SENDING SPECIMENS FOR MICROBIOLOGICAL EXAMINATION AT THE CENTRAL VETERINARY LABORATORY

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INTRODUCTION

Microbes are organisms which are alive but which are too small for us to see, except through a microscope. They can live in tissues and fluids of the bodies of animals. Sometimes they are useful, but sometimes they cause diseases.

Bacteria, fungi and viruses are all microbes that can cause diseases in the animals in which they live. Samples of tissues and fluids from sick animals can be examined in the laboratory, to see if microbes are the cause of the disease.

This article discusses how you can collect and send specimens for microbiological examination at the Central Veterinary Laboratory.

A Veterinary Specimen Advice Card must be sent with any material submitted to the laboratory. For more details about this, see HARVEST, Volume 6 (3): 134-138.

TESTS USED IN MICROBIOLOGY

Four kinds of technique are used in microbiology:

1. Growing (culturing) microbes from a tissue sample, to identify them. Examples are culturing bacteria from abscesses (sores containing pus) or growing fungi from a skin disease such as ringworm.

2. Testing serum (serology); for example, the Rose Bengal (RBT) and Complement Fixation (CFT) tests for brucellosis, or Agar Gel Diffusion (AGDT) tests for many viruses.

3. Staining smears to show the microbes. Examples of this are the Gram stain for bacteria, and other stains for fungi.

4. Tests in animals, for example, for toxins (poisons) made by bacteria (e.g. botulism).

How to collect specimens for these tests is discussed below.

SPECIMENS FOR GROWTH AND IDENTIFICATION OF MICROBES

Specimens must be collected without touching hands, faeces (manure), or dirt of any kind. This is because if they do touch dirt, faeces, etc., other bacteria from the dirt will grow on the sample, hiding those which caused the disease. These unwanted bacteria are called contaminants. Their presence indicates that the sample has not been collected properly, but has become contaminated.

Specimens for growth and identification of microbes from an animal which has died, must be collected as soon as possible after death. This is because very soon after an animal dies, bacteria from the intestines spread through the animal's body as autolysis (decay) begins. If this happens, these contaminant bacteria will be found on the specimens, and the microbes which caused the death of the animal may not be found.

Specimens for growth and identification of microbes may be either whole tissues or swabs.

Whole tissues

Whole tissues for microbiological examination should be collected without contamination and should be about the size of a matchbox. Collect samples from any organ that looks abnormal, and put only one tissue in each sample jar. (Also collect thin slices of each tissue and put these together in a jar of 10% formalin, for histopathology, as discussed in the earlier article on pathology. See HARVEST, Volume 6 (4): 206-209.

Whole fresh tissues should be sent chilled, on ice, but not frozen. Freezing kills many microbes so that they cannot be grown from the samples.

Fresh tissues must also be sent fast, or the microbes will die. Phone the Central Veterinary Laboratory (25 3588) with the flight details and consignment number, so that they can be collected at once. Try not to send them on Fridays, but keep them in the bottom of a refrigerator, and send them first thing on Monday.

For skin diseases, a sample of skin can be sent, as described for whole tissues above. If you suspect a fungal disease (e.g. ringworm), send hairs. these should be collected by pulling them from the edge and the centre of the affected area, and sent in a specimen jar or an envelope.

Swabs

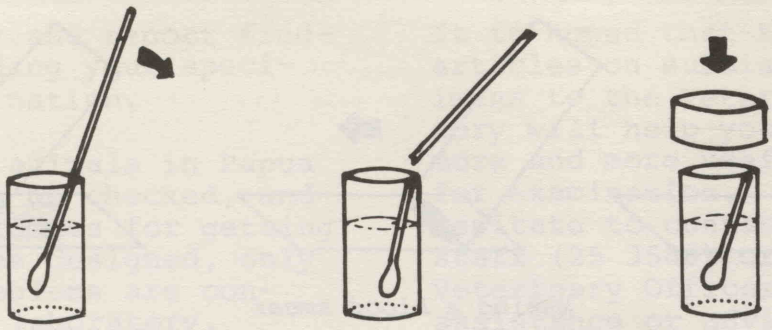
Swabs are sterile buds of cotton wool on the end of a small stick, inside a plastic container. They are used to collect samples of fluid (e.g. pus, tissue fluid). Like whole fresh tissues, they must be collected without contamination and sent as quickly as possible to the laboratory.

When using a swab, do not touch the cotton wool - hold it by the stick and dip the cotton wool bud into the fluid to be examined. Use a new swab for each kind of fluid.

Do not let the swab touch any other fluids or tissues, or any kind of dirt. If you do, contaminant bacteria will spoil the sample. Put the swab back into its container, seal it, and label it with details of what the fluid is, where it was taken from, and when it was taken.

Send swabs as soon as they have been used, because they dry out very quickly and the microbes will then die. If they do not arrive at the laboratory in under 24 hours, they should be sent in a transport medium.

A transport medium is a special jelly on which the microbes can feed to keep them alive so that they can be grown and identified in the laboratory. Special transport medium is available from the laboratory, but if you do not have any, boiled or ultra-heat-treated milk can be used. (Boil the milk gently for two or three minutes, and



Transferring the swab to a container

put it into a sterile specimen jar.) Ultra-heat-treated (U.H.T.) milk is sterile until opened, so milk from a fresh carton of U.H.T. milk can be used as transport medium)

To put the swab into the container of transport medium (or milk) put the cotton bud in, as shown in the diagram. Break off the stick against the edge of the container, without touching any part of the swab that goes into the container. Seal the container and label it.

Both whole tissues and swabs must be sent cool, on ice. This is best done by putting the specimens in a plastic bag and placing them next to another plastic bag of ice. The whole parcel can be sent in an insulated container (e.g. an 'esky') or put in another plastic bag and wrapped in several layers of newspaper.

