Introduction to The Laboratory techniques

Laboratory techniques, (also known as clinical laboratory scientists) can be define as the branch of laboratory medicine which deals with the examination of tissues and excretion of the human body and body fluids by various electronic, chemical, microscopic and other medical laboratory procedures or techniques either manual or automated which will aid the physician in the diagnosis, study and treatment of disease and in the promotion of health in general.

Departments of Medical laboratory technology

Laboratory technology can be entering in all areas of the clinical laboratory application which include:

1. Clinical Chemistry

It is department performs hundreds of quantitative analysis on a variety of body fluids. Common chemistry procedures include testing for glucose, lipid profile, hormones, liver function test, renal function test, vitamin, enzymes, protein and electrolytes.

2. Blood Bank (Immunohematology)

The blood bank provides blood typing and compatibility testing for both donors and recipients and ensures the safety of the blood supply.

3. Immunology /Serology

Immunologic or serologic testing evaluates antibodies and /or antigens that may be indicative of many types of infectious disease, such as widal test, CRP, RF.

4. Microbiology

The microbiology section identifies microorganisms that may be causing disease (pathogens). The microbiology department also provides information regarding appropriate antibiotics to use as treatment for various pathogens, such as bacteria and fungi, mainly by culturing and biochemical test.

5. Urinalysis

It is the department screen urine samples for evidence of kidney disease or bladder infections (UTI).

6. Hematology

This department performs tests that are important in diagnosing many disorders such as anemia, polycythemia, leukocytosis, leukopenia, thrombocytosis, thrombocytopenia and leukemia.

7. General stool examination

An important test to see diseases related to the digestive system.

8. Seminal fluid analysis

Measure amount of semen a man produces and determine the number and quality of sperm in the semen sample.

9. Genetics

Perform testing on DNA, RNA or chromosomes to help identify the causes, diagnosis, treatment of genetic diseases or to identify mapping disease, by using many technique such as PCR, recombinant DNA technology, gene therapy, microarray analysis.

10. Electrophoresis

Used to separate molecules based on size (DNA, RNA and protein).

11. Chromatography

Used for separating a mixture of chemical substances into its individual components.

12. Histopathology

Used to detection tumor and cancer in cell tissue after surgery (biopsy).

Importance of medical Laboratory:

Clinical laboratories play an integral role as part of the health care team in delivering those services up to 85% of decisions about diagnosis and treatment are based on laboratory test results.

Sample collection requirements:

Sample collection and preservation will vary, depending on the test and the type of sample to be collected. The laboratory must carefully define a sample collection process for all tests it performs. The following should be considered when preparing instructions:

- Patient preparation: Some tests require that the patient be fasting. There may also be special timing issues for tests such as blood glucose, lipid profile. Do not take antibiotic during culturing.

- Type of sample required: Blood tests might require serum, plasma, or whole blood. Other tests might require urine, saliva, sputum, stool, CSF, plural fluid, peritoneal fluid. Microbiology testing deals with a variety of sample types, so specific information as to what is required for the test is needed.

- Type of container: The container for the sample is often very important, as it will affect volume and any needed additives such as anti-coagulants and preservatives. Some microbiology samples will require specific transport media to preserve microorganisms.

- Sample labeling: All requirements for labeling of the sample at the time of collection will need to be explained in detail in the instructions for collection.

- Special handling: Some samples may require special handling, such as immediate.

General Methods to the Diagnose

Microbiology is the study of microorganisms, including those that are causing infectious diseases. It comprises several disciplines specializing in different types of microorganisms: viruses (virology), bacteria (bacteriology), fungi (mycology) and parasites (parasitology).
Pathogens are organisms that can cause disease. The different types of pathogens and the severity of the diseases that they cause are very diverse.

Spread of pathogens by variety of different ways:
1. Direct skin-to-skin contact or during sex can lead to sexually transmitted infections (STIs)
2. Coughing or sneezing can cause pathogens to spread through tiny droplets in the air.
3. Through the gut when a person consumes contaminated food or water.
4. Bites from infected insects can also spread disease.

Pathogenicity it is the ability to cause disease, **Virulence** it is the degree of pathogenicity-pathogens.
To cause disease a pathogen must:
1. Gain access to the host.
2. Adhere to host tissues.
3. Penetrate or evade host defenses.
4. Damage the host, either or indirectly by directly- accumulation of microbial waste.

Pathogenesis is the process by which a disease or disorder develops. It can include factors which contribute not only to the onset of the disease or disorder, but also to its progression and maintenance.
Normal flora are the microorganisms that live on another living organism (human or animal) or inanimate object without causing disease. The human body is not sterile; we become colonized by bacteria from the moment we are born. We are covered with, and contain within our intestines, approximately one hundred trillion bacteria that form the normal flora of our bodies.
The Normal Microbiota of the human body provides benefits, which are discussed below;
1. Prevent Pathogens
Most importantly Normal Microbiota prevents the colonization of Pathogens by competing with them for essential nutrients and attachment sites.
2. Vitamin Synthesis
Some normal microflora are synthesis vitamins for their own need. Sometimes our body absorbs those vitamins as a nutrient.
Example: Some enteric bacteria of the human body produce Vitamin K and Vitamin B12. Lactic acid bacteria also produce certain B-vitamins.
3. Killing bacteria

Some normal flora antagonizes other bacteria by producing some substances which inhibit or kill nonindigenous species.

Example: few intestinal bacteria produce some substances which have highly specific bacteriocins that help to kill other bacteria.

4. Development of certain tissues

Normal flora also helps in the development of certain tissues.

Ex: Caecum and certain lymphatic tissues (Peyer's patches) in the gastrointestinal tract.

5. Production of Antibody

The normal flora of the human body also helps to stimulate the synthesis of normal antibodies.

The opportunistic pathogen is an infectious pathogen that is normally commensal in the body but can cause disease when the host's resistance is altered. They can be some viruses (referred to as "opportunistic viruses"), fungi (referred to as "opportunistic fungi"), parasites ("opportunistic parasites"), and bacteria ("opportunistic bacteria").

General Methods to the Diagnose of a Bacterial Infection

1. Obtain a specimen from the infected site.
2. Stain the specimen using the appropriate procedure, e.g., Gram stain or acid fast stain.
3. Culture the specimen on the appropriate media, e.g., blood agar plates. The plates should be streaked in such a manner as to obtain isolated colonies, i.e., a "pure culture." The plates should be incubated in the presence or absence of oxygen as appropriate.

* Note special features such as hemolysis and pigment formation.

4. Identify the organism using the appropriate tests, e.g. biochemical test (catalase, oxidase).
5. Genotypic method, PCR, DNA probes.
6. Antibody-based tests such as agglutination, or immunofluorescence.
7. Perform antibiotics susceptibility tests.

Types of Specimens

1. Blood Cultures

Blood cultures are performed most often when sepsis, endocarditis, osteomyelitis, meningitis, or pneumonia is suspected.

It is important to obtain at least (3-10) mL of blood samples in a 24- hour period because the number of organisms can be small and their presence intermittent. The site for venipuncture must be cleansed with 2% iodine to prevent contamination by members of the flora of the skin, usually *Staphylococcus epidermidis*. The blood obtained is added to 100 mL of a rich growth medium such as brain–heart infusion broth. If two bottles are

used, one is kept under anaerobic condition and the other is not. If one bottle is used, the low oxygen tension at the bottom of the bottle permits anaerobes to grow.
Blood cultures are checked for turbidity or for CO2 production daily for 7 days or longer. If growth occurs, Gram stain, subculture, and antibiotic sensitivity tests are performed.

2. Throat Cultures

Throat cultures are used primarily to detect the presence of group A β-hemolytic streptococci (*Streptococcus pyogenes*), an important and treatable cause of pharyngitis. They are also used when *diphtheria*, *gonococcal pharyngitis*, or thrush (*Candida*) is suspected.
Take a swab of the throat; it may be pharynx, tonsils and gums.
*Note that a Gram stain is typically not done on a throat swab because it is impossible to distinguish between the appearance of the normal flora *streptococci* and *S.pyogenes*.

3. Sputum Cultures

Sputum cultures are performed primarily when *pneumonia* or *tuberculosis* is suspected. If tuberculosis is suspected, an acid-fast stain should be done immediately.

