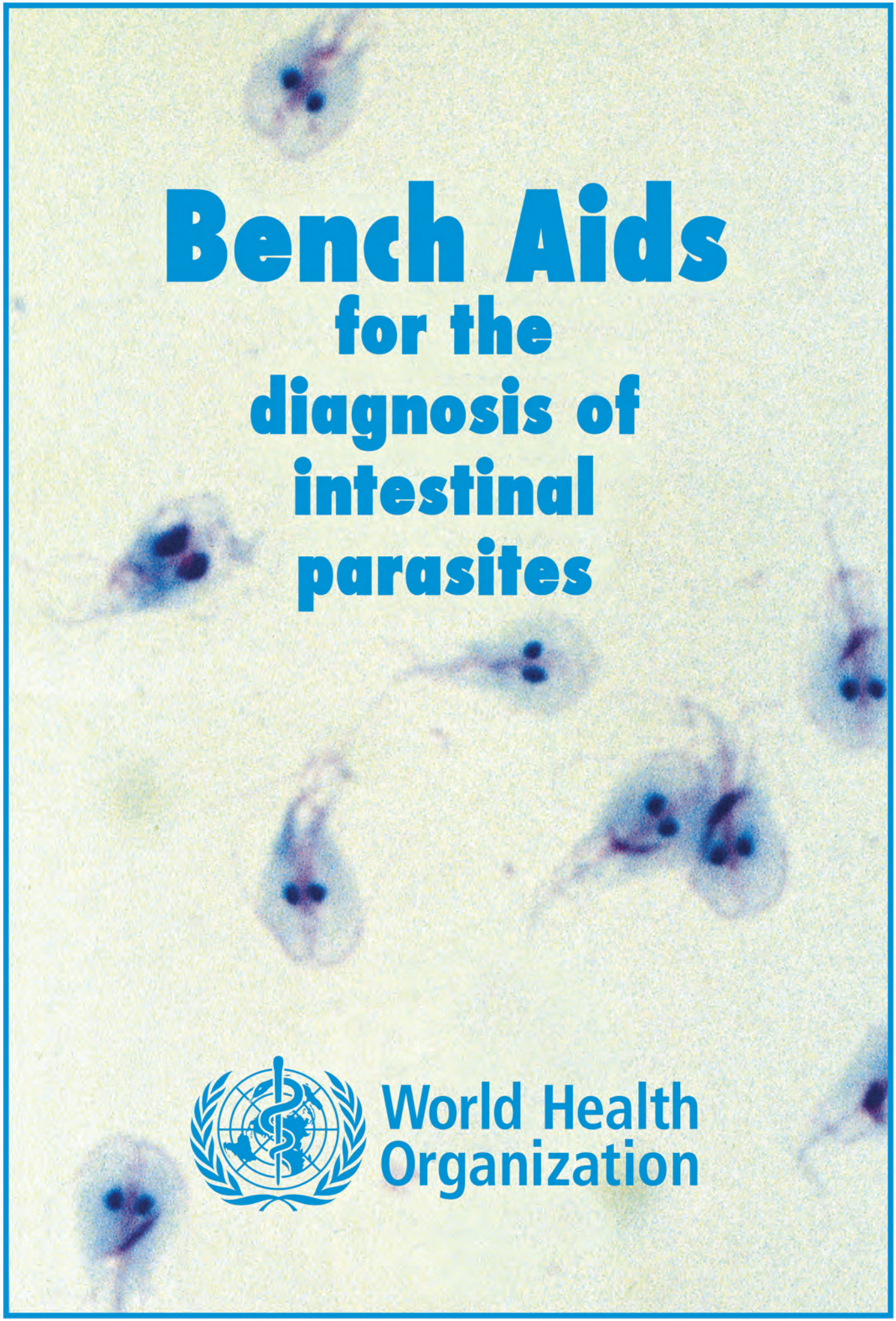

Bench Aids
for the diagnosis of intestinal parasites

World Health Organization

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Cover photomicrograph:
Cultured trophozoites of an isolate of *Giardia* spp. stained with Giemsa.
Courtesy of Nicolette Binz, WHO Collaborating Centre for the Molecular Epidemiology of Parasitic Infections, School of Veterinary Studies, Murdoch University, Western Australia.

