

# SENDING SPECIMENS FOR PATHOLOGICAL EXAMINATION

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## INTRODUCTION

The pathology section of the Central Veterinary Laboratory examines the health of animals by looking at samples of tissues, blood or other body fluids like urine and saliva.

When samples are sent for pathological examination, a Veterinary Specimen Advice Card must be sent too. As we said in an earlier article (see HARVEST Volume 6 (3): 134-138) this card should include notes on the history of the problem, the clinical signs shown by the sick animals, and any post-mortem (after death) findings.

This article shows in detail how to collect, store and send specimens for examination in the pathology section of the laboratory.

## HISTOPATHOLOGY

Histopathology is the study of fixed (preserved) tissues. By examining different tissues or parts of the body under a microscope, the type of sickness affecting an animal can be worked out.

There are 3 reasons for sending tissues. Most are sent after an animal has died, when an officer performs a post-mortem examination, and collects

samples of unusual-looking tissues. Some are sent from live animals after a biopsy (sample) such as a tumour (unusual swelling), is removed, and some are received from slaughtered animals when a meat inspector notices a lesion (abnormal tissue).

## How to 'fix' tissues

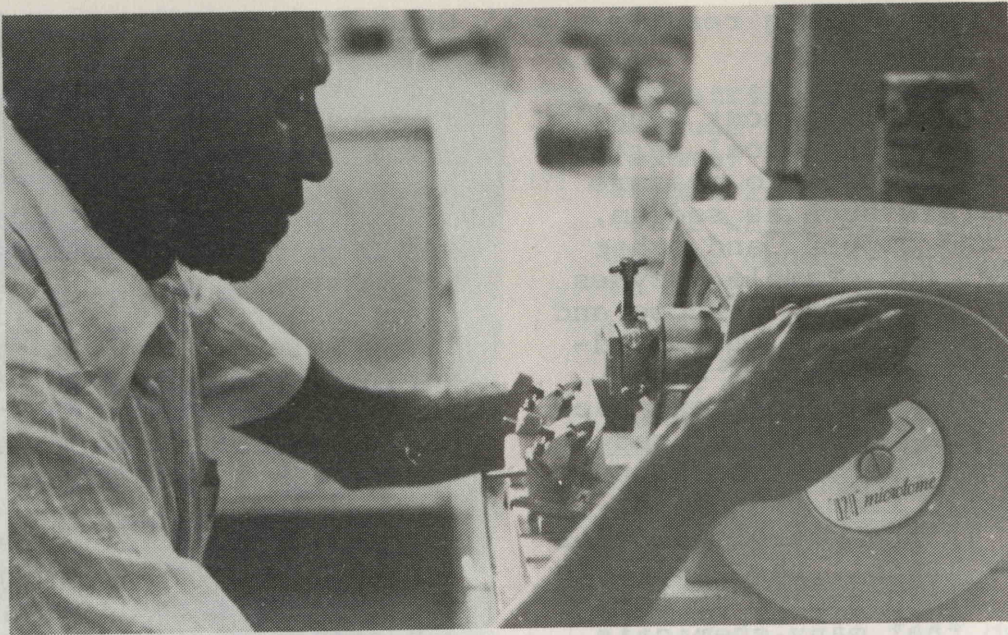
All tissues for histopathological examination must be fixed, in order to stop them decaying after death. Autolysis (post-mortem decay) will hide important changes in tissues so that a diagnosis may be missed.

To fix tissues, thin slices (less than 1 cm, or the width of the tip of a person's little finger) of tissues are placed into 10% formalin or 70% alcohol. Formalin is commonly used and 10% solution is made by mixing 1 part of commercial formalin with 9 parts of water. To allow enough formalin to fix all the tissues, make sure there is at least five times more formalin than tissue in each sample jar.