4. Spinal Fluid Cultures

Spinal fluid cultures are performed primarily when *meningitis* or *encephalitis* is suspected. There are three encapsulated organisms: *Neisseria meningitidis*, *S.pneumoniae*, and *Haemophilus influenza*, cultures are done on blood and on chocolate agar and incubated at 35°C in a 5% CO2 atmosphere. Hematin and nicotinamide adenine dinucleotide (NAD) (factors X and V, respectively) are added to enhance the growth of *H. influenzae*.

5. Stool Cultures

Stool cultures are performed primarily for cases of enterocolitis. The most common bacterial pathogens causing diarrhea are *Shigella*, *Salmonella*, and *E. coli* strains are also an important cause of diarrhea.
For culture of *Salmonella* and *Shigella*, a selective, differential medium such as MacConkey or Eosin methylene blue (EMB) agar is used. These media are selective because they allow gram- negative rods to grow but inhibit many gram- positive organisms.
*The sample should be taken directly and culturing directly on media to prevent contamination from outside.

6. Urine Cultures

Urine cultures are performed primarily when pyelonephritis most frequent cause of urinary tract infections is E.coli. Other common agents are Enterobacter, Proteus, and Enterococcus faecalis.

Urine in the bladder of a healthy person is sterile, but it acquires organisms of the normal flora as it passes through the distal portion of the urethra. To avoid these organisms, amid stream specimen, voided after washing the external orifice is used for urine cultures.
In special situations catheterization may be required to obtain a specimen.
Because urine is a good culture medium, it is essential that the cultures be done within (1) hour after collection or stored in a refrigerator at 4°C.
It is commonly accepted that a bacterial count of at least 100,000/mL must be found to conclude that significant bacteria is present (in as symptomatic persons).

7. Genital Tract Cultures

Genital tract cultures are performed primarily on specimens from individuals with an abnormal discharge or on specimens from symptomatic contacts of a person with a sexually transmitted disease. One of the most important pathogens in the genital tract is *Neisseria gonorrhoeae*, human papilloma virus, herpes simplex virus type 2.
*Specimens are obtained by swabbing from cervix (for women), Seminal fluid from men.
Non-gonococcal urethritis and cervicitis are also extremely common infections. The most frequent cause is *Chlamydia trachomatis*, which cannot grow on artificial medium but must be grown in living cells. Because *Treponema pallidum*, the agent of syphilis, cannot be cultured, diagnosis is made by microscopy and serology.

Staining

First: Gram Staining; Principle, Procedure and Results

Gram staining method, the most important procedure in Microbiology, was developed by Danish physician Hans Christian Gram in 1884. Gram staining is still the cornerstone of bacterial identification and taxonomic division.
This differential staining procedure separates most bacteria into two groups on the basis of cell wall composition:
1. Gram positive bacteria (thick layer of peptidoglycan 90% of cell wall) stains purple.
2. Gram negative bacteria (thin layer of peptidoglycan 10% of cell wall and high lipid content) stains red/pink.

Classic Gram staining techniques involves following steps:

1. Fixation of clinical materials to the surface of the microscope slide either by heating or by using methanol.
2. Application of the primary stain (crystal violet). Crystal violet stains all cells blue/purple
3. Application of mordant: The iodine solution (mordant) is added to form a crystal violet iodine (CV-I) complex; all cells continue to appear blue.
4. Decolorization step: it is distinguishes gram-positive from gram-negative cells. The organic solvent such as acetone or ethanol, extracts the blue dye complex from the

lipid-rich, thin walled gram negative bacteria to a greater degree than from the lipid poor, thick walled, gram-positive bacteria. The gram negative bacteria appear colorless and gram positive bacteria remain blue.

5. Application of counter stain (safranin): The red dye safranin stains the decolorized gram-negative cells red/pink; the gram-positive bacteria remain blue.

Second: Acid-Fast Stain; Principle, Procedure.

It is the differential staining techniques which was first developed by Ziehl and later on modified by Neelsen. So this method is also called Ziehl-Neelsen staining techniques.

This method is used for those microorganisms which are not staining by simple or Gram staining method, particularly the member of genus *Mycobacterium*, are resistant and can only be visualized by acid-fast staining.

Principle of Acid-Fast Stain

When the smear is stained with carbol fuchsin, it solubilizes the mycolic acid (long chain fatty acid) material present in the Mycobacterial cell wall (waxy) but by the application of heat, carbol fuchsin further penetrates through cell wall and enters into cytoplasm. Then after all cell appears red. Then the smear is decolorized with decolorizing agent (3% HCL in 95% alcohol) but the acid fast cells are resistant due to the presence of large amount of mycolic acid material in their cell wall which prevents the penetration of decolorizing solution. The non-acid fast organism lack the mycolic acid material in their cell wall due to which they are easily decolorized, leaving the cells colorless. Then the smear is stained with counterstain, methylene blue or malachite green. Only the decolorized cells are absorb the counter stain and take its color and appear blue while acid-fast cells retain the red color.

Procedure of Acid-Fast Stain

1. Prepare bacterial smear on clean and grease free slide, using sterile technique.
2. Allow smear to air dry and then heat fix.
3. Cover the smear with carbol fuchsin stain.
4. Heat the stain until vapour just begins to rise (i.e. about 60 C) for 5 minutes.
5. Wash off the stain with clean water.
6. Cover the smear with 3% v/v acid alcohol for 5 minutes or until the smear is sufficiently decolorized, i.e. pale pink.
7. Wash well with clean water.
8. Cover the smear with malachite green stain or methylene blue for 1–2 minutes.
9. Wash off the stain with clean water.
10. Wipe the back of the slide clean, and place it in a draining rack for the smear to air-dry.
11. Examine the smear microscopically using the 100X oil immersion objective.

Third: Capsule stain; Principle, Procedure.

Capsule stain is a type of differential stain which uses acidic and basic dyes to stain background & bacterial cells respectively so that presence of capsule is easily visualized. Capsule is synthesized in the cytoplasm and secreted to the outside of the cell where it surrounds the bacterium. Most of the capsulated bacteria have a capsule made up of polysaccharide layer but some bacteria have capsule made up of polypeptide, or glycoprotein.

Capsules are associated with virulence in several microorganisms, including *Streptococcus pneumoniae* and *Neisseria meningitides*, because capsules resist phagocytosis thus evading the host immune system.

Principle of Capsule Stain

Bacterial capsules are non-ionic, so neither acidic nor basic stains will adhere to their surfaces. Therefore, the best way to visualize them is to stain the background using an acidic stain (e.g., india ink, Nigrosine, congo red) and to stain the cell itself using a basic stain (e.g., crystal violet, safranin, basic fuchsin and methylene blue).

India ink method

In this method two dyes, crystal violet and india ink are used. The capsule is seen as a clear halo around the microorganism against the black background. This method is used for demonstrating *Cryptococcus*.

• The background will be dark (color of india ink).

• The bacterial cells will be stained purple (bacterial cells takes crystal violet-basic dyes as they are negatively charged).

• The capsule (if present) will appear clear against the dark background (capsule does not take any stain).

General Methods to the Diagnose

Types of media

Culture media contains nutrients and physical growth parameters necessary for microbial growth. All microorganisms cannot grow in a single culture medium, and in fact many can't grow in any known culture medium.

Obligate parasites are organisms that cannot grow in an artificial culture medium such as *Mycobacterium leprae, rickettsias, Chlamydias,* and *Treponema pallidum*. Bacterial culture media can be distinguished on the basis of composition, consistency and purpose.

Cultivation: is the process of growing microorganisms in culture by taking bacteria from the infection site and growing them in the artificial environment of the laboratory.

Classification of culture media used in Microbiology laboratory on the basis of consistency:

1. Solid medium

Medium contains agar at a concentration of 1.5-2.0% or some other agent. Solid medium has physical structure and allows bacteria to grow in physically informative such as colonies or in streaks. Solid medium is useful for isolating bacteria or for determining the colony characteristics of the isolate.

2. Semi solid media

They are prepared with agar at concentrations of 0.5% or less. Their consistency is similar to custard, and is useful for the cultivation of aerophilic bacteria or for determination of bacterial motility.

