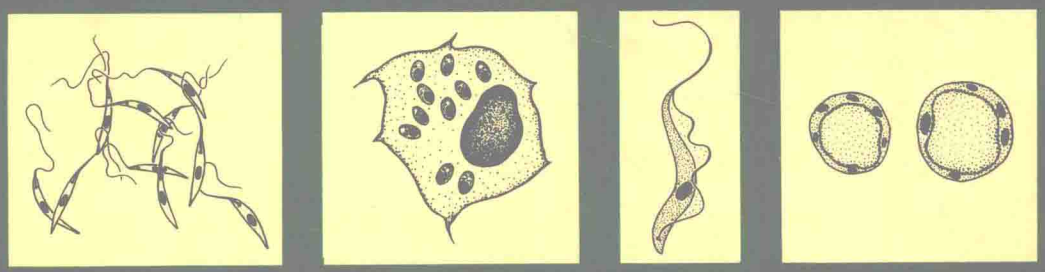
BASIC LABORATORY METHODS IN MEDICAL PARASITOLOGY



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Preface

This manual is intended as a practical guide for use by workers in laboratories in health centres and first-referral hospitals. Diagnostic methods are restricted to microscopy, though reference specimens may need to be sent for serodiagnosis.

The content of this manual is based on information contained in training material and manuals produced by the Hospital for Tropical Diseases, London, England; the Parasitology Training Section, Division of Laboratory Training and Consultation, Laboratory Program Office, Centers for Disease Control, Atlanta, Georgia, USA; the World Health Organization, Geneva, Switzerland; and the Pan American Health Organization, Washington, DC, USA. In particular, a number of illustrations were taken from the following publications: Brooke, M. M. & Melvin, D. M., *Morphology of diagnostic stages of intestinal parasites of humans*, 2nd ed. Atlanta, GA, US Department of Health and Human Services, 1984 (HHS Publication No. (CDC) 84-8116); Melvin, D. M. & Brooke, M. M., *Laboratory procedures for the diagnosis of intestinal parasites*, 3rd ed. Atlanta, GA, US Department of Health and Human Services, 1982 (HHS Publication No. (CDC) 82-8282).

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Contents

Introduction

Parasitic diseases are responsible for considerable morbidity and mortality throughout the world, and often present with nonspecific symptoms and signs. Most parasitic diseases cannot be diagnosed by physical examination alone, and laboratory investigation is necessary to decide whether or not the patient is infected with a parasite and, if so, what species of parasite is present. Thus the laboratory plays an important role in establishing the diagnosis of parasitic diseases and is therefore the key to the selection of the appropriate drug for treatment. Laboratory tests must be accurate and reliable if the results are to help the physician and benefit the patient.

This manual is a guide for the laboratory worker. Section 1 presents the techniques to be used when examining faeces, blood, urine, and other materials for the presence of parasites. Pitfalls and possible errors are pointed out and methods for avoiding these indicated. Quality control measures are also discussed. The laboratory worker must understand that only careful performance of the techniques required to recover and demonstrate parasites will make it possible for them to be seen clearly on microscopic examination.

Section 2 of the manual describes the morphological criteria used to identify parasites. Artefacts and problems of identification are also discussed.

Information about the equipment and reagents required is contained in four annexes. Annex 1 lists the materials and equipment needed in health centres and hospital laboratories at the primary health care level, Annex 2 gives the formulae and directions for preparing reagents, Annex 3 gives the formulae and directions for preparing culture media, and Annex 4 describes the procedure for cleaning and storing slides to be used for preparing blood films.

Laboratory safety

General principles

1. Each laboratory should have a written manual of safe laboratory methods, to be followed at all times.
2. The laboratory should have a first aid box and a designated first aider on the staff.
3. Non-laboratory staff must not be allowed to enter the working area of the laboratory.
4. Eating, drinking, smoking, and applying cosmetics should not be permitted in the laboratory.
5. Laboratory personnel should wear protective clothing, which should be removed on leaving the laboratory area.
6. Laboratory personnel should clean the benches with a detergent solution (soap) and disinfect the working surfaces after every working day, or after having spilt infectious material. The most commonly used disinfectants are:

   - 96% ethanol or isopropanol (irritant to skin),
   - 1% phenol solution (corrosive, caustic),
   - 0.5–1% hypochlorite solution (caustic, corrosive) (alkaline hypochlorite solution is more aggressive than neutral hypochlorite solution),
   - 1% formaldehyde or 2% glutaraldehyde solution (toxic and irritant to skin).

