Chapter-3

COLLECTION, PRESERVATION AND DESPATCH OF MATERIAL FOR LABORATORY EXAMINATION*

The success of laboratory examination depends mainly on the proper collection, preservation and despatch of suitable materials. Whereas the field veterinarian can view the entire carcass and note the condition of all the organs, the laboratory technician will have to depend upon only the materials supplied to him. It is, therefore, very necessary for the field veterinarians to supply for the laboratory examination all such materials that are likely to be of value in the diagnosis. The covering letter should contain all particulars of the specimens, preservatives used, history of the case and the time of animal's death and that of necropsy. The field veterinarian should also enclose a copy of the post-mortem examination report and mention, when possible, the disease suspected and the specific tests required. For quick disposal of the material, it is advisable to forward one copy of the covering letter by post and to enclose another in the parcel containing the specimen. All possible measures should be taken for specimens to reach the laboratory in the shortest possible time after their collection.

The materials required for diagnosis and the methods to be adopted for their collection and preservation depend on several factors such as the kind of examination required, the disease under investigation, the apparatus available, the atmospheric conditions and the length of interval between collection and laboratory examination. However, the general requirements are given in the following pages.

1. SMEARS

The glass-slides used for making smears should be absolutely clean and free from grease. Standard firms supply slides for ready use. If unclean, they should be washed in water and. after they have been drained and dried, immersed in a 5% solution of hydrochloric acid in alcohol for a few

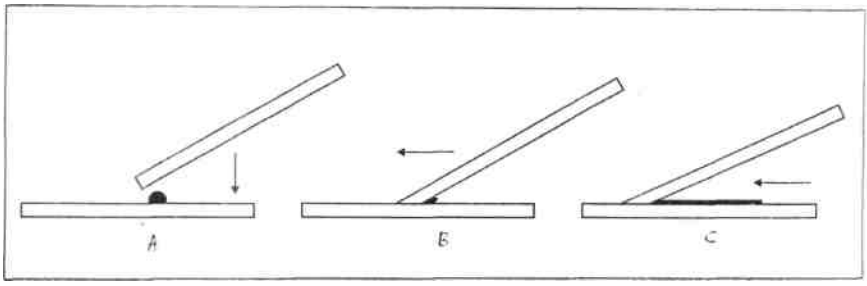
hours. The use of methylated spirit should be avoided for preparing this solution as it turns milky on contact with water. The slides should then be washed thoroughly in running water and kept in absolute alcohol or rectified spirit in a clean-stoppered bottle. When required for use, they may be removed with a pair of forceps and the alcohol burnt off by passing the

slide through a flame.

Blood

Blood films for microscopic examination should be thin. It is best to collect the blood from the tip of the ear in the case of a living animal or of an animal suspected to have died of anthrax. The procedure is as follows:

1. Clip the hair from the tip of the ear, if necessary.
2. Wipe away hair and dirt from the clipped area with a little dry cotton wool. Swab with methylated spirit and let it dry.
3. In a living animal, make a puncture at the tip of the ear with a pin that has been sterilised by flaming, while in a dead animal snip out a little piece of the skin at the tip and allow the blood to ooze from the wound.
4. Bring the surface of a slide in contact with the blood droplet or transfer the droplet to this slide by means of the edge of another slide. In either case, the blood should be deposited at a point on the slide about 1 cm from one end, while the quantity of blood removed from the wound should be just sufficient to spread within the middle half of the slide, leaving the ends blank.
5. Take another clean slide with a straight smooth edge at one of its ends for use as a 'spreader'. Place this edge just in front of the drop, holding the slide firmly, at an angle of about 45° on the lower slide, which may be placed on a table or some other flat surface. Now bring the lower end of the spreader slide in contact with the drop of blood, which will spread along the end of the slide by capillary attraction. Glide forward the upper slide evenly at a uniform pace so as to spread the droplet in a thin uniform film on the surface of the lower slide (See diagram). From animals suspected to have died of anthrax thick smears should be prepared, and it may be better to make two thin and two thick smears in each case for transmission to the laboratory. While making films, the slides should be protected from the direct sunrays, especially in summer months, to avoid rapid drying of the blood and the consequent formation of artefacts.



6. Dry the films in shade by waving them in the air.
7. In the case of films to be examined for protozoa or blood changes, immerse the slides in acetone-free methyl alcohol for about 10 minutes and then allow them to dry. Smears to be examined for bacteria should be fixed by repeatedly passing the slide, film upwards, through a flame until the glass is just uncomfortably hot when felt with the back of the hand.
8. Mark the slides with a grease pencil or fix labels on them to indicate the nature of the smears.
9. Place the slides back to back and wrap in a clean paper.
10. Enclose a brief description of the smears while packing for despatch.

