**CLINICAL PATHOLOGY**

I. What is clinical pathology?

A. Definition:

l. ***pathology***is the "branch of malicine that deals with the basis of disease, especially those structural and functional changes in organs and tissues caused by a disease". In general, it is the study of disease.

2*.* ***Clinical pathology***is a "subspecialty of pathology that deals with the use of laboratory methods for the diagnosis and treatment of disease". In general it is the study of disease in the clinical environment by use the **laboratory assays** that include:

1- Hematology

2- Microbiology

3- Clinical chemistry

4-Serology

5-Histopathology

6-Molecular biology

B. ***Veterinary clinical pathologists***are specialists in the regulation of basic microbiology, hematology (study of blood), clinical chemistry (study of physiologic , biochemical reactions),cytology (study of cells) ,immunology and histopathology.

II. Laboratory tests should be used with other diagnostic procedures. Before laboratory tests are used, two diagnostic procedures to clarify or classify pathologic problems are essential:

(1) Obtain case history.

(2) Obtain complete physical examination.

Some body systems (integument, nervous, skeletal and cardiovascular) are relatively evaluated via visual or imaging methods (physical examination, radiography and ultrasonography), whereas other body systems ( hemic, immune, urinary, and endocrine) are better evaluated by laboratory tests.

III. What are the major causes for analyzing patient samples via laboratory procedures?

A.To detect an unidentified pathologic state.

B.To define, classify or confirm a pathophysiologic disorders or disease state.

C.To eliminate (rule out) other possible causes of the disease.

D.To evaluate changes in a pathologic state

**SAMPLES**

**I. Blood samples :**

**A.** Most clinical laboratory assays are designed to detect or measure substances or cells in blood samples ; the substances or cell of interest is called the **analyte**.

**B.** Blood sample collection

l. Blood and major components are commonly used as samples for laboratory assay. Blood must be collected and processed properly so that assay results reflect the true composition of blood rather than artifactual changes.

2. Blood is composed of blood cells (erythrocytes, and five major leukocyts types and platelets) and plasma. Blood withdrawn from a blood vessel must immediately be mixed with an anticoagulant to prevent clot formation and to maintain cells and other components in suspension.

3. Analysis or processing of whole blood must be relatively rapid because the cells die within a few hours, and thus a sample will become unacceptable for analysis. Samples must be analyzed within minutes, usually within hours.

C. Anticoagulants used for blood sample collection:

**a. Calcium-binding agents** prevent Ca2+ from participating in the formation of a blood clot include:

1- Ethylene Diamine Tetra-acetic Acid (**EDTA**):

a- EDTA is the preferred anticoagulant for almost all routine hematologic tests, including the complete blood count (CBC) assays.

b- EDTA chelates Ca2+ and other divalent cations (Mg2+, Cu2+ and Pb2+) but the other anticoagulants do not. EDTA attaches to Ca2+ in six places so prevent coagulation of blood. Used in either a liquid or dry form. One drop of EDTA 10 % solution sufficient to prevent coagulation of 5 ml of blood.

2- **Citrate** (as sodium citrate or potassium citrate).

a- Citrate is the preferred anticoagulant for most tests of the coagulation system. Citrate's anticoagulant activity is achieved by its forming an ionic bond with Ca2+ .

b- Because it has low toxicity, citrate is also preferred for collection of whole blood to be used for **transfusions**.

3- **Oxalates**

a- Oxalate is used for a few laboratory tests; for example used for glucose and lactate evaluation assays. Generally, oxalates alter morphologic features of leukocytes and erythrocytes and thus are unsuitable for hematologic samples.

b- Oxalate's anticoagulant activity is achieved by its forming an ionic bond with Ca2+.

**b. Heparin** : activates antithrombin III which then inhibits the activity of several coagulation factors (including thrombin). It is also forms an ionic bond with Ca2+,but its major action is through interfering with conversion of prothrombin to thrombin .

1- Used for several special laboratory assays (such as blood gas analysis) and can be used for many clinical chemistry assays.

2-It is used commonly in liquid or dry form.

3- Major disadvantages:

a- Alters morphologic features and staining of leukocytes.

b-Allows platelet clumps to form.

**D.** Plasma

1. Plasma is the fluid component of blood that is harvested after centrifugation of an anticoagulated blood samples. Plasma will contain the anticoagulant that can interfere with some assays.