3. Liquid (Broth) media

Medium contains specific amounts of nutrients but don't have trace of gelling agents such as gelatin or agar. It is useful for various purposes such as propagation of large number of organisms, fermentation studies, and various other tests. e.g. sugar fermentation tests, MR-VR broth.

Classification of Bacterial Culture Media based on the basis of purpose/ functional use/ application:

Many special purpose media are needed to facilitate recognition, enumeration, and isolation of certain types of bacteria. To meet these needs, numerous media are available:

1. General purpose media/ Basic media:

Basal media are basically simple media that supports most non-fastidious bacteria. Peptone water, nutrient broth and nutrient agar are considered as basal medium. These media are generally used for the primary isolation of microorganisms.

2. Enriched medium (Added growth factors):

Addition of extra nutrients in the form of blood, serum, egg yolk etc., to basal medium makes them enriched media. Enriched media are used to grow nutritionally exacting (fastidious) bacteria. Blood agar, chocolate agar, Loeffler's serum slope etc., are few of the enriched media.

3. Selective media:

Selective media are used to select for the growth of a particular "selected" microorganism from a mixture of bacteria. Any agar media can be made selective by addition of certain inhibitory agents that don't affect the pathogen of interest. Antibiotics, dyes, chemicals, alteration of pH or a combination of these are inhibitory agents.

Examples of selective media include:

1- Mannitol Salt Agar and Salt Milk Agar used to recover *S. aureus* contain 10% NaCl.

2- MacConkey's Agar used for Enterobacteriaceae members contains bile salt that inhibits most gram positive bacteria.

3- Lowenstein Jensen Medium used to recover *M.tuberculosis* is made selective by incorporating malachite green.

4- TCBS Agar used for isolating *V. cholerae* from fecal specimens have elevated pH (8.5-8.6), which inhibits most other bacteria.

5- Xylose–lysine–deoxycholate agar (XLD) agar is selective for *Shigella* spp. and *Salmonella* spp. contain salt and sodium deoxycholate, inhibits many gram negative bacilli and gram positive bacteria.

4-Enrichment culture medium:

It is used to increase the concentration of certain microorganisms before culture it on solid selective medium. Enrichment media are liquid media that also serves to inhibit other pathogens in the clinical specimen. Such as Brain-heart infusion, Gram negative broth, Thioglycollate broth.

5. Differential/ indicator medium (differential appearance):

Differential media contain compounds such as of dyes, metabolic substrates etc, that allow groups of microorganisms to be visually distinguished by the appearance of the colony or the surrounding media, usually on the basis of some biochemical difference between the two groups.

Examples of differential media include:

1. Mannitol salts agar (mannitol fermentation = yellow)
2. Blood agar (various kinds of hemolysis i.e. α, β and γ hemolysis)

3. MacConkey agar (lactose fermenters, pink colonies whereas non-lactose fermenter produces pale or colorless colonies.
4. TCBS (Vibrio cholera produces yellow colonies due to fermentation of sucrose)
5. Xylose–lysine–deoxycholate agar (XLD) agar is differential between *shigella* spp. and *salmonella* spp.(xylose fermenter and decarboxylation of lysine produce H2S).

6. Transport media:

Clinical specimens must be transported to the laboratory immediately after collection by using transport media. It is help by prevent drying of specimen, maintain the pathogen to commensal ratio and inhibit overgrowth of unwanted bacteria. Examples of Transport media:

1- Boric acid for urine
2- Polyvinyl alcohol and buffered formalin for stool
3- Cary Blair transport medium used to transport feces from suspected cholera patients.
4- Pike's medium is used to transport streptococci from throat specimens.
5- Sodium polyanethol sulfonate (SPS): Used to blood, bone marrow and synovial fluid.

7. Anaerobic media:

Anaerobic bacteria need special media for growth because they need low oxygen content, reduced oxidation–reduction potential and extra nutrients. Media for anaerobes may have to be supplemented with nutrients like hemin and vitamin K. Before use the medium must be boiled in water bath to expel any dissolved oxygen and then sealed with sterile liquid paraffin.

Robertson Cooked Meat (RCM) medium that is commonly used to grow *Clostridium* spp. contains a 2.5 cm column of bullock heart meat and 15 ml of nutrient broth.

General Methods to the Diagnose

Tissue culture:

It is the process by which cells are grown under controlled conditions, generally outside of their natural environment. Cell culture conditions can vary for each cell type, but artificial environments consist of a suitable vessel with substrate or medium that supplies the essential nutrients (amino acids, carbohydrates, vitamins, minerals), growth factors, hormones, and gases (CO_2, O_2), and regulates the physio-chemical environment (pH buffer, osmotic pressure, temperature). Antibiotics (e.g. penicillin and streptomycin) and antifungals (e.g.amphotericin B) can also be added to the growth media. Most cells require a surface or an artificial substrate (adherent or monolayer culture) whereas others can be grown free floating in culture medium (suspension culture). The lifespan of most cells is genetically determined, but some cell culturing cells have been "transformed" into immortal cells which will reproduce indefinitely if the optimal conditions are provided.

Immortalized cell lines: are cells that have been manipulated to proliferate indefinitely and can thus be cultured for long periods of time. Immortalized cell lines are derived from a variety of sources such as tumors. Because immortalized cells continuously divide, they eventually fill up the dish or flask in which they are growing. By passaging (also known as splitting), scientists transfer a fraction of the multiplying cells into new dishes to provide space for continuing proliferation.

Components of cell culture

	Component	**Function**
1	Carbon source (glucose/glutamine)	Source of energy

2	Amino acid	Building blocks of protein
3	Vitamins	Promote cell survival and growth
4	Balanced salt solution	An isotonic mixture of ions to maintain optimum osmotic pressure within the cells and provide essential metal ions to act as cofactors for enzymatic reactions, cell adhesion etc.
5	Phenol red dye	PH indicator. The color of phenol red changes from orange/red at pH 7-7.4 to yellow at acidic (lower) pH and purple at basic (higher) pH.
6	Bicarbonate /HEPES buffer	It is used to maintain a balanced pH in the media

Typical Growth Conditions

Parameter	
Temperature	37 °C
CO2	5%
Humidity	95%

Applications of cell culture:

1- Virology and Vaccine Production; Cell culture with mammalian cells offers a host for viruses to replicate, development, and conditions required for their infectious cycle. Also the attenuated viruses used in vaccines against polio, measles, chicken are raised in animal cell cultures.

2- Biological Products; produced by recombinant DNA (rDNA) technology in animal cell cultures include enzymes, synthetic hormones, immunobiologicals (monoclonal antibodies, interleukins, interferon), blood clotting factors and anticancer agents.

3- Pharmaceutical proteins that do not require post; translational modification can be produced by bacteria or yeast (insulin, albumin, growth hormone).

4- Cellular agriculture which aims to provide both new products and new ways of producing existing agricultural products.

5- Tissue Regeneration and Transplantation

Embryonic stem cells and adult stem cells have the capacity to regenerate and differentiate into specialized cell types that can be used as replacement tissues or organs.

Biochemical test

Catalase test

The enzyme catalase mediates the breakdown of hydrogen peroxide (H2O2) into oxygen and water and bubble production. It is used to determine whether a gram-positive cocci. The presence of the enzyme in a bacterial isolate is evident when a small inoculum is introduced into hydrogen peroxide.

Coagulase test

Coagulase is an enzyme that clots blood plasma. This test is identifying the coagulase positive *Staphylococcus aureus*. The clotting mechanism involves modified or derived thrombin molecule, to form a coagulase-CRF complex. This complex in turn reacts with fibrinogen to produce the fibrin clot.

Oxidase test

It is to determine the presence of bacterial cytochrome oxidase using the oxidation of the substrate tetramethyl-p phenylenediamine dihydrochloride to indophenol. A positive test (presence of oxidase) is indicated by the development of a dark purple colored end

product such as *Pseudomonas* and *Neisseria*. No color development indicates a negative test and the absence of the enzyme.

