Collection of different samples

Each veterinary diagnostic laboratory offers a unique set of diagnostic tests that is subject to frequent changes as better tests become available. The protocols for sample collection and submission are therefore also subject to change. Most diagnostic laboratories publish user guidelines with preferred protocols for sample collection and submission, but the following broad recommendations are fairly standard .Regardless of the type of submission, a detailed case history should be included with the samples to assist laboratory personnel in determining a diagnosis.

A detailed case history should include:

- owner's name
- species
- breed
- sex
- age
- animal identification
- clinical signs
- gross appearance (including size and location) of the lesion(s)
- previous treatment (if any)
- time of recurrence from any previous treatment
- morbidity/mortality in the group

If a zoonotic disease is suspected, this should also be clearly indicated on the submission form to alert laboratory personnel. The submission form should be placed in a waterproof bag to protect it from any fluids that might be present in the packaged materials.

The starting point for the laboratory investigation of an animal disease is the taking of samples .Samples should be taken with care, to avoid undue stress or injury to the animal or danger to the operator. Where appropriate, samples should be collected

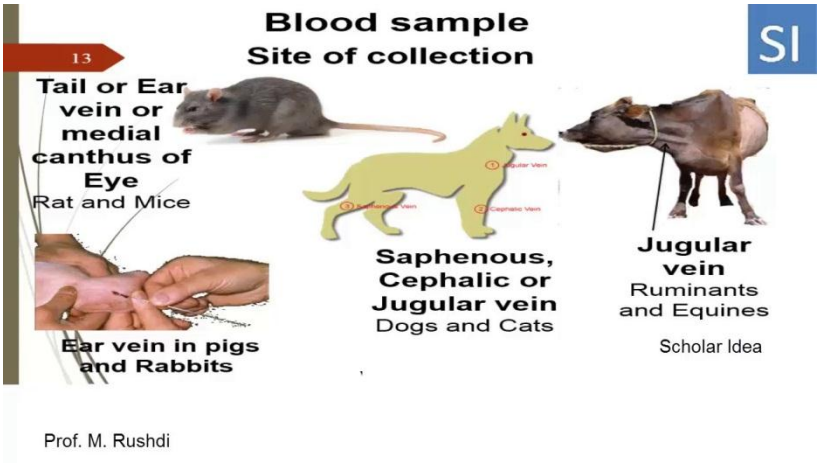
aseptically, and care should be taken to avoid cross-contamination between samples. The samples should be carefully packaged, labelled, and transmitted to the laboratory by the fastest practicable method, with the appropriate temperature control.

Sample collection from live animals

a) Blood

Blood samples may be taken for **haematology o**r for **culture** and/or **direct examination** for bacteria, viruses, or protozoa, in which case it is usual to use anticoagulants, such as ethylene diamine tetra-acetic acid (EDTA) or heparin. They may also be taken for **serology**, which requires a clotted sample. Blood plasma is also used for some procedures. **Steps of blood samples collection as below**:

- A blood sample is taken, as cleanly as possible, by venipuncture. Blood may be taken by syringe and needle or by needle and vacuum tube (not easy in delicate veins but convenient in strong veins).
- Small quantities of blood are conveniently obtained by pricking with a triangular, solid-pointed needle.
- Ideally the skin at the site of venipuncture should first be shaved (plucked) and swabbed with 70% alcohol and allowed to dry.
- For samples that are collected with anticoagulant, thorough mixing, using gentle agitation only, is necessary as soon as the sample has been taken.
- It may also be necessary to make a smear of fresh blood on a microscope slide; both thick and thin smears may be prepared.
- For polymerase chain reactions, EDTA is the preferred anticoagulant.
- For serum samples, the blood should be left to stand at ambient temperature (but protected from excessive heat or cold) for 1–2 hours until the clot begins to contract. The clot can then be ringed round with a sterile rod and the bottles placed in a refrigerator at 4°C. After several hours, or overnight, the sample can be centrifuged at about 1000 ***g*** for 10–15 minutes and the serum can be decanted or removed with a pipette.