2. **Plsma** has two major components:

1-**Water**: about 92- 95 % of plasma volume; 100 ml of plasma contains 92- 95 ml of H2O.

2-**Solids**: about 5-8 % of plasma volume. Most solids are proteins on a weight per volume (weight/volume) basis. Other solids are glucose,lipid,hormons, urea, electrolytes, and other chemicals.

3. Generally, the chemical composition of plasma is very similar to interstitial fluid in most tissues. Plasma and interstitial fluid are the extracellular fluids , one intravascular and other extravascular.

**E**. **Serum**

1. serum is the fluid component of blood that is harvested after centrifugation of a coagulated (clotted) blood sample. The clotting involves platelets and coagulation proteins.

2. Serum has the same composition as plasma except serum does not contain most of the coagulation proteins. The major protein that is absent in serum but present in plasma is fibrinogen.

3. During the clotting process, substance released from cells alter the analyte concentrations in serum. For example, platelets release K+, and thus serum K+ is greater than plasma K+ .

**II. Urine Samples**

A. Urine is the most common sample analyzed by laboratory assays. Urine must be collected and processed properly so that the assay results reflect the true composition of the product of the urinary system.

B. To prevent artifactual changes in urine, it should be processed soon after collection.

**III. Milk Samples:** milk is the sample analyzed during subacute mastitis.

**IV. Other Body Fluid samples**

A. Pleural fluid, peritoneal fluid, synovial fluid, and cerebrospinal fluid samples are collected to characterize body cavity effusions, joint diseases and central nervous system disorders respectively.

**The basic clinical pathology lab. :**

 In this lab. should contain the following:

A-Equipment:

1-Microscope

2-Microhematocrit centrifuge (12000 rpm)

3-Standered clinical centrifuge(with tubes about 15 cc)

4-Refractometer

5-Differential cell counter

6-Interval timer

7-Hemocytometer

8-Tubes,racks and glassware

9-Distilator

B-Chemicals:

1-Red and white blood cell diluting fluids.

2-Blood stains:wright,Giemsa , leishman stains,new methylin blue stain.

3-Stains for bacteria:gram, acid fast stains.

4-Kits for fluid analysis for detection proteins glucose,bilirubin,keton bodies, hormones and enzymes.

5-Sodium chloride,zink sulfate for preparation of flotation fluid.

6-Additional supplies include:

 a-formaline(10%).

 b-anticoagulantes.

 c-distilled water.

 d-oil immertion

**The Complete clinical pathology lab. :**

A-Equipments:

1-Spectrophotometer.

2-Water bath.

3-Automated cell counter.

4-pH meter.

5-Balance.

6-Incubator.

7-Bunsen burner.

8-Bacteriology inoculating loop.

9-Sensitivity disc dispenser.

B-Glassware and disposable plastic supplies:

1- Cuvettes.

2-Volumetric flasks.

3-Volumetric pipettes.

4- Micropipettes.

5- Glassware for cell count.

C-Kits for clinical biochemistry include:

1-Alkaline phosphatase.

2-Alanine-amino transferase.

3-Aspartate-amino transferase.

4-Serum creatinine.

5-Blood urea nitrogen.

6- Serum calcium, cholesterol and bilirubin.

7- Serum enzymes and hormons.

D-Chemical reagents

E-Bacteriology media include:

 a-Isolation media:

 1-Phenylethyl alcohol agar or streptococcal agar for Streptococcus.

 2-Staphylococus-110 or mannitol salt agar for Staphylococcus.

 3-Bile esculin agar for Listeria.

 4-SS agar , selenite broth and macConkey agar for Salmonella.

 5-Thiol medium for Campylobacter.

 6-Sabouraud medium for fungi.

 b-Differentiation media:

 1-MR-VP medium.

 2-Nitrate reduction medium

 3-Urea broth

 4-Triple sugar agar

 5-Indol production medium.

 6-Gelatin liquefaction medium

F-Antigens for serology:

 1-Brucella antigens.

 2-Leptospira antigens.

 3-Salmonella group antiserum.

 4-Feline leukemia virus antigens for ELISA.

G-Antibiotic sensitivity test need:

 1-Disposable petri dish and specific medium.

 2- Sterile tubes and broth.

 3- Sterile swabs.

 4-Disc of different antibiotics.

 5-Forceps .