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Identification of intestinal parasites

The goal of the microscopist in the diagnosis of intestinal parasitism is to ascertain the presence of parasites in faeces, whether they be minute protozoan cysts or large helminth eggs, and to identify them correctly. In some cases, the organisms are present in sufficient quantities to be found by direct examination of a small amount of faeces, i.e. the direct smear (see Plate 1). The addition of a drop of Lugol's iodine solution to the preparation will often bring out important morphological features of the parasites which will aid in their identification.

The identification of protozoan trophozoites and cysts in unstained faecal smears is a challenge even for the experienced microscopist and even under ideal conditions of collection and preparation of specimens. Trophozoites degenerate very rapidly so that faecal specimens must be examined promptly, permanent smears prepared for staining, or the specimen preserved in a special fixative such as merthiolate-formalin-iodine (MIF) as quickly as possible. Although direct examination of MIF-preserved material is useful, the microscopist must be experienced in the recognition of parasites in wet mounts.

Permanent-stained faecal smears are recommended for identification of protozoan parasites. Smears can be prepared from fresh faeces or from faecal material preserved in polyvinyl alcohol (PVA) or in sodium acetate-acetic acid-formalin (SAF). Other preservatives for faeces, such as 10% formalin, are not recommended for preparation of stained smears. The most commonly used permanent stains are trichrome and iron haematoxylin. Trichrome is easy to use and is especially good for smears made from fresh faeces or from PVA-preserved material; it is not recommended for use following SAF preservation. Iron haematoxylin is more difficult to use procedurally but gives excellent results on all types of faecal smears. In some instances, the use of more specialized staining techniques, such as acid-fast stains, will better demonstrate small coccidian organisms such as *Cryptosporidium* and *Cyclospora*. Even the minute spores of microsporidian species can be detected in faeces with modified or special staining procedures.

Most protozoan parasites are readily identified in permanent-stained smears. Even the most subtle and delicate features of these parasites can be visualized. As will be noted in the photomicrographs, the staining of organisms and faecal elements may vary considerably, even when the same stain is used. This may be due to many factors, including the age of the specimen when fixed, the fixative used, the thickness of the smears, and the time for destaining. We have attempted to provide the diagnostic features of all the common protozoan parasites using dichotomous keys and photomicrographs of all stages of each of the parasites as they appear unstained or stained in one or more of the stains described above.

In general, the diagnosis of intestinal helminths is less difficult than that of the intestinal protozoa. Helminth eggs are often easier to find and identify because of their size and their distinctive morphological features. While direct smears of fresh faeces will often demonstrate helminth eggs, it is usually more efficient for laboratories to do a simple concentration (see Plate 2) to avoid overlooking parasites that may be present in very small numbers. In some situations, such as large community-based surveys, specific objectives are limited to the detection of schistosome or soil-transmitted nematode (*Ascaris*, *Trichuris* and hookworm) infections. A modification of the direct smear procedure, the Kato-Katz technique (see Plate 3), is especially useful for field surveys for these infections because it also gives an estimation of the intensity of infection. We have provided images of the most common intestinal helminth parasites as they appear in faeces or, in some cases, in Kato-Katz preparations.

Finally, it is of the utmost importance that the microscopist is able to measure objects in the microscopic field. Accurate estimation of the size of organisms is important for correct diagnosis. Eyepiece reticules are available for virtually all microscopes. The reticule can be calibrated with the aid of a stage micrometer using the instructions provided overleaf.

Further reading

Ash LR, Orihel TC. *Parasites: a guide to laboratory procedures and identification*. Chicago, ASCP Press, 1991.
Ash LR, Orihel TC. *Atlas of human parasitology*, 3rd ed. Chicago, ASCP Press, 1990.
Basic laboratory methods in medical parasitology. Geneva, World Health Organization, 1991.

