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Collection and Preservation of Laboratory Samples

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2 **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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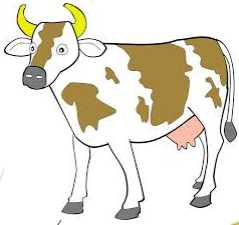
General Precautions for sample collections

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General Precautions for sample collections


Animal




Container



Samples



Clinician



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General Precautions for sample collections

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Animal

1. Animal should be controlled using the suitable methods.
2. Animal should take sufficient rest after transportation and before sampling.
3. Avoid as much as possible exposure of the animal to external stress before sampling.
4. Hygienic removal of animal excreta that may carry infection.

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General Precautions for sample collections

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Animal**5. In herd problems:**

- A. Select animals that correctly represents the diseased condition.
- B. An animal in advanced stage of the disease is most desirable.
- C. Collect specimen from more than one diseased animal.
- D. Collect samples from one or two recently died animals.

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General Precautions for sample collections

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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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General Precautions for sample collections

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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. Insure that collected samples are characteristic for the disease as seen in the field.

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General Precautions for sample collections

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Samples

4. Avoid contamination of the specimen with intestinal content, hair or dirties.
5. Sufficient quantity of material must be provided to permit thorough examination.
6. Samples should be examined directly after collection or preserved.
7. Each sample must be labeled and easily identifiable.

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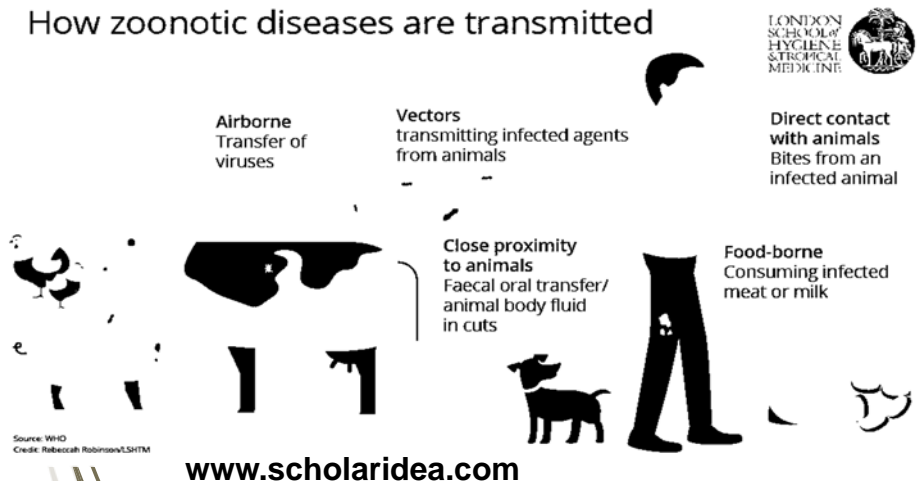
General Precautions for sample collections