Urease test

This test is used to determine the ability of an organism to produce the enzyme urease, which hydrolyzes urea and produces ammonia and CO2. The formation of ammonia alkalinizes the medium, and the pH shift is detected by the color change of phenol red from light orange at pH 6.8 to magenta at pH 8.1. *H. pylori* is positive for urease.

Bacitracin test

This test is used to determine the effect of a small amount of bacitracin (0.04 IU) on an organism. *Streptococcus pyogenes* is inhibited by the small amount of bacitracin in the disk; other beta-hemolytic streptococci usually are not.

IMViC tests

These tests are useful in distinguishing members of Enterobacteriaceae.

Indole test:

The indole test screens for the ability of an organism to degrade the amino acid tryptophan and produce indole under the action of enzyme tryptophanase. A positive result is indicated by a pink/red layer forming on top of the liquid such as *E. coli*.

Methyl red MR test:

It is used to detect the ability of an organism to produce and maintain acid end products from glucose fermentation. The pH indicator Methyl Red is added to the tube. Red color appears at PH lower than 4.2/ test is positive (mixed acid fermentation is used) such as *E. coli*. The solution remaining yellow (pH=6.2 or above)/ test is negative, meaning the butanediol fermentation is used.

Voges-Proskauer (VP) test:

It is used alpha-naphthol and potassium hydroxide to test for the presence of acetoin, an intermediate of the 2,3-butanediol fermentation pathway. A pinkish-red color indicates a positive test such as *Vibrio*.

Citrate test:

This test uses Simmon's citrate agar to determine the ability of a microorganism to use citrate as its sole carbon source. The agar contains citrate and ammonium ions (nitrogen source) and bromothymol blue as an indicator. The citrate agar is green before inoculation, and turns blue as a positive test indicator, meaning citrate is utilized such as *Klebsiella*.

Hematology

Hematology is it a study of the blood, it's formed Elements, hematopoietic System, and there disorders.

Blood is pumped by the heart through blood vessels, which extent throughout the body. Blood helps to maintain homeostasis in several ways:

1. Transport of gases, nutrient and waste Products.
2. Transport of processed molecules (vitamin D).
3. Transport of regulatory molecules (hormones, enzymes).
4. Regulation of PH and osmosis (7.35-7.45).
5. Maintenance of body temperature.
6. Production against foreign substances such as microorganism and toxin.
7. Clot formation.

Blood is composed of pale yellow fluid called plasma in which are suspended red cells (erythrocytes), White cells (leukocytes) and platelets (thrombocytes). Plasma forms about 55%Of blood volume and contains water 95% and many solutes Including protein , of digestion , and waste Products of excretion. Blood represent about 8% of total weight and has an average volume of 5 liters in women and 6 liters in men.

Blood cells formation (Haemopoiesis or Haematopoiesis) from embryo to adult:

1. Yolk sac (from six day to six week tor gestation)
2. Liver and spleen(from six week to birth)
3. Bone marrow at birth and remains so throughout the life.

The common ancestral of all mature blood cells in humans is the totipotent stem cells. This cells can differentiate to form lymphoid or myeloid pluripotent stem cells ,these stem cells retain the dual capacity for self-renewal and differentiation.

The difference between blood plasma and blood serum blood plasma and blood serum are both devoid of blood cells and platelets the difference in the clotting factors. Blood plasma still contains the proteins (fibrinogen and other clotting factors) while blood serum has no clotting factors because the clotting factors will be converted to a clot (fibrinogen converted to fibrin).

1- Hemoglobin

The hemoglobin consist of 4 globin molecules, and 4 heme molecules .it is percent within red blood cell, almost one-third of the weight of a red cell. Their primary function is the carriage of oxygen from the lungs to the tissues. A vital secondary role is the facilitation of reverse transportation of carbon dioxide. They also play a role in blood buffering.

The healthy range for hemoglobin is:

For men, 13.5 to 17 grams per deciliter

For women, 11.5 to 15.5 grams per deciliter

Clinical significance

A- Anemia: it is defined as reduction in the concentration of hemoglobin in R.B.C, anemia is functionally defined as an insufficient oxygen to peripheral tissues to meet physiological needs.

Lower than normal results (Anemia)

There are many forms of anemia, each with different causes, which can include:

1. Iron deficiency
2. Vitamin B12 deficiency or folate deficiency
3. Bleeding
4. Cancers that affect the bone marrow, such as leukemia
5. Kidney disease
6. Liver disease
7. Hypothyroidism
8. Thalassemia

B- Polycythemia (raised hemoglobin value) is disease state in which the proportion of blood volume that is occupied by red blood cells increases.

Higher than normal results (Polycythemia)

If your hemoglobin level is higher than normal, it may be the result of:

1. Lung disease
2. Dehydration
3. Living at a high altitude
4. Heavy smoking
5. Burns
6. Excessive vomiting
7. Extreme physical exercise

2- Pocked Cells Volume (PCV), Hematocrit (HCT)

Hematocrit (GCT) is defined as the volume occupied by erythrocyte in a given volume of blood. The hematocrit may also be referred to as packed cell volume (PCV). This is benefit test to diagnosis of certain blood disorders. The HCT test is part of the CBC. This test indirectly measures the RBC mass. The results are expressed as the percentage by volume

of packed RBCs in whole blood (PCV). It is an important measurement in determination of anemia or polycythemia.

Principle of (PCV)

Anticoagulant is added to a sample of blood and centrifuged in haematocrit tube. Red blood cells and other cellular elements of blood being heavier than plasma get packed towers the bottom of the tube by the centrifugal force and the plasma is separated. The reading of the percentage of the blood that is made up of red blood cells is noted.

Normal value

Men 40-50%.

Women 36-44%.

Clinical implications

1- Reduce PVC occurs in pregnancy, severe anemia, spleen disease and Kidney disease.
2- Increase PCV occurs in high altitude, burns, dehydration, and polycythemia.
3- Yellow color plasma occurs in jaundice.
4- Turbid plasma occurs in hemolytic anemia.
5- Decrease in number or size of red blood cells, and decrease the amount of space they occupy, result increase in PCV.

Hematology

3- Erythrocyte sedimentation rate (ESR)

It is blood test that can show inflammation in the body. Inflammation is the immune system's response to injury, infection, and many types of conditions, including immune system disorders, certain cancers, and blood disorders (arthritis).

Erythrocytes are red blood cells. To do an ESR test, a sample of a blood is places in a tall, thin test tube and measures how quickly the red blood cells settle or sink to the bottom of the tube. Normally, red blood cells sink slowly. But inflammation makes red blood cells stick together in clumps. These clumps of cells are heavier than single cells, so they sink faster.

If an ESR test shows that your red blood cells sink faster than normal, it may mean you have a medical condition causing inflammation. The speed of test result is a sign of how much inflammation has. Faster ESR rates mean higher levels of inflammation. But an ESR test alone cannot diagnose what condition is causing the inflammation.

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Mechanism

- ✓ ESR is determined by the interaction between factors that promote (fibrinogen) and resist (negative charge of RBCs- that repel each other) sedimentation.
- ✓ Normal RBCs settle slowly as they do not form rouleaux or aggregate together. Instead, they gently repel each other due to the negative charge on their surfaces.
- ✓ Increased rouleaux formation contributes to high ESR.
  Plasma proteins, especially fibrinogen, adhere to the red cell membranes and neutralize the surface negative charges, promoting cell adherence and rouleaux formation.

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Normal value:

Men : 0-15 mm/hr
Women : 0-20 mm/hr
Children : 0-10 mm/hr

****

The ESR is affected by three factors: Effect of plasma protein, the RBC size and number and technical factors

****

Clinical implications

A high ESR test result may be from a condition that causes inflammation, such as:

1. Arteritis
2. Arthritis
3. Systemic vasculitis
4. Polymyalgia rheumatic
5. Inflammatory bowel disease
6. Kidney disease
7. Infection
8. Rheumatoid arthritis and other autoimmune diseases
9. Heart disease
10. Certain cancers

A low ESR test result: means your red blood cells sank more slowly than normal. This may be caused by conditions such as:

1. A blood disorder, such as:
    a. Polycythemia
    b. Sickle cell disease (SCD)
    c. Leukocytosis, a very high white blood cell count (WBC)
2. Heart failure
3. Certain kidney and liver problems

Note: sickle cell anemia and spherocytosis have low ESR unlike other anemia's. (This is due to reduced rouleaux formation owing to the Abnormally shaped RBCs in this condition.