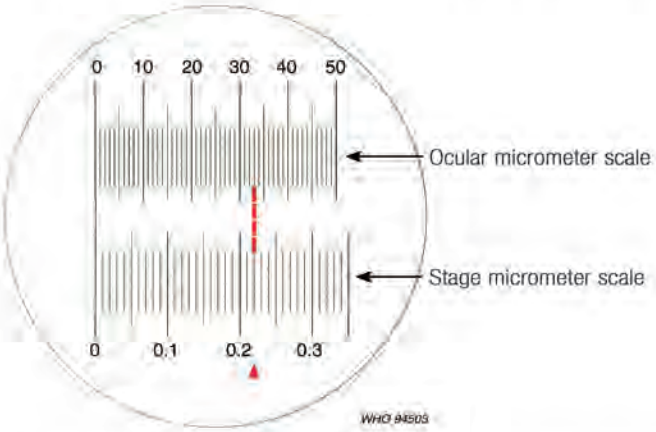
Calibration of ocular micrometer

In order to measure elements in the microscopic field, it is necessary to have a measuring scale in the eyepiece of the microscope. Before it can be used, however, the scale must be calibrated. Ocular micrometers are flat glass discs on which a line scale divided into 50 or 100 small divisions has been etched. These divisions will have different measurement values depending on the power of the microscope objective used. The measurement values are calculated using a stage micrometer etched with a known calibrated scale of 0.1 mm divisions subdivided into 0.01 mm divisions. To calibrate the ocular micrometer, proceed as follows:

1. Remove the eyepiece (10X or other) from the microscope and unscrew the top or bottom lens, depending on its construction. Place the ocular scale on the diaphragm within the eyepiece with the etched surface on the undersurface of the reticule. Screw back the lens and re-insert the eyepiece into the microscope.

2. Place the stage micrometer on the microscope stage and focus the low-power objective on some portion of the scale with the 10X eyepiece.

3. Adjust the stage micrometer by moving the stage so that the 0 line of the ocular micrometer is exactly superimposed on the 0 line of the stage micrometer.



4. Without moving the stage micrometer, find another point at the extreme right where two other lines are exactly superimposed. This second set of superimposed lines should be as far to the right as possible from the 0 lines. This distance will vary with the objective used. At higher magnifications, the thickness of the etched lines may be so great that you need to look for superimposition of either the left or right edge of the individual lines.

5. Count the number of division lines on the ocular micrometer between the 0 line and the point where the second set of lines is superimposed. In the example provided in the figure, this number, indicated by the dotted line, equals 33 ocular units.

6. Then count the number of 0.1 mm division lines between the 0 line and the second superimposed line on the stage micrometer; in the figure, this number, indicated by the arrowhead, equals 0.22 mm.

7. To calculate the length represented by one ocular unit:
   33 ocular units = 0.22 mm
   $$1 \text{ ocular unit} = \frac{0.22}{33} \text{ mm} = 0.0066 \text{ mm} = 6.6 \text{ µm}$$

   Thus, 1 ocular unit = 6.6 µm for this specific objective. Each objective on the microscope must be calibrated separately.