Pus

Prepare a thin smear by spreading the pus evenly on a side, with a sterile scalpel. In case this method fails to give a satisfactory smear the following procedure may be adopted:

1. Place a small quantity of the pus on the middle of a slide.
2. Place a second slide on the first one so that the pus is held between the two slides.
3. Press gently the two slides together so as to spread the pus.
4. Hold the opposite ends of the two slides in each hand and draw them apart, taking care not to lift one from the other as this will cause the formation of lumps and bubbles. If a thin smear is not obtained in the first attempt, the slides may be placed together again and the process repeated. 5. Dry the films by waving in the air, fix over a flame, label, pack and despatch.

Secretions and excretions

1. These can be collected in clean vessels and sent under refrigeration to the laboratory for detailed examination. It is always better to have a

styrofoam (Thermocol) box filled with ice to carry the various biological materials for culture and isolation of organism and for sero-

diagnosis.

2. The discharge can also be preserved using 10% neutral formalin. 2 or 3 ml of this solution will be sufficient for 3-5 ml of discharge.
3. Smears can also be immediately prepared from the discharges in clean slides and fixed rapidly by heat. These should be wrapped properly and kept.
4. For oozing lesions and cavities containing pus or other materials (as often seen in TB of lungs) - a small drop may be collected in a clean slide and uniformly spread using a tooth pick or swab and fixed immediately in heat.
5. In suspected cases of TB it is better to have material scraped out from the lesions (after evacuating the contaminated pus) collected on a clean slide and fixed.

Tissue

Tissue smears should be prepared as follows:

1. Cut out a small piece of the organ, hold it in a pair of forceps and rub its cut surface on the middle third of the slide.
2. If fluid accumulates on the cut surface, transfer a very small quantity of the fluid to a slide with a sterile scalpel and then spread it.
3. If the cut surface is very dry, scrape it with a sterile scalpel and spread the scraping evenly on a slide. This may be done with the help of normal saline, if necessary.
4. Caseating nodular and calcified lesions may be treated in the manner described for pus.
5. Fix and despatch as directed for blood smears.

(Note: Please also refer to Appendix-I for further details).

2. BLOOD AND BLOOD SERUM

Blood Serum

1. Select the appropriate vein of the ear and clean the skin with anaesthetic ether or methylated spirit.
2. Insert a sharp, large-bore, hypodermic needle into the vein and collect the blood directly into a round, screw-capped, wide-mouthed bottle of about 30-ml capacity. The needle and the bottle should be perfectly clean, sterile and dry. If on account of recent sterilisation, the needle or the bottle shows some moisture on it, rinse it thoroughly with physiological salt solution (0.9 g of sodium chloride in 100 ml of boiled water) to prevent haemolysis. A separate needle should preferably be used for each animal. But if this is not possible, the needle should be thoroughly washed out with the saline each time it is used for bleeding.
3. Do not fill the bottle more than half; set it gently on its side at an angel of 20° to the horizontal in a cool place on the premises where the blood has been drawn. Care should be taken to avoid agitation of the blood after collection.
4. When complete clotting has taken place, twist the bottle gently on its long axis through 180°. It generally takes 2 to 6 hours for the serum to separate.
5. Pipette the serum with sterile precautions into screw-capped or well-stoppered strong vials with mouths wide enough to admit a 1 ml graduated pipette.
6. Label the serum containers, pack and despatch.

   If drawn properly, the serum is clear and free from blood cells or haemoglobin. A haemolysed serum sample is unsuitable for laboratory examination.

Collection with Pasteur Pipette

The collection with Pasteur pipette should be made as follows:

1. Smear with a hot spatula the surface of the organ from which material is to be collected.
2. Pass the capillary portion of the pipette several times through a flame
3. to sterilise its external surface and allow it to cool.
4. Break gently the capillary end of the pipette with forceps, the broader end of the pipette having been fitted with a rubber teat.

5. Insert the sterilised end of the pipette into the tissue at the smeared spot, with the rubber teat kept pressed between the forefinger and the thumb. Relax the pressure on the rubber teat and draw the material into the pipette.
6. Hold the pipette at a slant so as to allow the fluid in the capillary to recede from the end. Seal the end.
7. Remove the teat from the pipette and seal the teat-end after removing the cotton plug. Continue to hold this point in the flame until the glass melts and forms a thick bead.
8. Hold the pipette with the hot end uppermost until quite cool.
9. When cooled, invert the pipette, so as to allow the fluid to collect towards the sealed broader end. Break the capillary end and seal it.

