within the A site and is ready to accept an aminoacyl-tRNA.

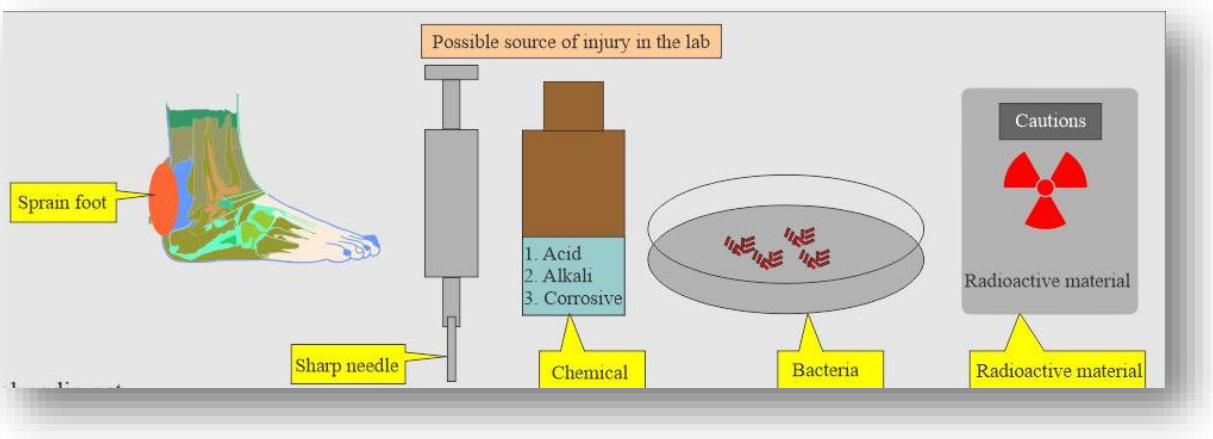

A
E site
P site
A site
Ribosome
f-met
tRNA
Amino acid
tRNA
arg
Initiation
Messenger
RNA codons
Start codon
B
Peptide bond
E site
P site
A site
f-met
arg
thr
Elongation
C
f-met
arg
thr
ala
arg
P site
A site
E site
arg
Release of
discharged tRNA
5'
3'

Clinical laboratory safety

The clinical laboratory has various types of safety hazards. Some of these may be very dangerous and life-threatening.
The people working in the laboratory must know the various types of hazards in the laboratory and know the possible precautions to avoid those accidents.
These dangers can be reduced by eliminating hazards where possible, establishing clean, safe work habits, taking proper precautions, and maintaining awareness of safety practices.

The main type of hazards in the laboratory are:

Possible type of agent	**Source of the hazard**	**Injury or effect**
Physical agents	A wet floor, heavy boxes, and people	Sprains falls, or strains
Sharp instruments	Needle, lancets, and damaged glass	Puncture, cuts, and exposure to pathogens
Fire / explosive	Fire from burners, organic chemicals	Burns
Chemicals	Reagents and preservatives	Carcinogens, Toxic materials, caustic agents
Electrical	Wet equipment, ungrounded equipment, irregular electric cords	Electrical shock
Biological	Infectious agents (bacteria, viruses, parasites, fungal)	Bacterial, viral, parasitic, fungal infections
Radioactive material	Radioisotope and radioactive agents	Radiation injury



Safety in the Clinical Laboratory: The possible source of injury in the lab

Biological factors (Infections):

1. Laboratory staff is always exposed to sources of potentially pathogenic microorganisms.
2. Hospitals are always filled with patients, some of whom have contagious diseases (Nosocomial infections).
3. Most of the samples, like blood, body fluids, urine, CSF, and tissues, have pathogenic organisms.
4. The spread of infections may be by:

    A. Direct contact with the laboratory staff to the patient sample.
    B. By inhalation of the infected material, e.g., by droplet infection, or if you are centrifuging the tube without capping it.
    C. By ingestion of contaminated foods and water.
    D. Then you may spread the infection to your colleagues.

5. Personal hand washing and wearing personal protective clothes and pieces of equipment are important.
6. Special precautions are needed while handling the sample of a patient with HBV, HIV, and HCV viruses.

    a) Wear gloves while collecting blood from the possible cause of HBV, HCV, or HIV.
    b) Wear a face mask if there is any possibility of splashing blood.
    c) Dispose of all the sharp needles and objects in the puncture-resistant containers.
    d) Never recap the used needle; better to dispose of it.

