COLLECTION AND PRESERVATION OF LABORATORY SAMPLES

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Objectives of sample collections:

1. For helping to establish a disease diagnosis.
2. For health surveillance and certification.
3. For evaluating the response to treatment.
4. For evaluating the health status of diseased cases before surgery.
5. For research and epidemiological studies.

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General Precautions for sample collections

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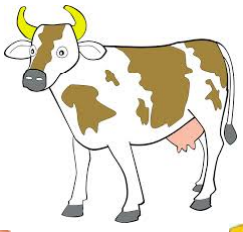
Sample collections

Animal



Container

Samples

Clinician

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Animal

1. Select an animal that correctly represents the diseased condition.
2. An animal in advanced stage of the disease is most desirable.
3. In herd problem, collect specimen from more than one diseased animal.
4. Collect samples from one or two recently died animals.

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Container

1. The container must be suitable for the collected sample.
2. The container must be clean and dry.
3. The container must be sterile in case of samples for bacteriological examination.
4. The container must keep the moisture content of the samples.

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Samples

1. The sample must be as fresh as possible and obtained and preserved in the correct manner.
2. The sample must be representative.
3. Insure that collected samples are characteristic of the disease as seen in the field.
4. Avoid as much as possible contamination of the specimen with intestinal content, hair or dirties.
5. Sufficient quantity of material must be provided to permit thorough examination.
6. sample must be examined directly after collection.
7. Each sample must be labeled and easily identifiable.

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Clinician

10. The clinician must protect himself (herself) from infection by wearing gloves, masks and glasses.
11. The clinician should include the following information with the sample:
    - Owner's name and address.
    - Description of animal species, age and sex.
    - Duration of the condition, mortality rate, number of animals affected and clinical signs observed.
    - Clinical diagnosis and tentative diagnosis submitted.
    - The clinician must request clearly the exact estimation he requires done and give his name, address and telephone number.
    - Types of samples

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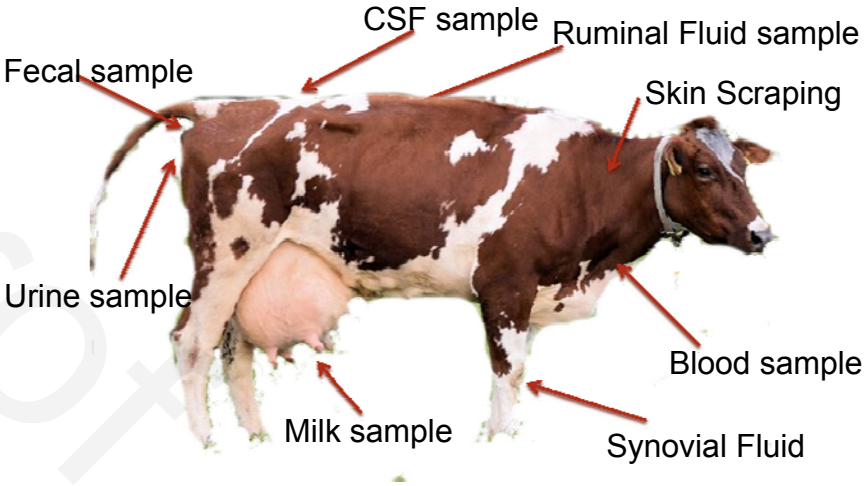
Types of samples



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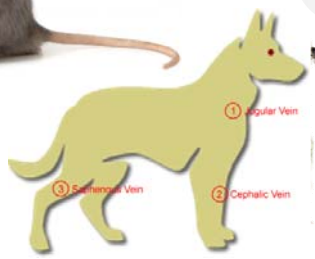
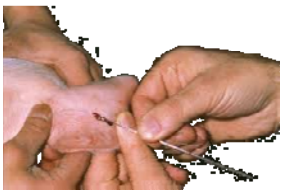
Blood sample

Site of collection

Tail or Ear vein medial canthus of Eye
Rat and Mice





Ear vein in pigs and Rabbits

Saphenous, Cephalic or Jugular vein
Dogs and Cats

Jugular vein
Ruminants and Equines

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Types of blood sample

Whole blood	Sample →	Blood + Anticoagulant
Serum	Sample →	Blood without Anticoagulant
Plasma	Sample →	Whole blood
Blood smear	Sample →	Whole blood or drop of blood
Blood swab	Sample →	Swab

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Anticoagulants

1. Ethylene Diamine Tetra-acetic acid (EDTA).
2. Heparin.
3. Ammonium and potassium oxalate mixture.
4. Sodium citrate.
5. Sodium fluoride and potassium oxalate mixture.