A good source of heat is essential for sealing the pipette properly. A Bunsen flame is quite suitable for the purpose but is rarely available in the field. However, the sealing can also be affected on a spirit lamp, provided the flame is kept steady by cutting out drafts of wind.

3. MATERIAL FOR HISTOPATHOLOGICAL EXAMINATION

The material should be properly fixed when it is in a fresh condition before being despatched. The aim of fixation is to set or fix the tissues in, as normal a condition as possible and to prevent post-mortem changes in them. Once the putrefactive or autolytic changes have set in, the tissues become unfit for histopathological examination, for they cease to represent the structures they had at the time of death. The following procedure is recommended for fixing tissues:

Use a fixative that would penetrate and kill the tissue cells before these have altered by autolytic or other changes. The quantity of the fixing solution used should not be less than 10 times the volume of the material. Cut the tissue into thin sections, each around 0.5 cm in thickness so that the fixative may penetrate and kill the cells quickly. In case it becomes necessary to send a large mass of material for examination, it should be accompanied by some small slices of the organ or lesion cut out and fixed separately. The most commonly employed fixing reagent for general histological work is a 10% solution of formalin in normal saline, which not only fixes the tissues in 48 hours but also preserves them.

To avoid bulk in transit, the material, after proper fixation, may be transferred to a smaller bottle containing a sufficient quantity of the

preservative to cover the tissues. A layer of absorbent cotton wool should be placed over the material and also at the bottom of the container to prevent damage to the tissues by jolting during transit. The material selected for despatch should comprise representative portions of the lesions as well as portions of the apparently healthy tissues surrounding the diseased area. The material that is likely to yield the most useful information concerning the pathology of the condition under investigation is usually the youngest portion of the lesion at the junction of the diseased and healthy tissues. Sections taken only from the centre of a large lesion are apt to be misleading.

4. MATERIAL FOR CULTURAL EXAMINATION

The material must be collected with aseptic precautions and dispatched in sterile containers to prevent contamination from extraneous sources. In the case of dead animals, the specimens should be taken soon after death, for otherwise putrefactive bacteria invade the tissues and render them unsuitable for examination.

Solid tissues such as liver, spleen and kidney, may be forwarded, if fresh, without a preservative, on ice, when the examination is to be carried out within a short time of their collection. If however, the examination is to be delayed by a few days, it is preferable, especially in summer months, to preserve the tissues in a 25% glycerine saline. While forwarding the material in this preservative, it would be better also to send larger pieces of the tissues, so that, if necessary, cultures may be attempted from their central areas where the glycerine has penetrated the least.

Liquid material such as inflammatory exudates, heart blood and cerebrospinal fluid may be taken either in sterile swabs or sealed in pipettes. When large quantities of the material are required for laboratory examination, they should be removed from the body with sterile pipettes and collected in tubes or bottles, with strict aseptic precautions throughout the procedure. If peritoneal fluid is required, an area on the abdominal wall is seared thoroughly with a hot spatula and a sterile forceps is used for holding the cavity open and sterile pipettes for drawing the fluid. Similarly, for collecting heart blood, the surface of the organ should be well seared and a sterile pipette inserted into it for drawing the blood. ' For taking swabs of the contents of a closed abscess in an animal, clip the hair from the area and paint it with tincture of iodine. Open the abscess with a sterile scalpel and take swabs from the wall as well as from the contents of the abscess and keep the swabs in sterile tubes.

To collect wound discharge, the wound should be thoroughly cleaned with warm water and soap, and sterile non-antiseptic cotton wool or gauze dressing applied. The material should be collected after about 24 hours by inserting a sterile swab underneath the dressing. This is usually done in living animals.

5. VIRUS MATERIAL

Virus material for examination may be forwarded in 50% glycerol saline, or preferably in a medium containing equal parts of pure glycerol and M/ 25 buffered phosphate. As the viruses are generally short-lived, it is particularly important that the material should reach the laboratory in the shortest possible time after collection, preferably over ice in a thermos flask.

Preparation of 50% glycerol in phosphate-buffered saline (PBS) pH 7.5

1. Put the following chemicals in a flask

NaCl	8.00 g
KC1	0.20 g
KH_2PO_4	0.12g
Na_2HPO_4 (anhydrous)	0.91 g

2. Add distilled water to 1,000 ml
3. The PBS solution may be sterilised by autoclaving at 8-kg pressure for 35 minutes.
4. Add equal quantities of PBS and glycerol. The glycerol should be of analytical reagent grade and neutral. Prior to its being added to PBS, it should be autoclaved at 5-kg pressure for 10 minutes.