Most important in the case of prick or bleeding:

Don’t stop the blood immediately; the best is to bleed under the tap water as much as possible and then use disinfectants and stop the bleeding

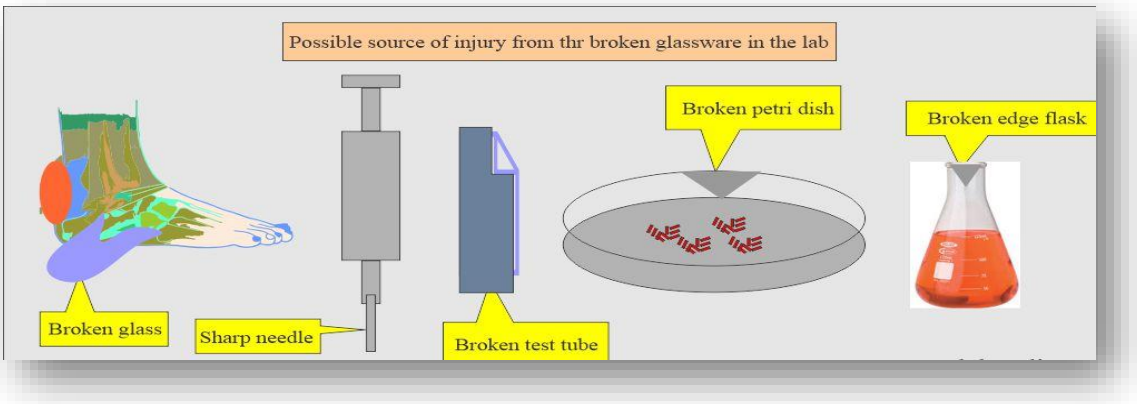
Physical hazards:

These are quite common, and these need to be avoided e.g.

1. Running in the rooms and hallways.
2. When the floor is wet, be careful.
3. Take care while lifting heavy objects.
4. Ladies should keep their long hair tight and on their backside.
5. Avoid loose jewelry.
6. Wear closed-toe shoes that provide maximum support.
7. Try to keep the working area neat and clean.
8. Also, keep the working area well-organized.

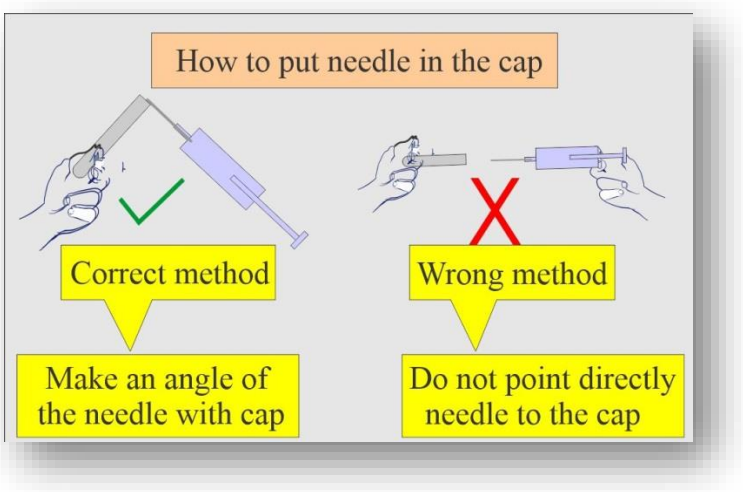
Sharp edge instruments:

1. Sharp edge instruments like a needle, broken glasses, and lancets give rise to biological hazards like:
    1. Give rise to blood-borne pathogens and disease
    2. These may give rise to bleeding.
    3. Dispose of all these sharp edges articles in a safe place, like metal containers.
    4. Cut the needle of the syringe with the cutter.
    5. Also, crush the plastic syringes.
    6. Broken glassware:
        I. Beakers, test tubes, and flasks with broken edges are hazards in the lab.
        II. Remove all these broken edges of glassware.



Safety from the broken glassware

- If you want to close the needle in the syringe, then follow the following diagrammatic method.