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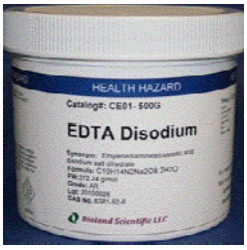
Ethylene Diamine Tetra-acetic acid (EDTA)

Dose: 1mg/ml blood



Mode: Binding ionized calcium

Advantages:

- ✓ Hematological analysis.
- ✓ No effect on leukocyte staining affinity.
- ✓ Preserve the blood sample for 24 hours.

Disadvantage

- ❖ Higher concentration of salt withdraws water from red cells and reduces PCV values.

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Heparin

Dose: 0.1 ml of 0.75 % solution/ 5ml blood

Mode: Inhibit thrombin

Advantages:

- ✓ Suitable for Haematocrit determination.
- ✓ For measuring acid base balance.

Disadvantage

- ❖ Preserve the blood sample for 8 hours.
- ❖ More expensive.

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AMMONIUM AND POTASSIUM OXALATE MIXTURE

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.

Mode: Binding ionized calcium

Advantages

•It is cheaper than EDTA.

Disadvantages

•It doesn't prevent clumping of platelets.
•It is poisonous

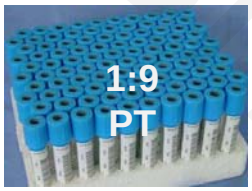
Sodium citrate

Dose: Sodium citrate 3.8% (1:4 or 1:9)

Mode of action: Binding ionized calcium.

Advantages

✓ Blood transfusion.
✓ ESR (1:4).
✓ Prothrombin time (1:9)



Advantages

• Prevent the clotting for only few hours.
• Not suitable for hematological analysis.

Sodium fluoride and potassium oxalate mixture

Amount: 4 parts sodium fluoride to 5 parts of potassium oxalate. 0.5 ml of 2.25 % /5 ml blood.

Mode of action: Binding ionized calcium.

Advantages

✓ Blood glucose level --- Inhibit the glycolytic enzymes

Disadvantages

✓ Poisonous.

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Preservation of samples

To keep samples until time of examination in a state similar to that when you obtained it.

Types of preservatives

I. Physical preservatives

1. Refrigeration:

Refrigeration of the samples is recommended when laboratory examination will be performed within hours by using:

a) Refrigerator **b) Natural ice**

2. Freezing

A) Dry ice (solid Co_2).

B) Deep Freeze.

II. Chemical preservatives

1. Fixing solutions.
2. Anticoagulants.
3. Bactericidal solutions.

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Physical preservatives

1. Refrigeration

Ice will preserve samples for 18 – 24 hours during the winter months and only for 8 – 12 hours during the summer.

2. Freezing

Suitable for samples used for isolation of bacteria or virus and for samples used for chemical and toxicological studies.

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II. Chemical preservatives

1. **Fixing solutions** ⟹ Formalin 10%.

2. **Anticoagulants.**

3. **Bactericidal solutions.**
   a) Formalin 10 %.
   b) Glycerin 50–100% for viral isolation.

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Causes of specimen spoilage

1.Autolysis.

2.Haemolysis.

3.Fragmentation.

4.Drying (Desiccation).

5.Decomposition.

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Autolysis

It means digestion of the sample by its own enzymes.

Causes:

- High temperature and is directly related to worm climate.
- Time between collection in the field and receipt at the laboratory.
- Using small amount of liquid preservative.

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Haemolysis

It means the breakdown of the RBCs.

- 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 will rupture cells and collapse the vein.
- Failure to remove the needle from the syringe, when transferring blood from a syringe to a container.
- Extreme heat or cold.

Fragmentation

It means breaking the sample into small pieces mostly encountered with tissue samples

Causes:

- Forcing a specimen into a small bottle.
- Cutting the specimen with dull knife or with scissors.

Drying (Desiccation)

Drying occurs in certain types of samples such as blood, serum, exudates or pus.

The principle causes are:

- Too small sample.
- Too large container.
- Storage of samples in an opened container.

Decomposition

Decomposition arise from lack of cleanliness on the part of collection and include:

- Contamination with soil, faeces or intestinal contents.
- Long time in shipment.
- High temperature.
- Bacterial contamination.