Accurate laboratory diagnosis is based on a valid sampling process.

**Purpose of sampling:**
For helping to establish a disease diagnosis.
For health surveillance and certification.
For monitoring the response to treatment or vaccination.

**Valid sampling process composed of:**
- Selection of appropriate sample
- Proper collection and handling of different types of samples
- Proper labeling of the sample
- Proper preservation of the samples
- Avoidance of spoilage of samples
I- Selection of appropriate samples

- Selection of representative animals
  - Animal that correctly represent the disease condition
  - Animal in advanced stage of the disease
  - In herd problem, samples from several animals with different stages of the disease
  - Collect samples from one or two recently died animals

- Selection of representative samples
  - collected samples must be related to the diseased condition.
  - More than one type of samples could be used if applicable.
  - Avoid sample contamination.

- Use of appropriate container for sample collection (Clean and dry).

- Use of appropriate preservative for shipping of samples.
II- Proper handling of different types of samples

Type of samples

Blood, Milk, Urine, Feces, Ruminal juice, Saliva and sputum and throat swap, Pus and exudates, Semen, preputial wash, Vaginal discharge, Skin scraping and hair, ...........
1- **Blood samples:**

- Venous blood is preferred for most hematological examinations.
- They are collected aseptically in different forms using special venule,
  vacutainer tubes or plastic syringes.

**Site of collection:**

<table>
<thead>
<tr>
<th>Animal</th>
<th>Veins</th>
</tr>
</thead>
<tbody>
<tr>
<td>Horse</td>
<td>Jugular vein.</td>
</tr>
<tr>
<td>Donkey</td>
<td>Jugular vein.</td>
</tr>
<tr>
<td>Buffaloes</td>
<td>Jugular vein.</td>
</tr>
<tr>
<td>Sheep &amp; Goot</td>
<td>Jugular vein.</td>
</tr>
<tr>
<td>Dog</td>
<td>Cephalic vein, lateral saphenous vein.</td>
</tr>
<tr>
<td>Cat</td>
<td>Ear vein, femoral vein.</td>
</tr>
<tr>
<td>Pig</td>
<td>Ear vein, anterior vena cava.</td>
</tr>
<tr>
<td>Rabbit</td>
<td>Marginal ear vein.</td>
</tr>
<tr>
<td>Rat and mouse</td>
<td>Tail vein.</td>
</tr>
</tbody>
</table>
Precautions during collection of blood samples:

- The site of collection of blood must be clean, dry and sterile.

- All the equipment used in collection must be clean, dry and sterile.

- The blood sample must be collected on the wall of the tube to avoid the hemolysis.

- Gentle mixing of the blood sample with the anticoagulant.

- Avoid contamination of the blood samples with hairs or dirties.

- The blood sample must be transferred directly to the laboratory under suitable conditions.
Errors occur during collection of blood samples:

- Using wet needle or syringe.
- Taking too much time in collection of blood.
- Failure to mixing the blood sample with anticoagulant immediately after collection or filling vials to the top. not properly mixed with blood.
- Vigorous mixing of the blood sample.
- Excessive negative pressure when collecting sample with a syringe will rupture cells and collapse the vein.
- Failure to remove the needle from the syringe, when transferring blood from a syringe to a container.
Types of blood samples

A) Whole blood.  B) Serum.
E) Blood swab.

A) **WHOLE BLOOD**

(Anti-coagulated blood)

Blood mixed with anticoagulant usually dipotassium salt of EDTA and used for:

1. Hematological picture
2. Blood culture.
3. Blood smear
5. Blood transfusion.
B- SERUM and PLASMA

- For biochemical analysis and serological examination

  - **Serum** from coagulated blood (Whole blood without anticoagulant)
  - **Plasma** from non-coagulated blood (whole blood with anticoagulant)

**STORAGE OF PLASMA OR SERUM**

- Samples may be stored at 4 °C in a refrigerator for up to 4 days or in freezing compartment for up to 1 week.
- If stored for longer periods, samples should be placed into deep freeze at -20 °C.
D) BLOOD SMEAR

Significance of the blood film
- Identification of animal species.
- Morphological classification of anemia.
- Diagnosis of bacterial diseases.
- Diagnosis of blood parasites.
- Deferential leucocytic counts (DLC).

E) Blood swabs
- For bacterial culture.
- Blood swab is taken from heart blood of small animals.
- In Anthrax, blood swab obtained from venous blood by tampon
Tests performed on blood samples

A- Hematological examination:
- Total erythrocytic count.
- Hematocrit (Packed cell volume).
- Determination of blood hemoglobin.
- Platelets count.
- Coagulation time.
- Total leucocytic count.
- Differential leucocytic count.
- Erythrocytic sedimentation rate.
- Bleeding time.
- Blood grouping.