Safety in the Clinical Laboratory: How to put the needle in the cap to avoid prick

Fire and explosives:

1. In the laboratory, there are so many explosive and volatile chemicals used in the routine.
2. Flammable chemicals should be kept in the safety cabinet and explosion-proof refrigerators.
3. Should keep the compressed gas cylinders away from the heat.
4. Whenever fire erupts, should take the following measures:
    a) Rescue anyone in immediate danger.
    b) Activate the institutional alarm.
    c) Close all the doors of the fire area.
    d) Try to extinguish the fire.
    e) Fire blankets should be available in the lab.
    f) May use Multiple purpose fire extinguishers.

Chemicals hazards:

1. These should be labeled as carcinogenic, poisonous, or corrosive.
2. Strong acids and alkalis are the most common corrosive chemicals to which lab technicians are exposed.
3. **When mixing the acid and water:**
    The first step measure the water and then add the acid slowly. Because by the addition of the acid, there will be heat production. If you add water to acid, that may burst out.
5. In the case of chemical spills, the best option is to flush the area with water.
6. Remove the contaminated clothes as soon as possible.

Toxic fumes:

1. There are solvents whose fumes (vapors) are toxic.
2. Extracts with chlorinated hydrocarbons, chemicals that cause damage to the liver.
3. Few solvents cause bone marrow depression with leucopenia, thrombocytopenia, and anemia.
4. There are a few chemicals that are carcinogenic.
5. **Precautions:**
    1. In the case of organic solvents, use a hood and work in a well-ventilated area.
    2. Avoid contamination of the skin because these can get absorbed slowly. Wash immediately with soap and water.

Some of Carcinogenic chemical agents:

1. Ethyleneimine
2. 2-Acetylaminofluorene
3. Benzidine
4. Methyl chloromethyl ether
5. Nitrobiphenyl.

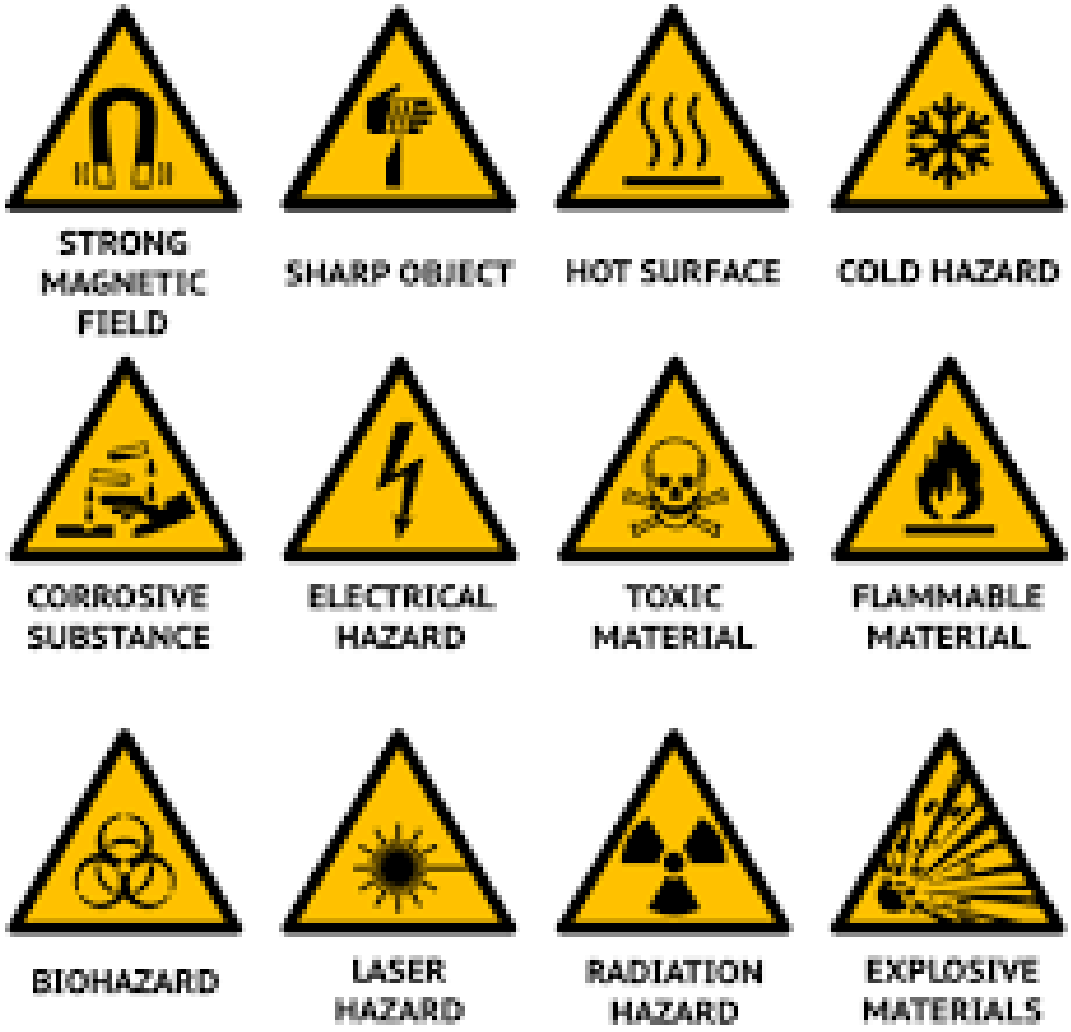
Electrical hazards:

1. There are so many pieces of equipment working on electricity.
2. Avoid wet hands to operating the equipment.
3. Look after the damaged wires and avoid overloading the circuits.
4. If any equipment becomes wet, then immediately unplug the wires and let them dry before using it.
5. Try to ground all the equipment.
6. If accidents occur and someone has an electric shock, immediately shut off the electricity without touching the person or equipment.

Radioactive materials:

Radioactive material sign

1. This is needed in cases of using radioactive material like radioisotopes.
2. Mostly the radioactivity in the laboratory is very minimal and very little dangerous.
3. The best option is to wear a measuring device.
4. Should avoid exposure to pregnant ladies because that will cause harm to the fetus.



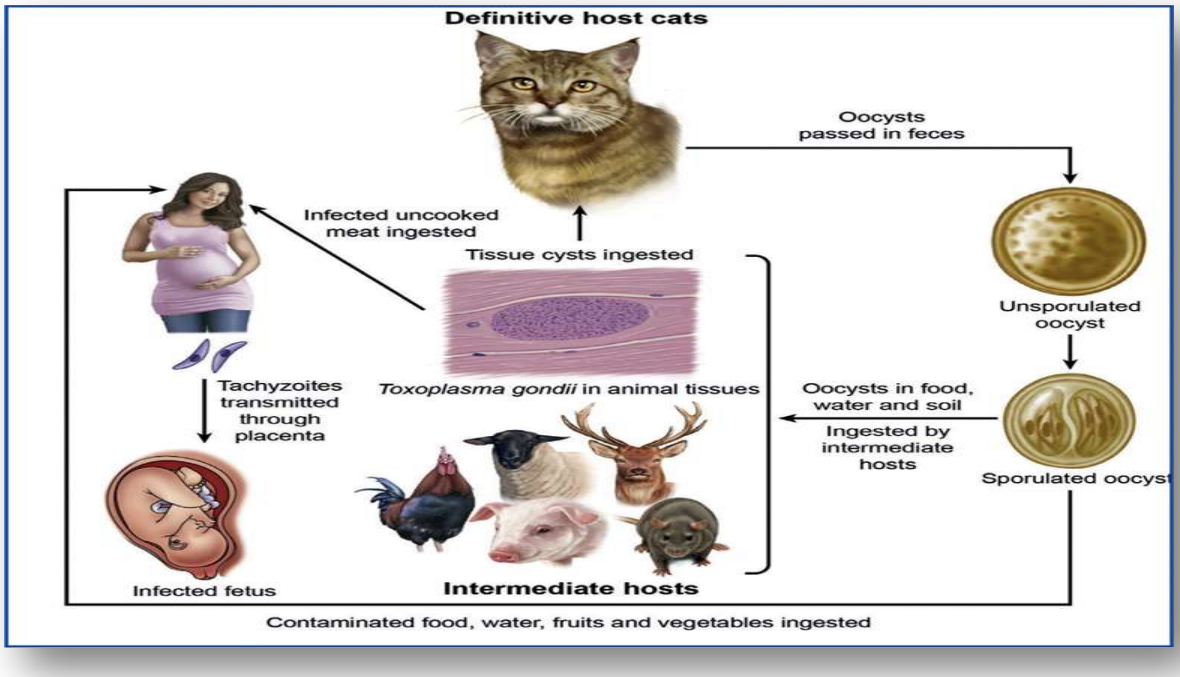
Hazards symbols

ANALYSIS OF TOXOPLASMOSIS

Causal Agents

Toxoplasma gondii is a protozoan parasite that infects most species of warm-blooded animals, including human, and causes toxoplasmosis.
The only known definitive hosts for *Toxoplasma gondii* are members of family Felidae (domestic cats and their relatives). Unsporulated oocysts are shed in the cat's feces.

LIFE CYCLE



Life cycle of Toxoplasma gondii.

Humans can become infected by any of several routes:

- Eating undercooked meat of animals harboring tissue cysts.
- Consuming food or water contaminated with cat feces or by contaminated environmental samples (such as fecal-contaminated soil or changing the litter box of a pet cat).
- Blood transfusion or organ transplantation.
- Transplacentally from mother to fetus.

Laboratory Diagnosis

The diagnosis of toxoplasmosis may be documented by:

- Serologic testing for the routine diagnosis.
- Observation of parasites in patient specimens.
- Isolation of parasites from blood or other body fluids, by intraperitoneal inoculation into mice or tissue culture.
- Detection of parasite genetic material by PCR.

The diagnosis of toxoplasmosis is typically made by serologic testing. A test that measures immunoglobulin G (IgG) is used to determine if a person has been infected. If it is necessary to try to estimate the time of infection, which is of particular importance for pregnant women, a test which measures immunoglobulin M (IgM).

Toxo—Latex Test

Principle

Toxo-latex test is a rapid slide agglutination procedure developed for the direct detection of antibodies anti-toxoplasma in human serum .