8. When all objectives have been calibrated, prepare a simple chart that displays the calibration factor for each one.

Bench Aids for the Diagnosis of Intestinal Parasites

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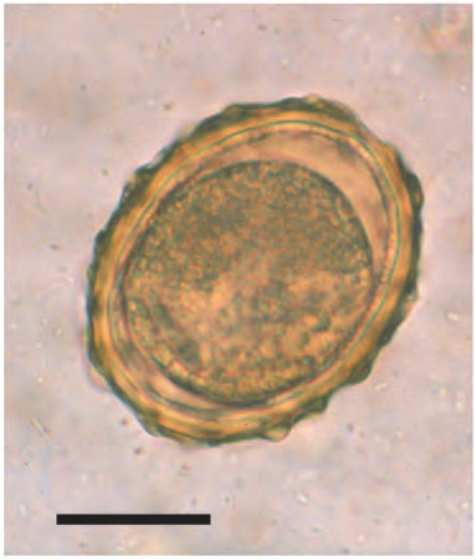
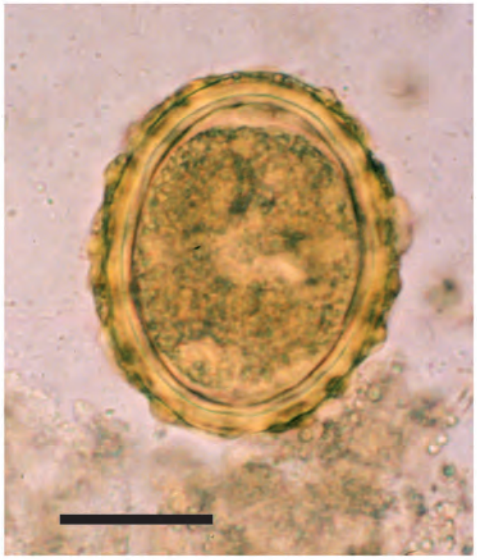
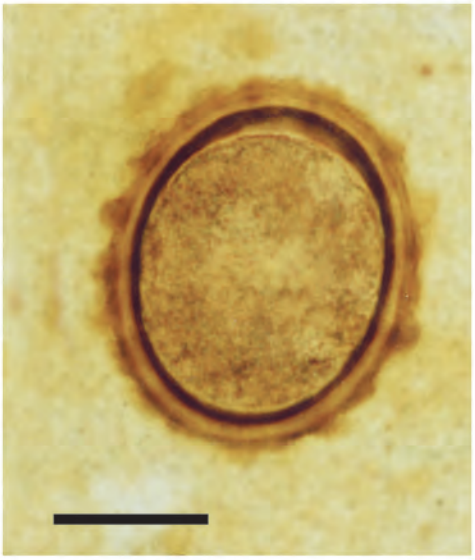
Plate 1 - Helminths

Note: all measuring bars = 25 µm



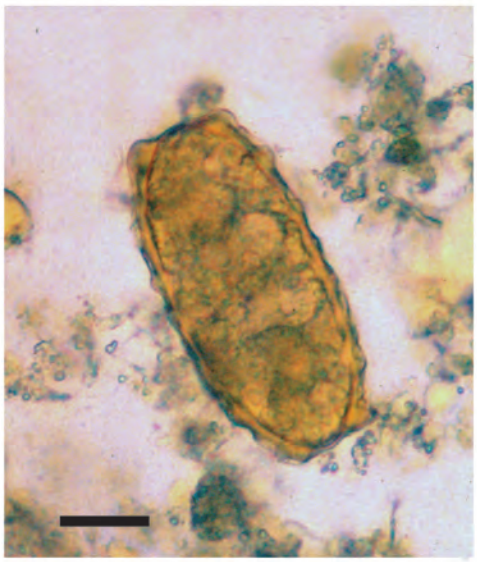
Normal fertile ***Ascaris lumbricoides*** eggs measure 55-75 µm by 35-50 µm, are golden yellow to brown in colour and are in the single cell stage when passed in the faeces. The egg has conspicuous mamillations on its surface.