B- Biochemical examination (plasma or serum):
Serum is preferred since it is less likely to show hemolysis than plasma and contain no anticoagulants which draw water outside the cells.

C- Bacteriological examination.
Collected blood is directly injected into the bottle containing the culture media.

D- Parasitological examination.
Stained blood smear is commonly used.
Diagnosis of both intra-cellular and extra-cellular blood parasites.

E- Toxicological examination.

F- Serological examination:
For estimation of the antibody level against certain disease-producing organisms.

G- Blood transfusion
2- Milk samples

During collection of milk sample avoid external contamination by:
- Thorough cleaning of udder and teat orifice.
- Collection in clean and sterilized containers.

Separate sample collected from each quarter (diseased or normal).

To avoid misdiagnosis, samples collected in the following order (LF, LH, RH, RF).

Immediately following collection, milk sample kept in refrigerator till time analysis.

**Indication of milk sample:**
- Examination of chemical and physical characteristics.
- Bacteriological examination for bacterial count and detection of mastitis causative agent.
- Detection of Brucella antibodies (Abortus Bang ring test – ABR test)
3- Urine sample

**Urine sample can be collected through:**

- Clean catch method after stimulation of animal urination.
- Catheterization for less contaminated samples.
- Cystocentesis for aseptic sample.

**Indication of urine sample:**

- Routine urine analysis.
- Bacterial examination.
- Parasitic examination.
4- Fecal sample

*In large animals:* fresh sample collected directly from animal by back racking using plastic bag.

*In small animals:* samples collected with:
- Finger covered with gloves.
- Sterile fecal spoon or fecal swap.
- Rectal enema with worm water.

Collected fecal sample should be examined directly or refrigerated.

Preservation include addition of 10% formalin

**Indication of fecal sample:**
- Examination of internal parasite infestation.
- Evaluation of digestive system.
- Bacteriological exam. (isolation of Salmonella or smear for acid fast bacilli).
- Chemical and toxicological examination.
Skin scraping for diagnosis of mange:

- Scraping of the periphery of the lesion till oozes of blood using dry or moist scalpel (mineral oil or glycerin).
- Skin scraping collected in screw capped bottle, test tubes or Petri dishes.
- 10% NaOH added for maceration of tissues and clearance of parasite.

Hair sample for diagnosis of Ring worm:

- Pull a tuft of hair but not cut to obtain the root of the hair.
- Wrap in a paper or put in envelop or in clean test tube.
Other types of samples:

- Ruminal juice
- Saliva and sputum and throat swap
- Pus and exudates
- Semen
- Preputial wash
- Vaginal discharge
III- Proper labeling of the sample

1- **Data of the animal**
   - Type, Sex, Age

2- **Data of the owner**
   - Name, Address, Date

3- **Data of the physician**
   - Name, location

4- **Data of the samples**
   - Type of sample.
   - Preservatives or other chemicals used with the sample

5- **Tentative clinical diagnosis and main clinical signs**

6- **Desired examinations**
IV- Proper preservation of samples

Proper preservation is to keep samples till time of analysis in a condition similar to that when obtained first time.

Type of preservatives:
- Physical preservatives
- Chemical preservatives
A- Physical preservatives:

1- Refrigeration:
By keeping the specimen in the refrigerator at 4°C.
Suitable for short time preservation (few hours)

2- Natural or dry ice:
- Natural ice:
  * can preserve specimens from 12 to 24 hours.
  * Specimen placed in water tight container and surrounded by ice.
- Dry ice: (Solid CO₂)
  * Dry ice can provide longer preservation.
  * Specimen placed in plastic bag or water tight container and dry ice wrapped in a paper and placed in the box.
  * Avoid direct contact with the specimen.
  * Dry ice is not to be used in air proof container to avoid explosion from the volatile gases and pressure.
3- *Freezing*:

- Freezing provide the longest preservation time.
- Suitable for preservation of specimens to be used for bacteriological examination.
- Used for preservation of serum and plasma samples for long time (deep freezing).
- Not to be used for parasitic examination of feces or hematological examination of whole blood.
1- **Fixing solutions**:

- 10% aqueous solution of formalin or 95% ethyl alcohol.
- 10 times volume of fixative should be added to the specimen.
- Penetrates the tissues and results in Harding and preservation for long time.
- Suitable for histological examination of tissues.
- Fixing solution for viral examination composed of normal saline (0.85% NaCl) containing 1% gelatin.