Site: The following lists of veins are the most appropriate blood collection sites from different species of veterinary importance

Jagular vein- the most commonly used site in the horse, cattle, sheep, goat, camel and large wild mammals: used occasionally in small animals. Vacutainer tube, vacutainer needle, syringe, needle, needle holder, and disinfectant should be used for blood sample.

Procedure

• Placement of the thumb of the left hand in the jagular furrow to occlude and anchor the jagular vein, while manipulating the syringe
and needle with the right hand

• Clipping the site of sampling especially in long haired animals is recommended

• The veins are more clearly out lined when the site is rubbed with alcohol

Cephalic vein - The most commonly used site for collection of small amount of blood in the dog. By constricting the area on the dorsal aspects of the fore limb at the level of the elbow which can be raised beginning just above the carpal vein.

Ear vein- can be used in small dog, pig, cat and small lab animals (small dog, rabbit, guinea pig, and monkey).

• A marginal vein on the dorsal side of the ear is selected

• Remove the hair by shaving, clipping, or other method

• Swab the skin with alcohol or other

• Place the left index finger under the ear at the point of applying stylet syringe

• Gentle aspiration is used when using a syringe in small animals like rabbit, to avoid collapse of the vein

Toe or Toe nail – can be used in small dog, puppy, guinea pig etc

• Clip the hair away

• Disinfect the capillary bed of the nail

• Cut in to just short of the base of the nail

• Take 20-40 drops as well as 80 drops (4ml) of blood

Tail – can be used in pig, cattle, sheep, rat, and mouse

• Veinipuncture –of the coccygeal vein on the ventral side

• Amputation – commonest method used in the rat and mouse. A small transverse incision with a razor blade produce drops of blood

Heart – may be used in animals like bird, fish, and others

Femoral or Tibial vessels – used in dog, cat, small mammals, rat etc

Mammary vein – used for dairy cattle. The vein appears at the anterior border of the mammary gland lateral from the linea Alba and runs forward passing through a foramen in the abdominal wall posterior to ribs.

Anterior vena cava – used for the pig

A needle (4½ – 6 in. and 17 to 20 gauge needle) is inserted just anterior and slightly lateral to the cariniform cartilage and a line from the cartilage to the base of the ear.

Retro orbital Venus plexus – used for the rat, mouse, guinea pig

• A best method for obtaining a large quantity of blood (1ml) in mouse

• This technique is reported to be less traumatic than others

Wing vein or comb – used for birds

• After the feathers in the axillary region are plucked, the alar vein is seen running from beneath the pectoral muscle then along the ventral surface of the humerus In most animals up to 0.5 ml/kg blood collection has no any adverse effect. The total volume of blood collection differs in different animals.

Vacutainer tubes

a. Red-stopper tubes – are for tests requiring clotted blood

b. Lavender stopper tubes – contain EDTA in concentrated liquid or desiccated powder form

c. Green stopper tubes – contain heparin and are used for blood gases, PH, (CO2, O2)….

d. Gray stopper tubes – contain oxalates, fluorides, or citrates

e. Yellow stopper tubes – available with Acid Citrate Dextrose (ACD) solution or physiological saline solution.

Anticoagulants

The most important anticoagulants that can be used during blood collection with different mode of action are listed below.