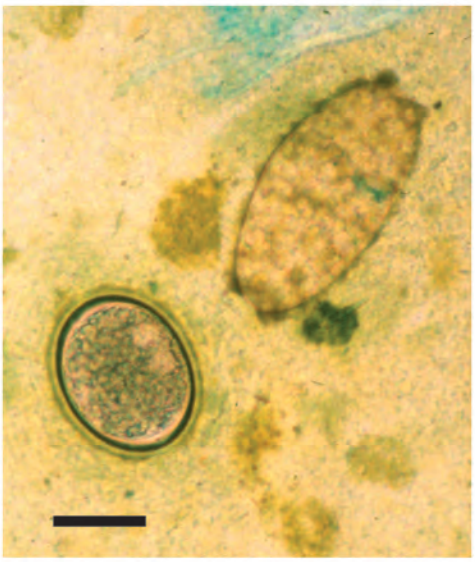




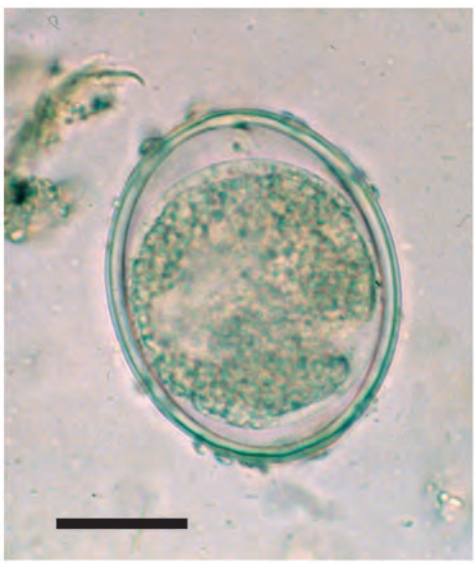
Typical fertile ***A. lumbricoides*** egg as it appears in a Kato-Katz preparation.

Typical infertile ***A. lumbricoides*** eggs in faeces. These eggs are elongated and much larger in size (85-95 µm by 43-47 µm), have thins shells and a grossly irregular mamillated layer. The content of the egg is usually granular and lacks any organization.

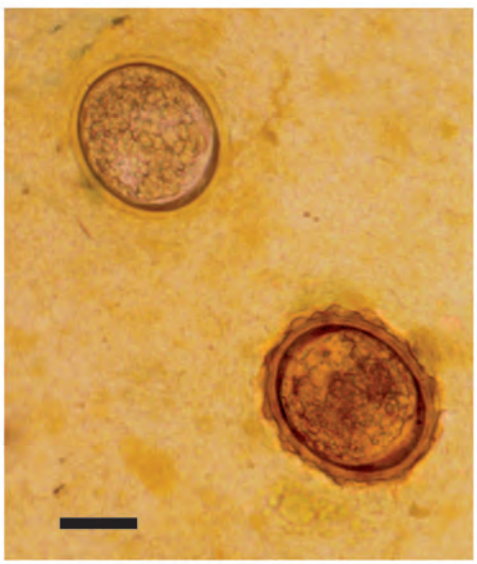




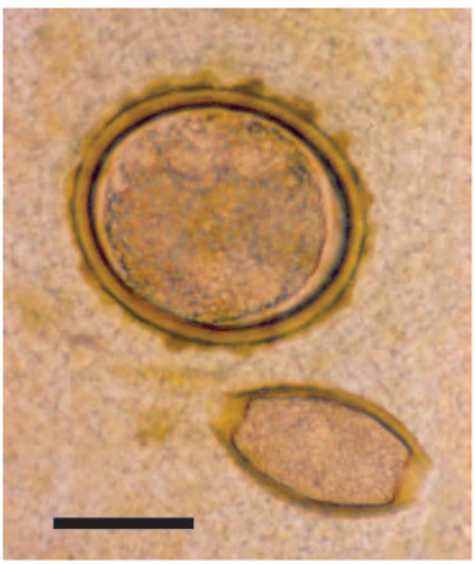
Fertile (lower left) and infertile eggs of ***A. lumbricoides*** in a Kato-Katz preparation.



A. lumbricoides. Sometimes, normal fertile eggs lack the mamillated layer and are referred to as "decorticated" eggs.



Normal and decorticated (upper left) ***A. lumbricoides*** egg in a Kato-Katz preparation.



A. lumbricoides (upper) and ***Trichuris trichiura*** (lower) eggs in a Kato-Katz preparation.

Direct faecal smears - saline and iodine wet mount preparations

Materials and reagents

1. Wooden applicator sticks or matches
2. Microscope slides (75 x 25 mm)
3. Coverslips
4. Pens or markers for indelible labelling
5. Dropping bottles containing:
   isotonic saline solution (0.85%; 8.5 g/l)*
   Lugol's iodine (1% solution)

* For reagent preparation, consult the WHO publication, *Basic laboratory methods in medical parasitology*, 1991 (ISBN 92 4 154410 4).