2- **Bactericidal solution**:

- Chemicals used when keeping bacterial growth to the minimum is desired.
- Not to be used with specimens for bacteriological examination.
- This include:
  - * formalin 10%: for fecal and urine samples.
  - * Phenol 0.5%: for serum sample.
3- Anticoagulants

- Chemical substances added to blood samples to keep blood in liquid form.
- The best anticoagulant is the one which prevent coagulation with least cellular damage.
- The choice of anticoagulant depends on the type of examination to be carried out.

*The most important anticoagulants are:*

- Ethylene Diamine Tetra-acetic acid (EDTA).
- Heparin.
- Ammonium and potassium oxalate mixture.
- Sodium citrate.
- Sodium fluoride and potassium oxalate mixture.
<table>
<thead>
<tr>
<th>Anticoagulant</th>
<th>Mode of action</th>
<th>Advantages</th>
<th>Disadvantages</th>
<th>Amount</th>
</tr>
</thead>
</table>
| EDTA Disodium Dipotassium | Precipitation of calcium ions in blood | - Excellent preserving power.  
- Recommended for routine blood examination.  
- Doesn’t alter the erythrocyte size and allow excellent leucocytes staining.  
- Used in determination of creatinine, urea nitrogen, glucose, phosphorus and uric acid. | - Higher concentration of salt withdraws water from red cells and reduces PCV values.  
- Not suitable for determination of alkaline phosphatase (ALP) activity as it combine with Mg ions needed for activation of ALP.  
- Chloride estimation in EDTA blood always higher than that with other anticoagulants. | 1 mg / 1 ml blood.  
- For 5 ml blood, add 0.5 ml of 1% solution or 1 drop of 10% solution to each tube and allow the water to evaporate off. |
| Heparin | Interferes with conversion of prothrombin to thrombin | - Does not alter the erythrocyte volume (suitable for PCV).  
- Least effect on erythrocyte haemolysis.  
- Recommended for blood of cats. | - More expensive.  
- Unsuitable for smears for its poor stain affinity.  
- Its action stopped after 8 hr.  
- Cause the cells to stain bluish with Wrights stain. | - For 5 ml blood, add 0.1 ml of 0.75% solution and evaporate to dryness at room temperature. |
| Ammonium and Potassium oxalate mixture (Heller & Paul Mixture) | Binding of ionized calcium | - It is cheaper than EDTA.  
- Easy to prepare and use.  
- Little cellular distortion occurs if the sample is examined within the 1st hour.  
- Cause very little haemolysis or changes in the volume of RBCs. | - It doesn’t prevent clumping of platelets.  
- It is poisonous.  
- It can’t be used in estimation of the potassium, ALP or urea. | Amm. oxalate 1.2 gm.  
Pot. oxalate 0.8 gm.  
D.W. 100 ml.  
- 1 ml of the solution in a tube, then dry at 60°C. This is sufficient for 10 ml blood or, 2 mg / ml blood. |
| Sodium Citrate | Binding of ionized calcium | - It is employed in blood transfusion as citrate is metabolized and excreted rapidly. | - Interfere with many biochemical tests.  
- Prevent the clotting for few hrs.  
- Increased conc. cause shrinkage of cells. | - For blood transfusion:  
Tri-sodium citrate 1.32 gm.  
Citric acid 0.48 gm.  
Dextrose 1.4 gm.  
D. W. 100 ml. |
| Sodium Fluoride & Potassium oxalate mixture | Binding of ionized calcium | - For determination of glucose level, since it is effective in inhibiting the glycolytic enzyme which breakdown glucose in blood. | - It is poisonous.  
- Unsuitable for determination of ALP or urea determination. | 4 parts Sodium Fluoride  
5 parts Potassium Oxalate.  
- For 5 ml blood, add 0.5 ml of 2.25% solution of the mixture and evaporate off the water. |
V- Avoidance of sample spoilage

CAUSES OF SPECIMEN SPOILAGE

- Autolysis.
- Hemolysis.
- Fragmentation.
- Drying (Desiccation).
- Decomposition.
1- **Autolysis**
- It is the digestion of the sample by its own enzymes.
- It happens most often in samples of the digestive tract.
- It may occur in samples packed in borax or some other dry antiseptic powder.

**Autolysis is helped by:**
- High temperature and is directly related to worm climate.
- Time between collection in the field and receipt at the laboratory.

2- **Hemolysis**
- It is the breakdown of the cellular elements in the blood samples.

**Causes:**
- Using wet needle or syringe.
- Collection of the blood sample directly to the bottom of the tube.
- Vigorous mixing of the blood sample.
- Excessive negative pressure when collecting sample with a syringe.
- Failure to remove the needle from the syringe.
- Bacterial contamination.
- Chemical contamination.
- Extreme heat or cold.
3- **Fragmentation**
- It is breaking the sample into small pieces.
- *This results from*:
  - Forcing a specimen into a small bottle.
  - Cutting the specimen with dull knife or with scissors.