Anticoagulant / product	Mode of action	Amount required	Advantage	Disadvantage
EDTA/Ethylene diamine tetra acetic acid	Form insoluble Ca salts	10-20mg (1ml of 1 % solution dried at room temp. or at incubator	Recommended for routine hematological procedures, preserve cellular elements better	May shrinks cell because Na salt is less soluble
Heparin	Antithrombin and antithromboplastin	1-2mg (0.2ml of 1% solution)	Less effect on RBC hemolysis Used for blood gas analysis	May cause clumping of WBC, unsuitable for smears, as it interferes with stain ability of WBC expensive
Na citrate	Combine with Ca to form an insoluble Ca salt	10-20mg (1ml)	Can be used for blood transfusion	Interferes with many chemical tests, shrink cells
Potassium oxalate	Units with Ca to form insoluble calcium oxalate	20mg (2 drops of 20% solution dried in incubator	Very soluble	Causes shrinkage, it increase the volume of blood
Sodium oxalate	Units with Ca to form insoluble calcium oxalate	20mg	Used mainly for prothrombin time	Same as potassium oxalate

b) Faeces

At least 10 g of freshly voided faeces should be selected. Faeces for parasitology should fill the container and be sent to arrive at the laboratory within 24 hours. If transport times are likely to be longer than 24 hours, the sample should be sent on ice or refrigerated to prevent the hatching of parasite eggs. Screw top containers or sterile plastic bags should be used for shipment; avoid tubes with rubber stoppers as gas generated can result in blowing the stopper off the tube, ruining the integrity of the sample and contaminating other samples in the package. An alternative and sometimes preferable method is to take swabs from the rectum (or cloaca), taking care to swab the mucosal surface. The swabs should be visibly coated with faecal material; however, samples collected with a swab are inadequate for parasitology. Care should be taken when collecting swabs from small, delicate animals or birds to avoid injury to the animal; small swabs are commercially available that should be used. Swabs should be transported in appropriate transport medium. Faeces are best stored and transported at 4°C.

c) Skin

In diseases producing vesicular lesions, collect, if possible, 2 g of affected epithelial tissue as aseptically as possible and place it in 5 ml phosphate buffered glycerine or Tris-buffered tryptose broth virus transport medium at pH 7.6. Additionally, the

vesicular fluid should be sampled where unruptured vesicles are present; if possible, vesicular fluid should be aspirated with a syringe and placed in a separate sterile tube.

Plucked hair or wool samples are useful for surface-feeding mites, lice and fungal infections. Deep skin scrapings, using the edge of a scalpel blade, are useful for burrowing mites and, in birds, feather tips can be taken for detection of viral antigen where Marek's disease is suspected.

d) Genital tract and semen

Samples may be taken by vaginal or preputial washing, or by the use of suitable swabs. The cervix or urethra may be sampled by swabbing. Samples of semen are best obtained using an artificial vagina or by extrusion of the penis and artificial stimulation. The sperm-rich fraction should be present in the sample and contamination by antiseptic washing solutions should be avoided. Specific transport media and conditions are often required.

e) Eye

A sample from the conjunctiva can be taken by holding the palpebra apart and gently swabbing the surface. The swab is then put into transport medium. Scrapings may also be taken on to a microscope slide. The handles of metal-handled swabs are useful for this, to ensure that sufficient cells are removed for '"microscopic examination. Mucopurulent nasal and lacrimal discharges are rarely useful.

f) Nasal discharge (saliva, tears)

Samples may be taken with Dacron, cotton or gauze swabs, preferably on wire handles as wood is inflexible and may snap. It may be helpful if the swab is first moistened with transport medium. The swab should be allowed to remain in contact with the secretions for up to 1 minute, then placed in transport medium and sent to the laboratory without delay at 4°C. Long protected nasopharyngeal swabs should be used to collect samples for some suspected viral infections.

g) Milk

Milk samples should be taken after cleansing and drying the tip of the teat, the use of antiseptics should be avoided. The initial stream of milk should be discarded and a

tube filled with the next stream(s), a sample of bulk tank milk can be used for some tests. Milk for serological tests should not have been frozen, heated or subjected to violent shaking. If there is going to be a delay in submitting them to the laboratory, preservatives can be added to milk samples that are being collected for serological testing. If necessary, milk for bacterial examination can be frozen.