## What to send

Tissue slices should include some normal as well as abnormal tissue, and samples of all unusual-looking tissue should be sent. If you do not see any lesions when you examine an





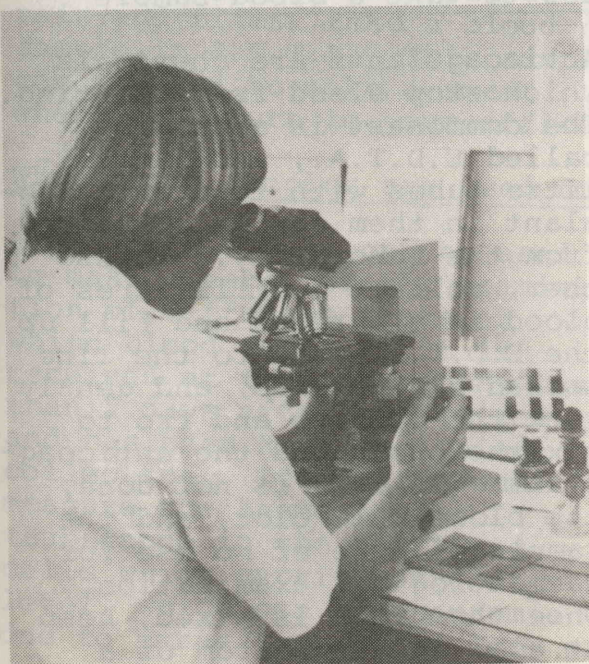
*Before examination under the microscope in the laboratory, tissue slices must be cut into very thin sections, using this machine.*

animal which dies of an unknown cause, send samples of as many parts of the body as possible. Many changes cannot be seen with the naked eye but may be seen under the microscope. Always include samples from the liver, lung, kidneys, and heart from mammals, and of the cloacal bursa (sac) and sciatic (leg) nerve as well from birds.

Remember to estimate how long the animal has been dead, and to describe the size, shape, colour, feel and place where you find any abnormalities. Always take faeces and stomach contents, as discussed in the earlier article on parasitology (see HARVEST Volume 6(3):139-144), in case worms are the cause of death.

#### CLINICAL PATHOLOGY

Clinical pathology or biochemistry is the study of the health of animals by examining the chemistry of serum, urine, peritoneal (from the lining of the abdomen) and other body fluids. Clinical pathology is a very precise science because many chemicals in body fluids are present in very, very small amounts. Therefore it is very important that samples sent for biochemical studies are collected without getting them wet or dirty, and then treating and storing them correctly. If samples are made dirty, or damaged by incorrect treatment and storage, any tests done on



*Preparing slides for histopathology*



them will give wrong results and will be useless.

Serum is the fluid most usually sent in for biochemical tests, and it can be used to measure many minerals such as sodium, calcium and copper, and other chemicals, for example enzymes and other proteins, glucose and other carbohydrates. The hardest thing in getting a good serum sample is to prevent haemolysis (the bursting of red blood cells) before the serum is separated from the blood sample. If the red blood cells haemolyse (burst) the chemicals they contain dissolve in the serum, so that many chemicals, especially phosphorus, will show high levels when the serum is tested, and the sample will be of little use.

#### How to take a serum sample

To collect a good serum sample, collect 10 ml of blood (for poultry 2-5 ml is enough) into a plain tube or syringe. Leave it to clot for 30 minutes at room temperature. Once the clot (jelly) has formed, separate it from the tube by gently tapping the side of the tube or by pressing around the edge of the clot with a pipette or wooden applicator stick.

Wait a further 5 or 10 minutes to allow the clot to settle, then gently pour or pipette off the serum (clear fluid), without any red blood cells. Put it into a new plain tube, and store it, if possible in a refrigerator.

If a centrifuge is available, spin the tube at 1,500 r.p.m. (revolutions per minute) for 3 minutes after the clot is separated and collect the serum in the same way. Urine, saliva and other body fluids should be collected as cleanly as possible and stored in a refrigerator.

#### Other fluids may be needed

Serum can be used to measure many minerals and other chemicals, and the levels found can help decide what condition is affecting a group of sick animals. Serum alone is enough to measure some minerals (for example copper and phosphorus). To measure some of the others, further specimens are required as well (for example serum, saliva and urine for measuring sodium).