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Clinician

10. The clinician must protect himself (herself) from infection by wearing gloves, boot, mask and glasses.

How zoonotic diseases are transmitted



General Precautions for sample collections

11 Clinician

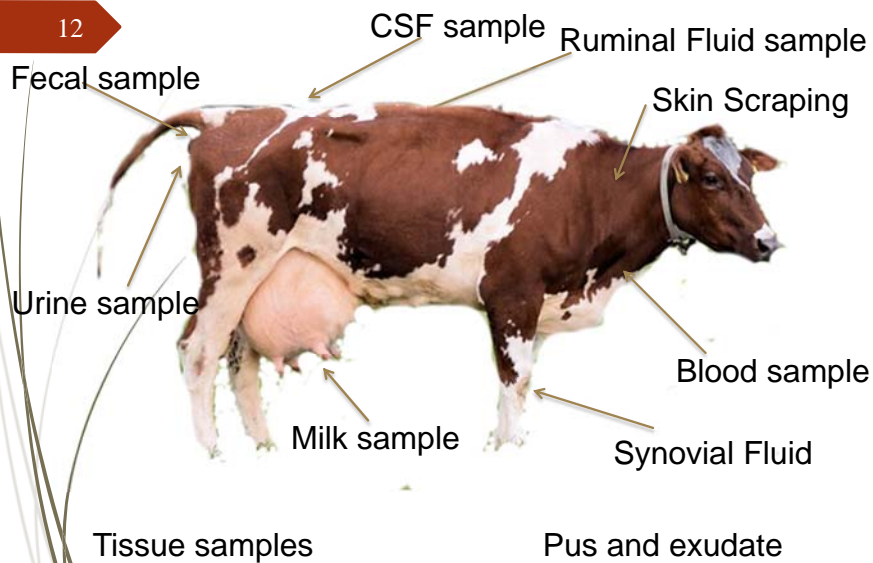
10. The clinician should include the following information with the sample:

- 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.

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Types of samples

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Blood samples

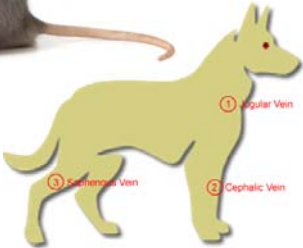
Site of collection

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Tail or Ear vein or 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 samples

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Whole blood

Serum

Plasma

Blood smear

Blood swab

Sample → **Blood + Anticoagulant**

Sample → **Blood without Anticoagulant**

Sample → **Whole blood**

Sample → **Whole blood or drop of blood**

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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Anticoagulants

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Ethylene Diamine Tetra-acetic acid (EDTA)

Dose: 1mg/ml blood

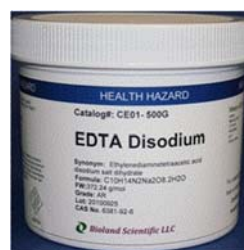
Mode: Binding ionized calcium

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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Anticoagulants

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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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Anticoagulants

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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

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Anticoagulants

Sodium citrate

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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)
- ✓ Bacteriological culture.

Advantages

- Not suitable for hematological analysis.





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Anticoagulants

Sodium fluoride and potassium oxalate mixture

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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

II. Chemical preservatives

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Preservation of samples

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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

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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, toxicological and molecular biology studies.



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II. Chemical preservatives

1. Fixing solutions \longrightarrow Formalin 10%.

2. Anticoagulants.

3. Bactericidal solutions.

a) Formalin 10 %.

4. Glycerin 50% for viral isolation.

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General precautions for preservation of samples

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1. Examine samples as much as you can directly after collection
2. Don't freeze samples for parasitological analysis.
3. Don't freeze whole blood samples for hematological analysis.
4. Keep samples that will be examined directly after collection under refrigeration.

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General precautions for preservation of samples

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5. Freeze samples for bacteriological analysis.
6. Don't add preservatives for samples designated for bacteriological analysis.
7. Don't freeze samples that will be examined physically or microscopically (Urine, CSF, peritoneal fluid, ruminal juice, synovial fluid...etc)
8. Refers to special precautions for different samples before collection.

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Causes of specimen spoilage

1. Autolysis.
2. Haemolysis.
3. Clotting of blood samples.
4. Fragmentation.
5. Drying (Desiccation).
6. Decomposition.

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Causes of specimen spoilage

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Autolysis

It means digestion of the sample by its own enzymes.

Causes:

- High temperature and is directly related to warm climate.
- Long time between collection in the field and receipt at the laboratory.
- Using small amount of 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.

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Clotting of blood samples

It means formation of clots in the whole blood sample.

- Delay in mixing the blood sample with the anticoagulant.
- The amount of the collected blood sample is larger than the concentration of the anticoagulant.
- Taking long time in collection of the blood sample.

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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.

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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.

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Decomposition

Decomposition arise from lack of cleanliness on the part of collection

Causes:

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

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