****

4- Red Blood Cell manual count

Red blood cells count using haemocytometer, a haemocytometer is a counting-chamber device originally designed and usually used for counting blood cells.

Red cell diluting fluid must be have:

1- Anti-coagulant
2- Anti-hemolysis
3- Anti-aggregation
4- Anti-rouleaux

****

Normal value:

Men (4.7-6 million/ microliter)

Women (4-5 million/microlitre)

Children (4.2-5 million/microliter)

Infant (4-5 million/microliter)

****

Clinical implication

Higher value (polycythemia)

- Physiological: gender, age, high altitude.
- Pathological: bone marrow disease, lung disease, heart disease, Kidney disease.

Lower value (anemia)

- Excessive bleeding
- Decreased red blood cell production
- Increase red blood cell destruction

Hematology

5- White blood cell count

White blood cells (leukocytes) are dividing in to two groups: granulocyte and agranulocyte. The granulocytes receive their name from the distinctive granules that are present in the cytoplasm consist of (neutrophils, basophiles and eosinophils). Agranulocytes consist of lymphocytes and monocytes, do not contain distinctive granules and have non lobular nuclei. White blood cells are formed in the bone marrow, and they are transport in the blood to different parts of the body where they are needed.

Normal value 4000 – 11000 cells/mm

Clinical implication

An increase in the W.B.C called leukocytosis, occur most commonly as from:-

1. Bacterial infection such as appendicitis, tonsillitis, ulcers, pertussis.
2. Tissue injury.
3. Toxin.
4. Drug.
5. After splenectomy.
6. Tissue necrosis.

A decrease in the number of W.B.C called leucopenia which is usually due to:-

1. Bacterial infection such as typhoid fever, brucellosis.
2. Bone marrow depression.
3. Some viral infection.
4. Certain drugs such as radio therapy and chemotherapy.

****

6- Platelets count

Platelets (thrombocytes) are the smallest of the formed elements in the blood, it is round or oval flattened disc shaped structures. It is produced in the bone marrow by fragmentation of the cytoplasm of megakaryocytes. Platelet activity is necessary for a blood clotting and vascular integrity and vasoconstriction and the adhesion and aggregation activity that occurs during the formation of platelet plugs in small vessels.

Normal count: 150,000 - 400,000/mm3

Clinical Implications

An increased number of platelet (thrombocytosis) occurs in:-

1. Hemolytic anemia
2. Malignancies
3. Renal failure
4. Infection
5. Iron-deficiency anemia
6. Splenectomy
7. Trauma
8. Hemorrhage
9. Autoimmune disease.

A decreased number of platelet (thrombocytopenia) occurs in:-

1. Thrombopoietin deficiency.
2. After massive blood transfusion (dilution effect).
3. HIV infection.
4. Leukemia.
5. Renal insufficiency.
6. Hypersplenism.
7. Alcohol toxicity.
8. Idiopathic thrombocytopenic purpura.
9. Drug cancer chemotherapy and radiation.

****

7- Blood Group

The differences in human blood are due to the presence or absence of certain protein molecules called antigens and antibodies. The antigens are located on the surface of the RBCs and the antibodies are in the blood plasma. The ABO and Rhesus (Rh) systems are the most important ones used for blood transfusions.

Not all blood groups are compatible with each other. Mixing incompatible blood group leads to blood clumping or agglutination, which is dangerous for individuals.

ABO blood grouping system

Blood group A

If you belong to the blood group A, you have A antigens on the surface of your RBCs and B antibodies in your blood plasma.

Blood group B

If you belong to the blood group B, you have B antigens on the surface of your RBCs and A antibodies in your blood plasma.

Blood group AB

If you belong to the blood group AB, you have both A and B antigens on the surface of your RBCs and no A or B antibodies at all in your blood plasma.

Blood group O

If you belong to the blood group O (null), you have neither A nor B antigens on the surface of your RBCs but you have both A and B antibodies in your blood plasma.

People with blood group O are called "universal donors" and people with blood group AB are called "universal receivers".

Blood Group	Antigens	Antibodies	Can give blood to	Can receive blood from
AB	A and B	None	AB	AB,A,B,O
A	A	B	A and AB	A and O
B	B	A	B and AB	B and O
O	NONE	A and B	AB,A,B,O	O

The Rhesus (Rh) system

Rh antigens are transmembrane protein with loops exposed at the surface of red blood cells.

RBCs that are "Rh positive" express the antigen designated D. 85% of the population is RhD positive, the other 15% of the population is running around with RhD negative blood.

A person with Rh- blood can develop Rh antibodies in the blood plasma if he or she receives blood from a person with Rh+ blood, whose Rh antigens can trigger the production of Rh antibodies.

A person with Rh+ blood can receive blood from a person with Rh- blood without any problems.

What happens when blood clumps or agglutinates after blood transfusion?

The red blood cells from the donated blood will clump or agglutinate. The agglutinated red cells can clog blood vessels and stop the circulation of the blood to various parts of the body. The red blood cells contain hemoglobin which becomes toxic when outside the cell, this can have can fatal.

Anti-A	Anti-B	Anti-D	Blood type
			A^+
			B^+
			AB^+
			O^-

Hematology

8- White blood cell differential counts:-

It is the relative percentage of the each type of white blood cells in peripheral blood. This test experiment is a part of blood routine test.

Neutrophil

1. The mature neutrophil is about 12-13 mm in diameter.
2. The nucleus normally contains condensed dark staining material arranged in to two to five lobes joined together by thick threads of chromatin.
3. Contain violet – pink granules.
4. Highly motile / very active.
5. Consist of 65% of white blood cells count.

Eosinophil

1. The eosinophil is about 14-16 mm in diameter.
2. The eosinophil usually has a two lobes nucleus.
3. The granules larger than those of neutrophil, and they are spherical, red-orange.
4. Cytoplasm is full of granules.
5. Found in lining of respiratory and digestive tract.
6. Consist of 0-5 % of white blood cells count.

Basophil

1. The Basophil is about 14-16 mm in diameter.
2. Basophil has numerous dark purple granules that typically overlie and obscure the nucleus.
3. Nucleus has three cover leaf lobes.
4. Consist of 0-1 % of white blood cells count.

Monocyte

1. The largest circulating cells normally found in the peripheral blood, measure about 14-20 mm in diameter.
2. Nucleus: large, irregularly, dark kidney shape and folded shape.
3. Cytoplasm is dull gray-blue and contains numerous diffuse, usually small and light-colored granules.
4. Nucleons: dark kidney shape.
5. Consist of 5-7 % of white blood cells count.

Small lymphocyte

1. About 90% the circulating lymphocyte are small cell (9-12 mm) in diameter.
2. Nucleus approximately 8.5 mm in diameter that contains dense chromatin clumps.
3. Cytoplasm: thin rim of cytoplasm surround nucleus, devoid from granules.
4. Consist of 25% of white blood cells count.

Large lymphocyte

1. About 10% of circulating lymphocyte and it is larger cell (12-16 mm) in diameter.
2. Cytoplasm is pale and more abundant than small lymphocyte, some large lymphocyte have reddish purple granules in the cytoplasm.

Common causes of altered leukocyte count

leukocyte count	Decreased	Increased
Neurtophil	Bone marrow disease Immune reaction Drug Hereditary	Infection Tissue destruction Inflammatory disease
Eosinophil	Bacterial infection	Parasitic infection Hypersensitivity infection
Basophil	Drug	Some viral infection Leukemia Allergic reaction
Monocyte	Drug	Chronic infection (tuberculosis) Rheumatic arthritis
Lymphocyte	Immunodeficiency Drug	Viral infection Some fungal infection Rare bacterial infection

9- Coagulation tests:

a. Bleeding time:

It is the time taken for a standard skin wound to stop bleeding, upon vessel injury, platelets adhere and form hemostatic platelet (plug). Bleeding time measure the ability of these platelet to arrest bleeding and there for measure platelet number and function. Bleeding time performed as a screening procedure used to detect both congenital and acquired disorders platelet function. So the duration of bleeding from standard puncture wound of the skin is a measure of function of platelet as well as the integrity of the vessel wall.

