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 (clinical chemistry, microbiology, hematology ,... ) for the diagnosis and treatment of disease". In general it is the study of disease in the clinical environment by use the laboratory assays.

B. ***Veterinary clinical pathologists***are specialists in the disciplines of basic pathology, hematology (study of blood), clinical chemistry (study of physiologic and biochemical reactions),cytology (study of cells), and surgical pathology (study of disease via microscopic analysis of tissue samples obtained during surgery).

C. Veterinary clinical pathologists and other laboratory professionals (medical technologists, medical laboratory technicians and veterinary technicians) often work in a clinical laboratory that limits its assay to: hematologic assays, clinical chemical assay, urinalysis, and clinical cytologic or histologic examinations. Other assays or diagnostic laboratory procedures includes specific laboratories (eg. microbiology, histopathology and toxicology) that are supervised by microbiologists, histopathologists. and toxicologists, respectively.

II. Laboratory tests should be used with other diagnostic procedures. Before laboratory tests are used, two diagnostic procedures are essential:

(1) Obtain case history.

(2) Obtain complete physical examination.

 With knowledge gained from these two basic procedures, a diagnostician can select diagnostic procedures to clarify or classify identified problems. Veterinarians frequently use the laboratory assays in conjunction with other diagnostic methods to identity or classify pathologic states that develop in domestic mammals. 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) a possible cause of the disease.

D.To assess changes in a pathologic state either due to natural progression of the disease or because of medical or surgical therapy.

**SAMPLES**

**I. Blood samples and specimens:**

**A.** Most clinical laboratory assays are designed to detect or quantify substances or cells in blood samples ; the substances or cell of interest is called the **analyte**. Obtaining useful results for the analyte requires appropriate samples. Whenever there is doubt about the appropriate sample for a particular test at particular laboratory, the laboratory should be contacted prior to sample collection.

**B.** Blood sample collection

l. Blood and major components are frequently 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 initiation of 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, rarely within days.

**C.** Anticoagulants used for blood sample collection:

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

1- Ethylene Diamine Tetra-acetic Acid (EDTA) (as Na2EDTA, K2EDTA, or K3EDTA)

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 (as lithium, ammonium, and potassium saIts).

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** (as lithium, ammonium, potassium, or sodium salts) 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 clotting as effects are slowly overridden by the coagulation system.

c- 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:

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

b. Solids: about 5-8 % of plasma volume. Most solids are proteins on a weight per volume (weight/volume) basis. Other solids are glucose, 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 one 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. To get the maximal amount of serum from the clotted sample, centrifugation should not be started prior to the retraction of the clot (which typically takes at least 30 min. if a clot activator is not present in the tube). If samples are centrifuged prior to clot retraction, some serum will be trapped in a soft fibrin clot.

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. 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.

MAJOR TYPES OF LABORATORY ASSAYS

I. Many laboratory tests or assays involve the analysis of body fluids (blood, serum, plasma, urine, peritoneal fluid, pleural fluid, cerebrospinal fluid, and synovial fluid), tissue samples, or feces. Most clinical laboratory procedures fall into one of three large groups (many procedures could be classified into more than one group).

**A. Clinical hematology assays**: Most assays are completed on whole blood samples.

1. Quantitation of cell concentrations in blood: total leukocyte, erythrocyte, and platelet count .

2. Semiquantitation of cell concentrations: calculated absolute leukocyte count and platelet estimation from blood film examination.

3. Defining or classifying cells by microscopic features: band or segmented neutrophils, reactive lymphocytes, polychromatophilic erythrocytes, poikilocytes, microcytes, hypochromic erythrocytes, leukemic cells.

4. Assessing the coagulation properties of blood: clotting times and platelet function assays.

**B. Clinical chemistry assays**: Most assays are completed on serum or plasma sample.

l. Detecting or quantifying the concentration of chemical substances.

a. Quantitative analysis : Results are close to the true concentration (e.g.:serum concentration of glucose, sodium, protein, creatinine and urea) and typically are reported with a specific numerical value.

b. Semiquantitative analysis: Results are within the ballpark (e.g., urine glucose, protein and bilirubin concentration by reagent pad chemistry assays) and may be reported as approximate numerical values or in a categorical scale (e.g ., 1+,2+ or 3+) that represents ranges of numerical values.

c. Qualitative analysis: Results indicate that a substance is or is not present (e.g ., fat detected microscopically in pleural fluid with a Sudan stain) and are reported as "present" or "absent" or as "positive" or "negative".

2. Detecting or quantifying the activity of chemical substances:

a. Quantitative analysis: results are close to true activity (e.g ., measured activities of serum enzyme such as alanine aminotransferase, alkaline phosphatase, lactate dehydrogenase and creatine kinase).

b. Qualitative analysis: Results indicate that activity is or is not present (e.g., heme's peroxidase activity or leukocyte esterase in urine).

**C. Clinical microscopy**

1. Clinical cytology: The study of cell populations and their microscopic feature in an attempt to define or classify abnormal tissue or fluid (e.g., lymph node aspirates to diagnose lymphoma or histoplasmosis, aspirates of a skin tumor to determine whether it is an inflammatory or neoplastic lesion and analysis of peritoneal fluid to determine if it is a transudate, exudate. or other type of effusion)

2. Surgical histopathology: The study of frozen or fixed tissue in an attempt to define or classify abnormal tissue (i.e. inflammatory, neoplastic and toxic disorder) and perhaps establish an etiologic diagnosis.

3. Urine sediment analysis: Microscopic examination of urine to detect or semiquantify the presence of leukocytes, erythrocytes, casts, bacteria. Crystals, or other structures.

4. Clinical parasitology: Microscopic examonation of fecal, urine, blood, or other sample to detect ova, larvae, or other microscopic forms of parasites.

The end 1st lecture