The assay performed by testing a suspension of latex particles coated with antigenic extract of toxoplasma gondii against unknown sample . the presence or absence f visible agglutination ,indicates the presence or absence of anti-toxoplsma gondii.

Sample

Fresh clear serum , samples should be stored at 2-8 ^{0}C up to one week or for longer period at -20 ^{0}C .

Procedure

I. Qualitative Test

1.The test reagent and samples to room temperature

2. re-suspend the antigen vial gently .

3.place one drop (50 µl) of the sample into one of the circles on the card

4.place one drop of control positive and one drop of control negative into two additional circles .

5.add one drop (50 µl) of toxo-latex reagent to each circle ,and mix them by disposable Pipette while spreading over the entire area .use separate pipettes for each sample .

6.rotat the slide slowly for a period 5

7. observe immediately under suitable light source for any degree of agglutination .

II. semiquantitative test

1.for each sample ,place 50 µl of 0.9 saline solution into each circles of a card

2.to circle one add 50 µl of sample to the saline solution and using the same tip, mix them .

3. transfer 50 µl of the mixture to the saline solution in the second circle .

4. continue with the 2-fold serial dilution in a similar manner up to the sixth circle ,and discard 50 µl from this circle .

Final sample dilutions will be 1:2 ,1:4 ,1:16 ,1: 32 ,1:64.

5. observe immediately under suitable light source for any degree of agglutination .

READING THE RESULTS :

Toxoplasmosis Laboratory Diagnosis		
IgG Result	**IgM Result**	**Report/interpretation for humans***
Negative	Negative	No serological evidence of infection with *Toxoplasma*.
Negative	Equivocal	Possible early acute infection or false-positive IgM reaction.
Negative	Positive	Possible acute infection or false-positive IgM result.
Equivocal	Positive	Possible acute infection with *Toxoplasma*.
Positive	Negative	Infected with *Toxoplasma* for six months or more.
Positive	Equivocal	Infected with *Toxoplasma* for probably more than 1 year.
Positive	Positive	Possible recent infection within the last 12 months.

NOTES
1.The sensitivity of the test may be reduced at low temperatures. The best results are achieved at 15-25ºC.
2.Delays in reading the results may generate in over-estimation of the antibody present.