Normal value: 1 to 5 minutes

Prolonged bleeding time occurs in:

1. Blood vessel defect
2. Platelet aggregation defect.
3. Thrombocytopenia (law platelet count)

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b. Clotting time:

It is the time required for a sample of blood to coagulate in vitro under standard conditions. There are various methods for determining the clotting time, the most common being the capillary tube method. It is affected by calcium ion levels and many diseases.

Normal value: 2-7 minute

Prolonged clotting time occurs in:

1. Liver disease.
2. Vitamin K deficiency.
3. Hypofibrinogenemia.
4. Hemophilia.
5. Deficiency of prothrombin.

****

c. Prothrombin time test (Pt):

It directly measures a potential defect stage II of the clotting mechanism (extrinsic coagulation system) through analysis of the clotting ability of five plasma coagulation factors (prothrombin, fibrinogen, factor V, factor VII and factor X).

In addition to screening for deficiency of prothrombin, the PT is used to evaluate dysfibrinogenemia, the drug effect, liver failure and vitamin K deficiency.

Normal value: 11- 16 second

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d. Activated partial thromboplastin time (APTT):

The PTT: a one stage clotting test, screens for coagulation disorders. Specifically it can detect deficiencies of the intrinsic thromboplastin system. The APPT is used to detect deficiencies in the intrinsic coagulation pathway (factor XII ,XI , IX , X , V, II and I) Prolonged APPT occurs in Hemophilia, heparin therapy, warfarin therapy, liver disease.

Normal value: 21 - 35 seconds.

Polymerase chain reaction (PCR)

Polymerase chain reaction is a technique used in molecular biology to amplify a single copy or a few copies of a segment of DNA across several orders of magnitude, generating thousands to millions of copies of a particular DNA sequence by using PCR thermo cycle.

PCR is now a common and often indispensable technique used in clinical and research laboratories for a broad variety of applications, these include:

1. DNA cloning for sequencing.
2. Gene cloning and manipulation.
3. Gene mutagenesis.
4. Functional analysis of genes.
5. Diagnosis and monitoring of hereditary diseases.
6. Analysis of genetic fingerprints for DNA profiling.
7. Detection of pathogens in nucleic acid tests for the diagnosis of infectious diseases.

A basic PCR set-up requires several components and reagents,[8] including:

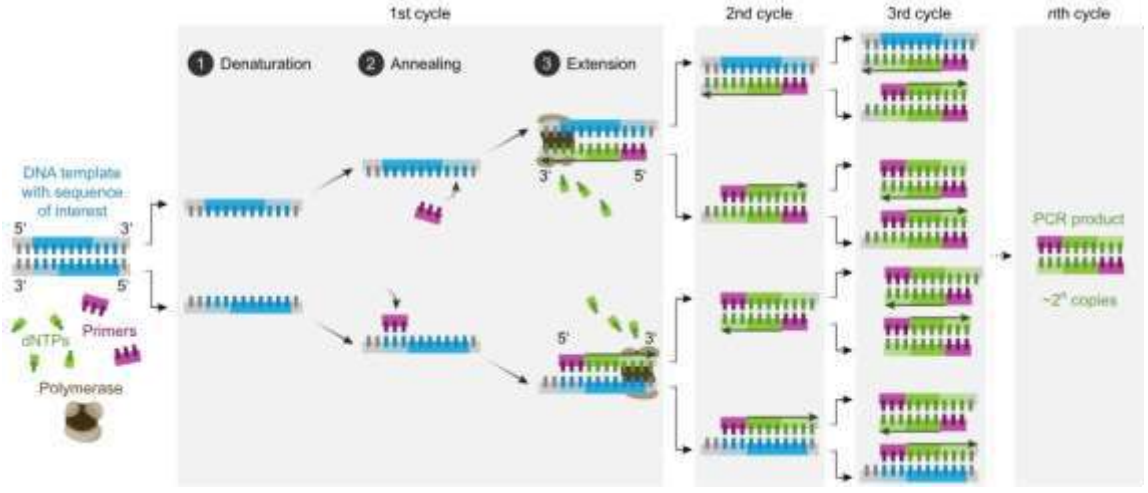
1. A DNA template that contains the DNA target region to amplify.
2. A DNA polymerase; an enzyme that polymerizes new DNA strands; heat-resistant Taq polymerase is especially common, as it is more likely to remain intact during the high-temperature DNA denaturation process.
3. Two DNA primers that are complementary to the 3' (three prime) ends of each of the sense and anti-sense strands of the DNA target (DNA polymerase can only bind to and elongate from a double-stranded region of DNA; without primers, there is no double-stranded initiation site at which the polymerase can bind); specific primers that are complementary to the DNA target region are selected beforehand, and are often custom-made in a laboratory or purchased from commercial biochemical suppliers.
4. Deoxynucleoside triphosphates, or dNTPs (sometimes called "deoxynucleotide triphosphates"; nucleotides containing triphosphate groups), the building blocks from which the DNA polymerase synthesizes a new DNA strand.
5. A buffer solution providing a suitable chemical environment for optimum activity and stability of the DNA polymerase.
6. Bivalent cations, typically magnesium (Mg) or manganese (Mn) ions; Mg2+ is the most common, but Mn2+ can be used for PCR-mediated DNA mutagenesis, as a higher Mn2+ concentration increases the error rate during DNA synthesis; and monovalent cations, typically potassium (K) ions.

Procedure PCR

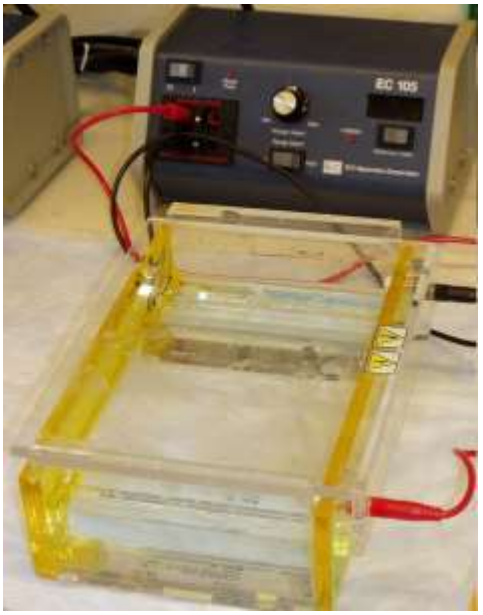
1. **Denaturation:** This step is the first regular cycling event and consists of heating the reaction chamber to 94–98 °C for 20–30 seconds. This causes DNA melting, or denaturation, of the double-stranded DNA template by breaking the hydrogen bonds between complementary bases, yielding two single-stranded DNA molecules.

2. **Annealing:** The reaction temperature is lowered to 50-65°C for 20-40 seconds, allowing annealing of the primers to each of the single-stranded DNA templates. Two different primers are typically included in the reaction mixture: one for each of the two single-stranded complements containing the target region. The primers are single-stranded sequences themselves, but are much shorter than the length of the target region, complementing only very short sequences at the 3' end of each strand.

3. **Extension/elongation:** The temperature at this step depends on the DNA polymerase used; the optimum activity temperature for Taq polymerase is approximately 75-80 °C, though a temperature of 72°C is commonly used with this enzyme. In this step, the DNA polymerase synthesizes a new DNA strand complementary to the DNA template strand by adding free dNTPs from the reaction mixture that are complementary to the template in the 5'-to-3' direction, condensing the 5'-phosphate group of the dNTPs with the 3'- hydroxy group at the end of the nascent (elongating) DNA strand. The precise time required for elongation depends both on the DNA polymerase used and on the length of the DNA target region to amplify. With each successive cycle, the original template strands plus all newly generated strands become template strands for the next round of elongation, leading to exponential (geometric) amplification of the specific DNA target region.



The processes of denaturation, annealing and elongation constitute a single cycle. Multiple cycles are required to amplify the DNA target to millions of copies.