Scabs from pock diseases may be forwarded in a dry specimen tube without the addition of preservative.

6. PARASITIC MATERIAL

Specimens of Insects, Ticks and Free living Mites

Winged specimens should be killed with chloroform in a cyanide-killing bottle and pinned in entomological store-boxes or on the under side of the cork inside a specimen tube. Specimens of ticks, mites, eggs and larvae of insects may be sent in 70% alcohol. However, in case ticks and mites are engorged with blood, the abdomen may be punctured with a needle and

the parasite boiled in 10% sodium hydroxide to remove the blood and soft tissues.

Mange Mites

Scrapings from mangy skin should be forwarded in dry containers sealed properly to prevent mites from crawling out. The following procedure is recommended for collection of mange scrapings:

1. Select an area where the skin lesions are most marked. In cases of very light infection, the area where the animal likes being scratched is likely to yield the most suitable material for examination.
2. Clip the hair from the selected area and moisten it with 10% solution of caustic potash or even plain water.
3. Scrape the area with a blunt knife. A sharp scalpel should not be used, for it may cut the skin.
4. Collect all loose scabs, scurf, hair etc. in a large test-tube. The scraping should be continued until blood oozes from the scraped area as it is only when this stage is reached that the area is likely to yield samples of the different forms of mites harboured by it. Each time a little quantity of blood collects on the scalpel, lightly scrape an adjacent area with it to cover up the blood with scurf and loose hair.
5. Wipe the scalpel against the inner rim of the tube and then detach the adherent mass with the knife to make it drop down into the tube. The blood will thus be prevented from adhering to the knife or to the mouth of the tube.
6. Scrapings at least sufficient in quantity to cover a rupee coin should be collected from all cases suspected for mange. Negative findings are sometimes due to insufficient or only superficial scrapings having been examined for the presence of mites.

Helminths

The alimentary canal is best examined for the presence of helminths by mixing the contents of its different parts with water; allowing it to stand for 10-25 minutes, decanting away the supernatant fluid and making a careful search for the parasites in the sediment. For examination of liver and lungs, give these organs several cuts and squeeze the tissue in a basin of water to liberate the parasites, if any. The parasites may then be picked up with a pair of fine forceps. The subsequent procedure should be as follows:

1. After collection, wash the parasites quickly in 0.9% saline.
2. For fixing, transfer to steaming 5% formalin, not less than 10 times the

quantity of the parasites. Glycerol may be added at the rate of 5% for keeping the worms soft.

3. Transfer after 24 hours to a smaller quantity of the fixing fluid or 70% ethyl alcohol for despatch.

For preservation of faeces containing helminths, the faecal matter, if not already thin, may be brought to the consistency of porridge by the addition of water. Bring it to boil and add 10 times its volume of 5% formalin. Mix well and let it stand. Decant off the supernatant fluid and preserve the sediment in cold 5% formalin.

Filarioid worms are very liable to burst and as such should be placed immediately in a 10% solution of formalin without washing in saline, unless they are soiled with blood.

It is difficult to remove nematode larvae or small adult nematodes which are in tissues. However, by placing the tissues in a dish of warm physiological saline, one can usually get those freed. The cestodes should be collected along with their heads as this would be of great help in proper identification. In case the heads are attached to the intestinal wall, a piece of the intestine should be cut and placed into a dish of warm water at about 400C. The worms usually die fully extended in about an hour and the heads get free. If not, these might be dissected out.

DIRECTIONS FOR DESPATCHING PATHOLOGICAL MATERIAL BY POST

Postal regulations for despatch of pathological material should be strictly observed. The mouths of containers should be well sealed with molten paraffin wax, after securing the stoppers properly on to them with strong twine. Bottles or tubes containing dry material may be closed tightly with cotton-wool plug and the mouths then paraffined.

All containers should be properly labelled, wrapped in packing paper and packed in sufficient quantity of some absorbent material such as sawdust or cotton wool. The packing should be such as to prevent any possibility of the containers rocking in transit and also to provide for the complete absorption of fluids by the packing material in the event of breakage. All parcels should be conspicuously marked with waterproof marking pen **'Fragile—With Care'** and bear the words **'Pathological Specimen'.**

A packing note should invariably be enclosed in each parcel to indicate the nature of the specimen and the examination required, and should also give the name and address of the sender. The outer wrapper of the packing

should bear a note of caution when the specimen is one of infectious material.