   Aldehyde and phenol solutions are active over a longer period. It is advisable to wipe the working areas with tissue soaked with a disinfectant solution rather than using a spray.
7. Laboratory personnel must always wash their hands before leaving the laboratory.

Handling specimens

Great care is needed in handling all laboratory specimens and rubber gloves should always be worn.

Blood samples. All blood samples must be regarded as potentially infectious. As very serious pathogens can be transmitted by blood (e.g., human immunodeficiency virus (HIV), hepatitis B virus) great care is required when collecting and processing samples. Particular risks are:

(*a*) Stabbing or cutting injuries—dispose of used needles or lancets in a container which can then be incinerated or buried in a disposable specimen container after soaking in disinfectant solution. Do not reuse lancets. Do not leave used lancets lying around the laboratory. Do not use chipped or cracked glassware.

(*b*) Contamination of damaged skin or of mucous membranes—cover any cuts with impervious dressings. Avoid spilling blood on to the skin or mucous membranes. Pipetting by mouth should be absolutely forbidden! If blood is spilt on to the skin, immediately wash the affected area with soap and water; if blood gets into the eyes, they should be irrigated with large amounts of water. Any blood spilt on to laboratory surfaces should be soaked with hypochlorite solution and then wiped up with a cloth impregnated with hypochlorite solution.

Stool samples. Skin contact must be avoided. When finished with, samples should be either (*a*) incinerated or (*b*) soaked in disinfectant solution and then buried in disposable specimen containers.

Urine samples. Skin contact must be avoided. Samples can be discarded via the sewage system.

Disposal of microscope slides

Slides should be discarded into a pot containing 1% hypochlorite solution and buried in disposable specimen containers, if they are not to be cleaned for reuse.

Section 1

Techniques of collection, preparation, and examination of samples

Care of the microscope

Do's

1. Do keep the microscope covered with a clean plastic or cloth cover when it is not in use.
2. Do take special care to protect the microscope from dust in hot dry periods.
3. Do take special care to protect the microscope lenses and prisms from fungal growth in hot humid periods. This can be done by:
   - keeping the microscope in an air-conditioned room,
     OR
   - storing the microscope in a special dehumidified room—an electric dehumidifier is about half the price of an air-conditioner,
     OR
   - connecting a number of 15 or 25 watt bulbs inside a cupboard with tightly fitting doors,
     OR
   - placing a 15 watt bulb in the individual microscope box which then acts as a warm cupboard,
     OR
   - in areas without electricity, placing a shelf to hold the microscope box about 30 cm over the chimney of the gas- or kerosene-operated refrigerator or freezer; an airtight bag and silica gel in its dry state (as indicated by its blue colour) will keep a microscope sufficiently dry to protect lenses from fungi.
4. Do clean the immersion oil from the immersion objective every day; use a soft cloth dampened with ethanol/ether (3 ml/7 ml) or benzine/ethanol/ether (2 ml/2 ml/1 ml) and polish with a clean, lint-free cloth.
5. Do clean the oculars with a soft, lint-free cloth; as an alternative, use lens tissue or facial tissue, if available.
6. Do use the microscope retaining screw fitted at the base of the microscope box to prevent damage to the instrument while in transit.
7. Do quote the model number and, if possible, the instrument and part number when ordering replacement parts.

Dont's

1. Don't use the tissue or cloth used for the oil immersion objective to clean the oculars.
2. Don't use alcohol to clean painted surfaces of the microscope.
3. Don't dismantle or try to clean parts of the microscope that are difficult to reach unless you have been trained to do so.
4. Don't leave the lens ports empty; use the appropriate cover or some sticking plaster to cover the empty port.
5. Don't exchange lenses from microscopes of different manufacture—even some models by the same manufacturer have different specifications.