Transmission of collected samples

1. A fundamental approach is to devise a 3-layer barrier to protect the sample. The sample is placed in an appropriate primary container (sealed jar/bag/tube). This is then enclosed in a secondary container, which includes some adsorbent material.
2. Note that items such as syringes, obstetrical gloves, and containers without sealable orifices are not suitable for shipment.
3. Liquid samples should not ship in plastic bags; a sealable jar should be used.
4. Waterproof markers should be used when labeling specimen bags and containers: the contents and patient identification are critical information.
5. The secondary container is then placed in the shipping box (tertiary container), which often houses coolant packages as well as various cushioning materials (eg, polystyrene foam) to protect the sample. The coolant materials should be sealed in plastic bags to prevent condensation damage. Coolant packs should not be placed directly onto samples, such as tubes of whole blood, that could suffer adverse effects if frozen in transit. Be sure to include the suitably protected submission form. The tertiary container is ideally a sturdy polystyrene refrigerator box or a cardboard box lined with a fitted polystyrene lining. If dry ice is used, this should be noted on the cardboard box label, and the lid should *not* be sealed with tape. Otherwise, CO_2 released from the dry ice could increase pressure and damage the package or contents.

2. Sample collection at post-mortem

Samples of tissue from a variety of organs can be taken at post-mortem. The equipment required will depend on the size and species of animal, but a knife, saw and cleaver will be required, and also scalpel, forceps and scissors, including scissors with a rounded tip on one blade, for opening intestines. A plentiful supply of containers and tubes of transport media appropriate to the nature of the sample required should be available, along with labels and report forms. Containers should be fully labelled with the date, tissue and animal identification. Special media may be required for transport of samples from the field. The operator should wear protective clothing: overalls, washable apron, rubber gloves and rubber boots. Additionally, if potential zoonotic diseases are being investigated, the post-mortem examination should be conducted in a biological safety cabinet; if this is not possible, an efficient face mask and eye protection should be worn. If rabies or transmissible spongiform encephalopathies (TSEs) are suspected, it is usual to detach the animal's head.

Tissues may be collected for microbiological culture, parasitology, biochemistry, histopathology and/or immunohistochemistry ,and for detection of proteins or genome nucleic acids. In addition buccal, oropharyngeal or rectal (cloacal) swabs may be collected. The person conducting the post-mortem examination should have sufficient knowledge of anatomy and pathology to select the most promising organs and lesions for sampling. Each piece of tissue should be placed in a fully labelled separate plastic bag or sterile screw-capped jar. Swabs should always be submitted in appropriate transport media. Sterile instruments should be used for collecting specimens for microbiological culture and care should be taken not to contaminate tissues with intestinal contents. Disinfectants should not be used on or near tissues to be sampled for bacterial culture or virus isolation .

The tissues may be sent to the laboratory dry or in bacterial or virus transport medium, depending on the type of specimen and the examinations required; swabs should be sent in transport medium. After collection, the samples for microbiological examination should be refrigerated until shipped. If shipment cannot be made within 48 hours, the samples should be frozen; however, prolonged storage at –20°C may be detrimental to virus isolation.

For histopathology, blocks of tissue not more than 0.5 cm thick and 1–2 cm long are cut and placed in neutral buffered 4–10% formalin, which should be at least ten times the volume of the tissue sample. For certain suspected diseases, larger portions of brain are required; the brain is sectioned using a sagittal cut, half is submitted fresh, on ice, and the other half is submitted in 10% buffered formalin. For scrapie, bovine spongiform encephalopathy and other TSEs. Store and pack formalin-fixed tissues separately from fresh tissues, blood and smears. Care should be taken to insure that formalin-fixed tissues are not frozen. Once fixed, tissues can be removed from formalin and, as long as they are kept moist and rotected (e.g. by wrapping in formalin-soaked paper towels, then sealed in screw-capped jars), they can be forwarded to the laboratory without formalin.