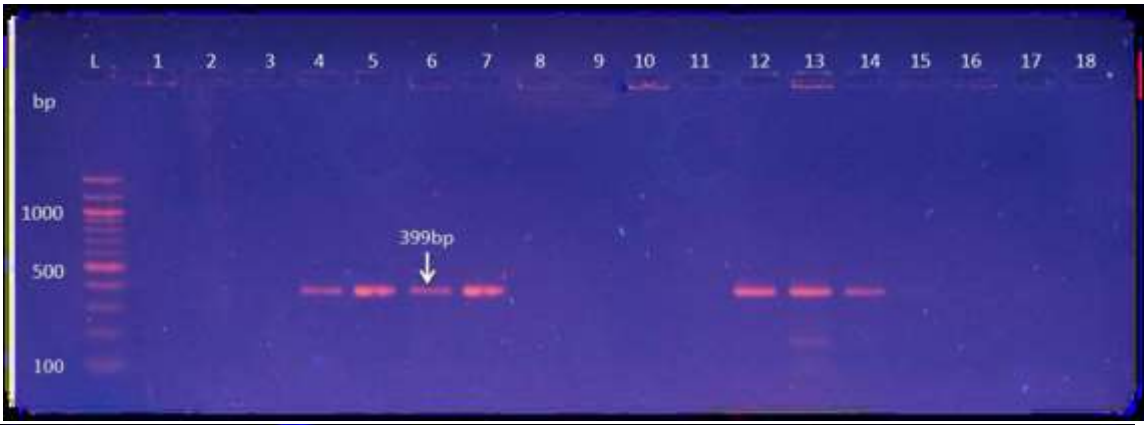
Gel electrophoresis is a method for separation and analysis of macromolecules (DNA, RNA and proteins) and their fragments, based on their size and charge. It is used in clinical chemistry to separate proteins by charge and/or size and in biochemistry and molecular biology to separate a mixed population of DNA and RNA fragments by length, to estimate the size of DNA and RNA fragments or to separate proteins by charge.

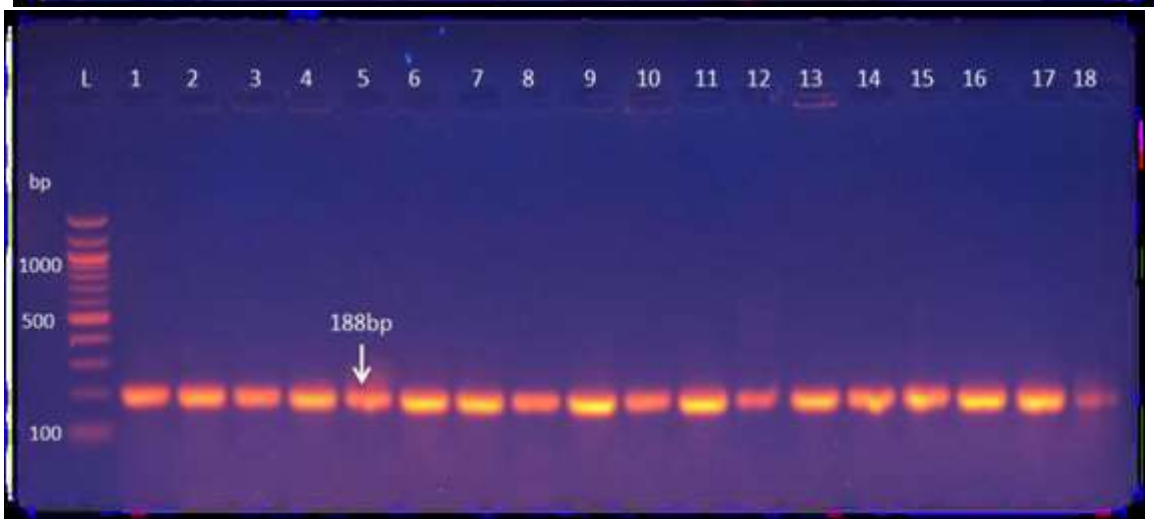
Nucleic acid molecules are separated by applying an electric field to move the negatively charged molecules through a matrix of agarose or other substances. Shorter molecules move faster and migrate farther than longer ones because shorter molecules migrate more easily through the pores of the gel. Gels can also simply serve to maintain the finished separation, so that a post electrophoresis stain can be applied. DNA Gel electrophoresis is usually performed for analytical purposes, often after amplification of DNA via polymerase chain reaction (PCR).

Ethidium bromide is an intercalating agent commonly used as a fluorescent tag (nucleic acid stain) in molecular biology laboratories for techniques such as agarose gel electrophoresis.

Buffers in gel electrophoresis are used to provide ions that carry a current and to maintain the pH at a relatively constant value. These buffers have plenty of ions in them, which is necessary for the passage of electricity through them.

A molecular-weight size marker, also referred to as a protein ladder, DNA ladder, or RNA ladder, is a set of standards that are used to identify the approximate size of a molecule run on a gel during electrophoresis, using the principle that molecular weight is inversely proportional to migration rate through a gel matrix.


L 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18
bp
1000
500
100
399bp


L 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18
bp
1000
500
100
188bp

Enzyme linked immune sorbent assay (ELISA)

Enzyme-linked immunosorbent assay (ELISA), also known as an enzyme immunoassay (EIA), is a biochemical technique used mainly in immunology to detect the presence of an antibody or an antigen in a sample.

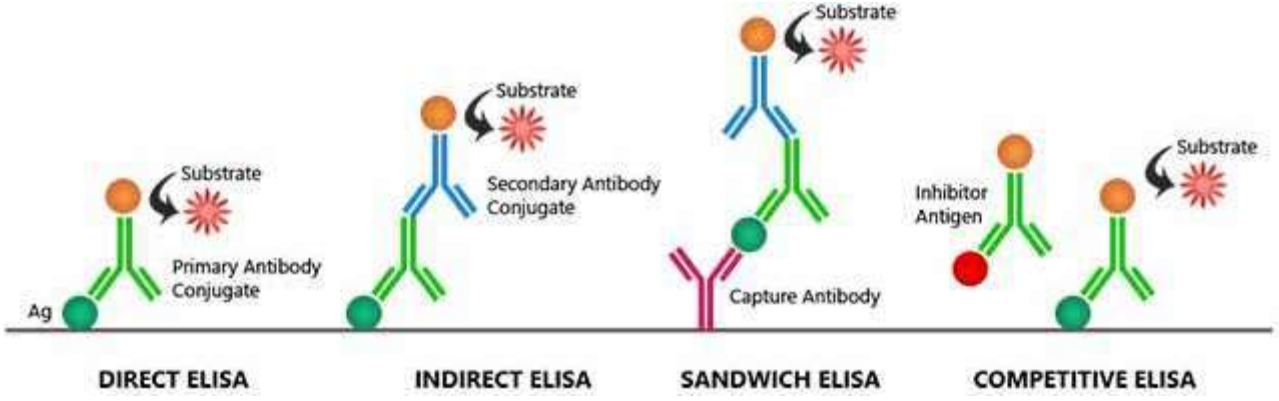
Basic ELISA principles

In an ELISA assay, the antigen is immobilized to a solid surface. This is done either directly or via the use of a capture antibody itself immobilized on the surface. The antigen is then complexed to a detection antibody conjugated with a molecule amenable for detection such as an enzyme or a fluorophore. Between each steps there is a step of incubation and washing.

ELISA Applications

1. Food Industry
2. Vaccine Development
3. Immunology
4. Humoral Immunity
5. Diagnosis
6. Cancer Detection

Types of ELISA Test



1. Direct ELISA

For direct detection, an antigen coated to a multi-well plate is detected by an antibody that has been directly conjugated to an enzyme.

Advantages

Fast and simple protocol**.**

Disadvantages

Less specific since you are only using one antibody.

2. Indirect ELISA

The antigen coated to a multi-well plate is detected in two stages or layers. First an unlabeled primary antibody, which is specific for the antigen, is applied. Next, an enzyme-labeled secondary antibody is bound to the first antibody.

Advantages

Amplification using a secondary antibody.

Disadvantages

Potential for cross-reactivity caused by secondary antibody.

3. Sandwich ELISA

Sandwich ELISAs typically require the use of matched antibody pairs, where each antibody is specific for a different, non-overlapping part (epitope) of the antigen molecule. A first antibody (known as capture antibody) is coated to the wells. The sample solution is then added to the well. A second antibody (known as detection antibody) follows this step in order to measure the concentration of the sample.