4- **Drying**
- Drying occurs in certain types of samples such as blood, serum, exudates or pus.
- *This results from*:
  - Too small sample.
  - Too large container.

5- **Decomposition**
- Slight over growth by bacteria or mold can make material unfit for examination.
- Bacterial or fungal enzymes frequently digest tissues and destroy both structural and cellular organization.
- *This results from*:
  - Contamination with soil, faeces or intestinal contents.
  - Long time in shipment.
  - High temperature.
  - Bacterial contamination.
Thank you
1. **Ethylene Diamine Tetra-acetic acid** (Disodium or dipotassium salt of EDTA)

*Mode of action*: Precipitation of calcium ions.

*Advantages:*
- Excellent preserving power, recommended for routine blood examination.
- Doesn’t alter the erythrocyte size and allow excellent leucocytes staining.
- Used in determination of creatinine, urea nitrogen, glucose, phosphorus and uric acid.

*Disadvantages:*
- Higher concentration of salt withdraws water from red cells and reduces PCV values.
- Not suitable for determination of alkaline phosphatase (ALP) activity as it combine with Mg ions needed for activation of ALP.
- Chloride estimation in EDTA blood always higher than that with other anticoagulants.

*Amount required:*
1mg / 1 ml blood. (for 5 ml blood, add 0.5 ml of 1 % solution or 1 drop of 10% solution to each tube and allow the water to evaporate off).
2. **HEPARIN**

- It is a natural anticoagulant, found abundantly in the liver from which its name is derived.

**Mode of action:**
Prevents blood coagulation by interfering with conversion of prothrombin into thrombin.

**Advantages:**
- Does not alter the erythrocyte volume (suitable for PCV).
- Least effect on erythrocyte haemolysis. - Recommended for blood of cats.

**Disadvantages:**
- More expensive. - Unsuitable for smears for its poor stain affinity.
- Its action stopped after 8 hr. - Cause the cells to stain bluish with wrights stain.

**Amount required:**
- For 5 ml blood, add 0.1 ml of 0.75 % solution and evaporate to dryness at room temperature.

(Can be used in liquid form as Heparine injection to coat the inside of the syringe).
3. AMMONIUM AND POTASSIUM OXALATE MIXTURE

*(HELLER AND PAUL MIXTURE)*

**Mode of action:** Binding ionized calcium.

**Advantages:**
- It is cheaper than EDTA.
- Easy to prepare and use.
- Little cellular distortion occurs if the sample is examined within the 1st hour of collection.
- Cause very little hemolysis or changes in the volume of RBCs.

**Disadvantages:**
- It doesn’t prevent clumping of platelets.
- It is poisonous.
- It can’t be used in estimation of the potassium, ALP or urea.

**Amount required:**
- Ammonium oxalate  1.2 gm.
- Potassium oxalate  0.8 gm.
- D.W.                          100 ml.

1ml of the solution in a tube, then dry at 60 °C. This is sufficient for 10 ml blood. or, 2 mg / ml blood.
4. SODIUM CITRATE

**Mode of action:** Binding ionized calcium.

**Advantages:**
- It is employed in blood transfusion as citrate is metabolized and excreted rapidly.

**Disadvantages:**
- Interfere with many biochemical tests.
- Prevent the clotting for only few hr.
- Increased concentration may cause shrinkage of cells.

**Amount required:**
For blood transfusion: Tri-sodium citrate 1.32 gm.
Citric acid 0.48 gm.
Dextrose 1.4 gm.
D. W. 100 ml.

- The strength of the stock solution is 3.8 %.
  (9 volumes of blood + 1 volume of sodium citrate solution and mixed immediately)
- Sodium citrate is also widely used in estimation of the ESR.
  (4 volumes of venous blood + 1 volume of sodium citrate solution)
5. SODIUM FLUORIDE AND POTASSIUM OXALATE MIXTURE

*Mode of action:* Binding ionized calcium.

*Advantages:*
- Most suitable for determination of glucose level, since it is effective in inhibiting the glycolytic enzyme which breakdown glucose in blood.

*Disadvantages:*
- It is poisonous.
- Unsuitable for determination the level of ALP or urea determination.

*Amount required:*
- It is better to use the mixture than sodium fluoride alone as it increase the anticoagulant effect.
- The mixture consists of: 4 parts Sodium Fluoride 5 parts Potassium Oxalate.
- For 5 ml. blood, add 0.5 ml of 2.25 % solution of the mixture and evaporate off the water.