SUBMISSION OF SERUM FOR SEROLOGY

Collect blood aseptically (without contamination) by first cleaning the dirt from the collection site (e.g. tail of cattle, ear of pigs, wing vein of poultry). If possible, clean the collection site with alcohol such as methylated spirits or with disinfectant, and wait until it dries.

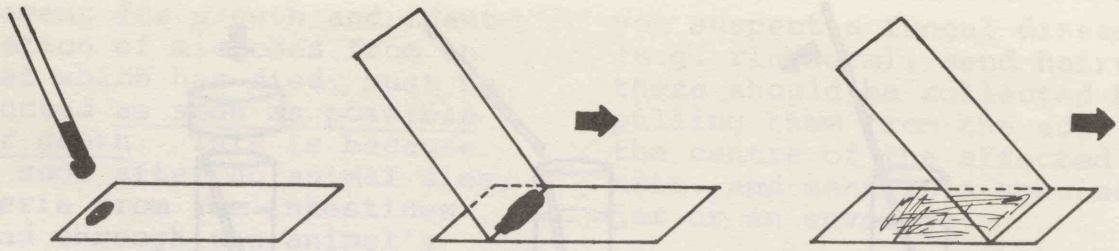
The blood can be collected by using a sterile needle and syringe, or by making a small cut using a scalpel blade and collecting the blood into a sterile tube.

Leave the blood to clot (form a blob of red jelly). Pour off the serum (clear fluid above the clot) into a sterile tube and seal it. Label each sample carefully, and send it to the laboratory immediately. If the serum cannot be sent at once, keep it cool in the bottom of a refrigerator or icebox.

SUBMISSION OF SMEARS

Smears can be made of blood, pus or other fluids (e.g. tissue fluid). To make good smears, it is important to have clean glass slides. The laboratory will supply clean slides wrapped in tissue paper, and these can be used without further cleaning. If you have slides that are not clean, they should be cleaned, preferably in alcohol. Hold slides by the edges to stop dust and grease from your hands from getting on the slides and spoiling the smear.

Collect a drop of the fluid to be smeared, in a syringe or on a scalpel blade or slide. Drop the fluid on the end of a clean glass slide you are holding flat. Dip the edge of another clean slide in this fluid and let the fluid spread along the edge of the slide. Then gently push one slide over the other,



Making a blood smear

as shown in the diagram. This will spread (smear) the fluid across the slide.

Make several smears of each fluid, and let them dry in the shade, away from dust or moisture. To stop decay, the slide can be fixed after it is dry, by dipping it in alcohol for one minute, and again allowing it to dry in the air without wiping it. Carefully wrap each smear in paper and label it, either on the paper or by putting a sticky label (e.g. Kwik Stik) on the slide, or by writing on the slide with a wax or chinagraph pencil. Do not put smears in a refrigerator or ice box, because if you do moisture will form on them and spoil them.

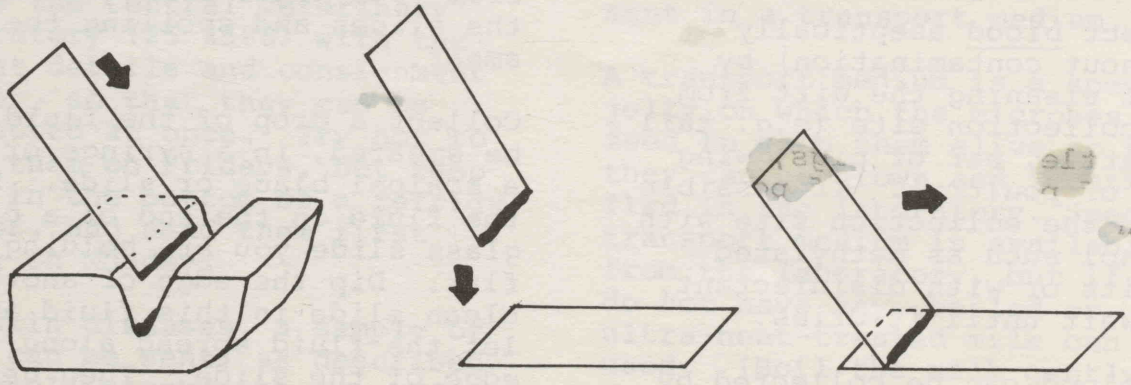
Smears can be made from tissue fluid. Cut open the tissue and touch the cut edge with the end of a clean glass slide, as shown below. Then put this edge with the tissue fluid on it against another clean glass slide which you are holding flat, and smear the fluid across it. Note that smears will only help to show if bacteria caused the disease, and are of little value in fungal or viral infections. Smears cannot be used for growth and identification of microbes, so, if possible, always send fresh tissues on ice, or a swab, with the smears.

SAMPLES FOR TESTS IN ANIMALS

In general, fresh tissues and intestinal contents, kept cool on ice are required for tests in animals. Most microbial diseases can be identified by one or more of the techniques outlined above, so that identification of microbial disease by inoculation of laboratory animals is not often done.

CONCLUSION

As described in earlier articles on the work of the veterinary laboratory HARVEST 6 (3):134-138, 139-143, and 6(4): 206-209) you should always send



Making a smear from tissue fluid

a full history and report findings when sending your specimens for examination.

The health of animals in Papua New Guinea can be checked, and suitable programmes for getting rid of diseases designed, only if disease problems are confirmed in the laboratory.

It is hoped that the series of articles on submission of specimens to the Veterinary Laboratory will help you to collect more and more useful specimens for examination. Please do not hesitate to contact laboratory staff (25 3588) or your Area Veterinary Officer for further assistance or advice on the use of the Veterinary Laboratory.