Advantages

1. Highest specificity and sensitivity.
2. Compatible with complex sample matrices.

Disadvantages:

1. Longer protocol.
2. Challenging to develop.

4. Competitive ELISA

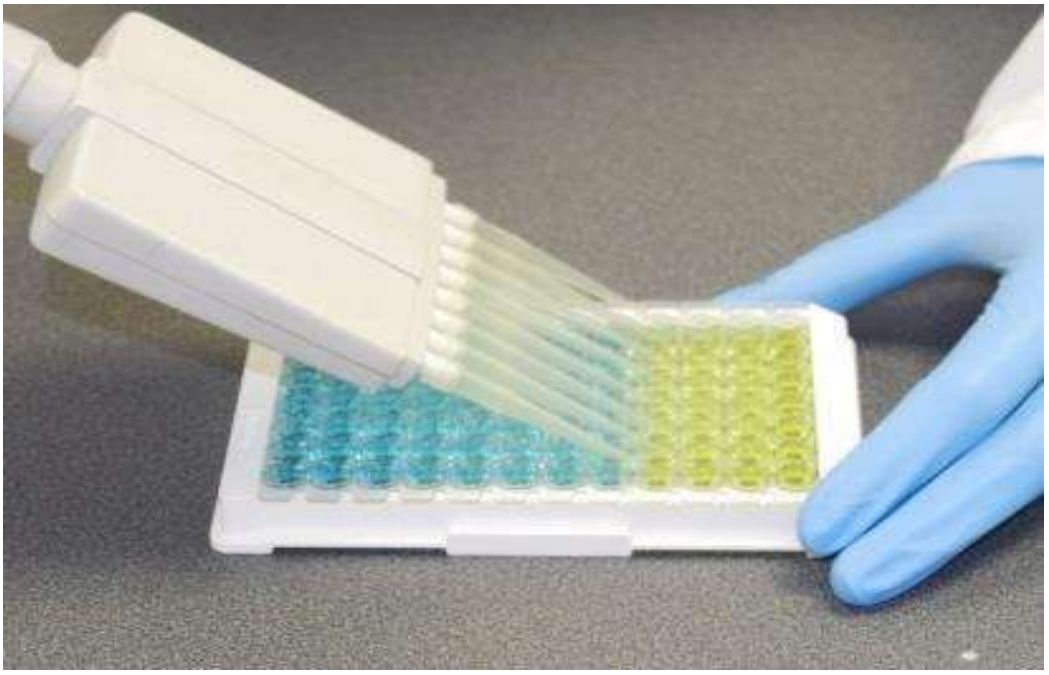
The key event of competitive ELISA (also known as inhibition ELISA) is the process of competitive reaction between the sample antigen and antigen bound to the wells of a microtiter plate with the primary antibody.

Advantages:

Ability to quantitate small molecules**.**

Disadvantages:

1. Less specific since you are only using 1 antibody.
2. Requires a conjugated antigen.



GENERAL URINE EXAMINATION (GUE)

Urine is a typically sterile liquid by-product of the body secreted by the kidneys through a process called urination and excreted through the urethra.

GUE is the important screening procedure in clinical medicine because it gives diagnostically important information about the presence of disease inside and outside the urinary tract by urine analysis screen for renal or urinary tract disease and help detect metabolic and systemic disease.

Urine composition is affected mainly by three factors, they are:

1. Nutritional status.
2. State of metabolic processes.
3. Ability of kidney to selectively handle the material presented to it.

METHODS OF URINE COLLECTION

1. Random collection taken at any time of day with no precautions regarding contamination. The sample may be dilute, isotonic, or hypertonic and may contain white cells, bacteria, and squamous epithelium as contaminants.

2. Early morning collection of the sample before ingestion of any fluid. This is usually hypertonic and reflects the ability of the kidney to concentrate urine during dehydration which occurs overnight.

3. Clean-catch, midstream urine specimen: this is preferred type of specimen for culture and sensitivity testing to reduce microbial contamination.

4. Catherization of the bladder through the urethra for urine collection is carried out only in special circumstances, i.e., in a comatose or confused patient. This procedure risks introducing infection and traumatizing the urethra and bladder, thus producing iatrogenic infection or hematuria.

5. Timed collection specimen (24h): usually used to measuring creatinine clearance, urine volume and to measure the amount of total protein in urine.

General Urine Examination (G.U.E): It includes two basic tests:

A-Macroscopic examination of the urine:

1. Volume

The normal range of urine output is 800 to 2,000 milliliters per day if you have a normal fluid intake of about two liters per day.

Abnormal volumes which are:

a) Polyuria >2000 mls./day, an abnormally high urine volume.

b) Oliguria <400 mls. /day, an abnormally low urine volume.

c) Dysuria: is any discomfort or painful associated with urination which often signifies an infection of the lower urinary tract.

d) Anuria: complete cessation of urine caused by presence of tumor or large renal stone (Calculus).

2. Specific gravity:

It is a test that shows the concentration of all chemical particles in the urine that compares the density of urine to the density of water, depends on the concentration of various solutes in urine like salt, Albumins, Sager. Normal value is (1.010-1.030). A low value may indicate diabetes.

3. Colour:

Normal urine color has a wide range of variation ranging from pale yellow, yellow, dark yellow, amber due to urobillin pigment appears in urine.

4. Appearance:

Normal appearance of urine is clear and abnormal cloudy or turbid implying to presence salt, albumin and pus cells.

5. Reaction (pH):

A urine pH test measures the acid-base (pH) level in your urine. A high urine pH may indicate conditions including kidney issues and a urinary tract infection (UTI). The average value for urine pH is (6.0).

6. Odor:

Fresh normal urine has a faint aromatic odor and leaving it for a long time leads to convert urea into ammonia which has foul odor.

7- Glucose

It's measures the amount of glucose in the urine. Normally, there shouldn't be glucose in the urine, so the presence of glucose could be a sign of diabetes.

8- Ketones

Ketones build up when the body has to break down fats and fatty acids to use as energy. This is most likely to happen if the body does not get enough sugar or carbohydrates as energy. Ketone urine tests to check for diabetes-related ketoacidosis.

9- Protein (Albumin)

A protein urine test measures the presence of proteins, such as albumin, in your urine. If urine protein levels is higher than normal may indicate several different health conditions, such as heart failure, kidney issues and dehydration.

10. Bilirubin:

Bilirubinuria: Is the presence of bile pigments bilirubin in the urine.

Causes: Liver diseases or injury, viral hepatitis and obstruction to biliary duct.

11. Urobilinogen:

Urobilinogen is formed from the reduction of bilirubin. Urinary urobilinogen may be increased in the presence of a hemolytic process such as hemolytic anemia. It may also be increased with infectious hepatitis, or with cirrhosis.

Causes: Hemolytic anemias, and liver disease such as hepatitis or cirrhosis.

12. Nitrite:

A positive nitrite test result can indicate a urinary tract infection (UTI). However, not all bacteria are capable of converting nitrate to nitrite.

B- Microscopic Urinalysis

1- Red Blood Cells

An elevated number of RBCs indicates that there's blood in the urine. However, this test can't identify where the blood is coming from.

2- White Blood Cells

An increased number of WBCs and/or a positive test for leukocyte may indicate an infection or inflammation somewhere in the urinary tract.

3- Epithelial Cells

It's normal to have a small number of certain types of epithelial cells in urine. A large number may indicate a urinary tract infection, kidney disease, or other serious medical condition.

4- Casts

They are solid and cylindrical structures formed by precipitation of debris in the renal tubules. A large amount of hyaline casts may indicate kidney damage due to decreased blood flow to the kidneys.

5- Bacteria

Bacteria are common in urine specimens because of the many normal flora of the vagina or external urethral and because of their ability to rapidly multiply in urine standing at room temperature.

6- Yeast

Yeast cells may be contaminants or represent a true yeast infection. Most often they are Candida, which may colonize bladder, urethra, or vagina.

7- Crystals

Common crystals seen even in healthy patients include calcium oxalate, triple phosphate crystals, amorphous phosphates, amorphous urates, uric acid.