Specially washed needles, syringes and tubes are needed for some of these tests, so you should telephone the Laboratory and arrange with the Senior Veterinary Pathologist or the Biochemist to forward the correct equipment if you wish to test animals for minerals or other chemicals.

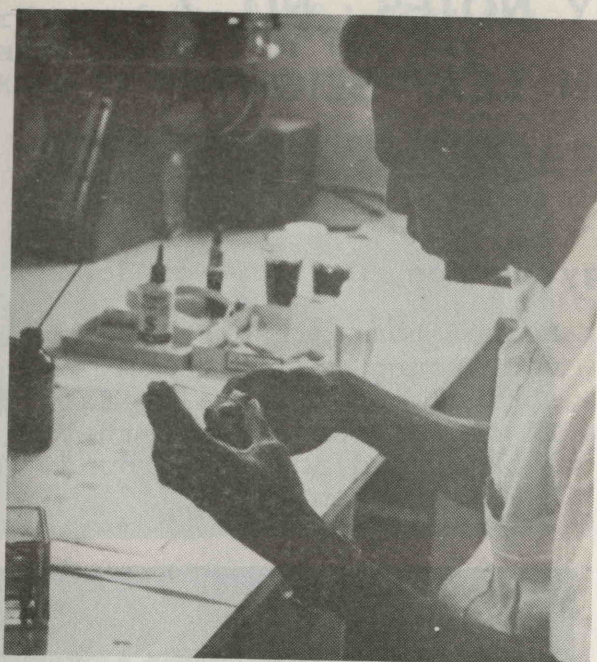
#### HAEMATOLOGY

Haematology is the study of blood. Blood can be sent for examination as either blood with an anticoagulant added, or as blood smears.

#### How to take a blood sample

Anticoagulants are chemicals which stop blood from clotting. The commonest is a chemical called E.D.T.A., and 5-millilitre tubes with this anticoagulant in them can be obtained from the Laboratory. To use them, collect 5 millilitres of blood in a syringe and fill up the E.D.T.A. tube to the line marked on the side, and gently rock the tube to and fro to mix the blood and the anticoagulant. If this is not done, the blood will clot, and the sample will be of no use for haematological examination. Once the blood is mixed, keep it cool in the bottom of a refrigerator, or in an 'esky' with an ice brick.





*Examining blood samples*

#### How to take a blood smear

Blood smears are made by spreading the blood across a clean glass slide, as described in the earlier article on parasites (see HARVEST Volume 6 (3): 139-144). You can either make a smear of a drop of fresh blood at the project where you are working, or, to avoid dust, rain and flies, take a blood sample in E.D.T.A. This sample can be used to make a smear when you return to your office.

#### What blood samples are used for

Blood in E.D.T.A. can be used to make smears for the examination of different types of white blood cells if it is received at the laboratory less than 5 days after collection, and has been kept cool since collection. It is also used to measure the haemoglobin (substance in the red blood cells which carries oxygen) level, which can show anaemia due to parasites, blood loss or

malnutrition. Other tests include the P.C.V. (packed cell volume) which measures if an animal is dried out or not, and total white cell count, which may be abnormally high or low in different diseases.

#### What blood smears are used for

Blood smears are used to count the percentage of different types of white blood cells present. This can give useful information about the animal's health. For example, high numbers of the type of white blood cell called neutrophils show that a bacterial infection is present, while the presence of a different type of cell, called eosinophils, usually indicates a parasitic infestation.

#### CONCLUSION

Specimens for histopathological examination are easy to take and submit in 10% formalin, and should be taken from lesions seen at slaughter or whenever an animal dies on a project. Specimens for clinical pathology must be taken carefully and stored correctly to be of any value, and further advice can be obtained from laboratory staff. Specimens of blood in E.D.T.A. are easy to collect for haematological examination, but must be sent quickly if worthwhile results are to be obtained.

If you need further information or supplies of specimen jars, specimen advice cards, tubes or glass slides for blood, please contact the Central Veterinary Laboratory at P.O. Box 6372, Boroko, or telephone 25 3588 or 25 4510.