Calibrating the microscope for measurement

Size is an important criterion for the identification of many parasites, particularly cysts and ova. Size can be determined using a blood cell counting chamber (Neubauer), or alternatively an eyepiece micrometer. Using an eyepiece micrometer, the procedure is as follows:

1. The eyepiece scale is divided into 100 small divisions.
2. The stage micrometer scale consists of 1 mm divided into 0.1 mm divisions and each 0.1 mm is divided in 0.01 mm.
3. Insert the eyepiece scale (a round glass disc) into the eyepiece by removing the uppermost lens and placing the scale on the field stop.
4. Insert the eyepiece into the microscope.
5. Place the stage micrometer on the microscope stage.
6. Focus the low-power objective on the stage scale.
7. Adjust the stage and eyepiece scales until the eyepiece scale and the stage scale are parallel.
8. Note the number of eyepiece divisions and its appropriate stage measurement, e.g., 50 eyepiece divisions = 0.75 mm; 10 eyepiece divisions = 0.15 mm.
9. From this reading, work out the value for one eyepiece division, as follows:

   50 eyepiece divisions = 0.75 mm
   1 eyepiece division = 0.75/50 = 0.015 mm
   OR
   10 eyepiece divisions = 0.15 mm
   1 eyepiece division = 0.15/10 = 0.015 mm.

10. Change the measurement value from mm to μm (1 mm = 1000 μm), e.g., 0.015 mm = 15 μm.
11. Repeat for all objectives and note the reading for each.
12. Calibration need be done only once for each microscope used.

Faecal specimens

Faecal specimens are examined for the presence of protozoa and helminth larvae or eggs.

The stages of protozoa found in stools are trophozoites and cysts. The stages of helminths usually found in stools are eggs and larvae, though whole adult worms or segments of worms may also be seen. Adult worms and segments of tapeworms are usually visible to the naked eye, but eggs, larvae, trophozoites, and cysts can be seen only with the microscope. In order to see these structures, the faecal material must be properly prepared and examined.

Collection of faecal specimens

Because of the fragile nature of many intestinal parasites, and the need to maintain their morphology for accurate identification, reliable microscopic diagnosis cannot be made unless the stool is collected properly.

1. Give the patient the following:

   – a waxed cardboard box with an overlapping lid, or a plastic cup or box with a tight-fitting lid, and
   – 2 applicator sticks.

   If waxed boxes or plastic cups are not available, tin boxes or glass jars can be used. Banana leaves and match boxes are not satisfactory containers for the collection and storage of stool specimens.
   In control programmes, it is often sufficient to examine a single specimen, but for patients, three specimens are usually required, at 3-day intervals, to detect all parasitic infections. A variety of substances may interfere with the examination of stool specimens for parasites (e.g., laxatives, antacids, ingested contrast media, certain antibiotics).
2. Tell the patient to pass the stool specimen directly into the container, or to pass the stool on to a piece of paper and use the applicator sticks to transfer it to the container. If paper is not available, the faeces can be passed on to a large, clean leaf, such as a banana leaf. However, the stool *must be transferred immediately to the specimen container*. It should not remain on the leaf, or be brought to the laboratory on the leaf.
3. Some organisms, especially amoebic trophozoites, will begin to disintegrate or change within a short time after passage and become unrecognizable. Warm temperatures will hasten these changes. Therefore, specimens must reach the laboratory very soon (i.e., within half an hour) after passage. If this is not possible, the specimen must be treated with preservatives (see pp. 28–29).
4. The container with the specimen should be labelled clearly with the following information:

   – patient's name or number
   – date of collection
   – time the patient passed the stool (ask the patient when he/she passed the stool).

5. The stool specimen must be large enough for satisfactory examination. The smallest quantity that should be accepted is about the size of a pigeon's egg. Urine and dirt should be excluded. Urine will destroy any amoebic trophozoites and dirt will interfere with the examination.
   If the specimen is too small, or if it is mixed with urine or dirt, it should not be accepted. Ask the patient to pass another specimen.