Procedure

1. With a wax pencil or other marker, write the patient's name or identification number and the date at the left-hand end of the slide.
2. Place a drop of saline in the centre of the left half of the slide and place a drop of iodine solution in the centre of the right half of the slide (Fig.1).
   (*Note*: iodine wet mount preparations are most useful for protozoa, less so for helminths).
3. With an applicator stick or match, pick up a small portion of faeces (approximately 2 mg which is about the size of a match head) and add it to the drop of saline: add a similar portion to the drop of iodine. Mix the faeces with the drops to form suspensions (Fig. 2).
4. Cover each drop with a coverslip by holding the coverslip at an angle, touching the edge of the drop, and gently lowering the coverslip onto the slide so that air bubbles are not produced (Fig. 3).
   (*Note*: ideal preparations containing 2 mg of faeces are uniform - not so thick that faecal debris can obscure organisms, nor so thin that blank spaces are present.)
5. Examine the preparations with the 10X objective or, if needed for identification, higher power objectives of the microscope in a systematic manner (either up and down or laterally) so that the entire coverslip area is observed (Fig. 4). When organisms or suspicious objects are seen, switch to higher magnification to see the more detailed morphology of the object in question.

Fig. 1

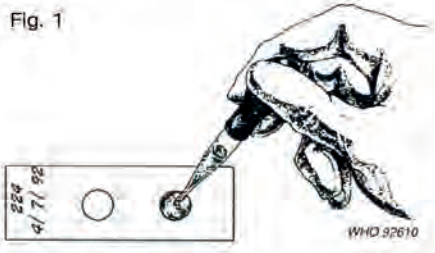


Fig. 2

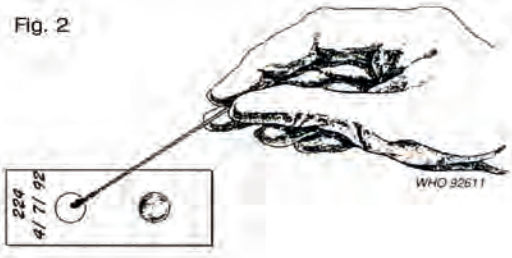


Fig. 3

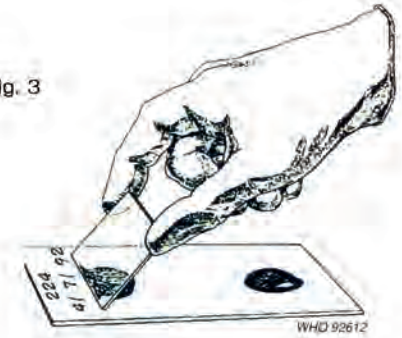
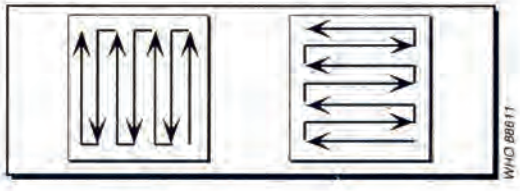


Fig. 4



Bench Aids for the Diagnosis of Intestinal Parasites

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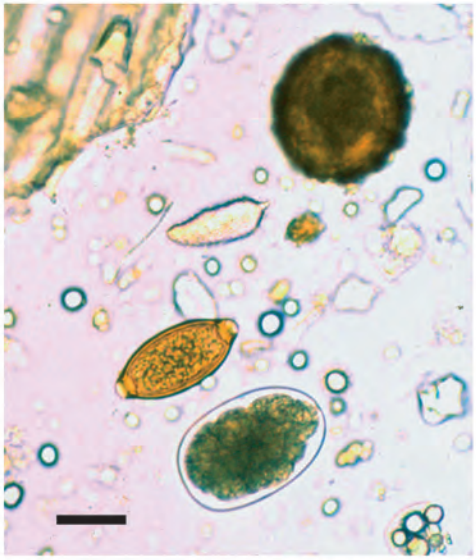
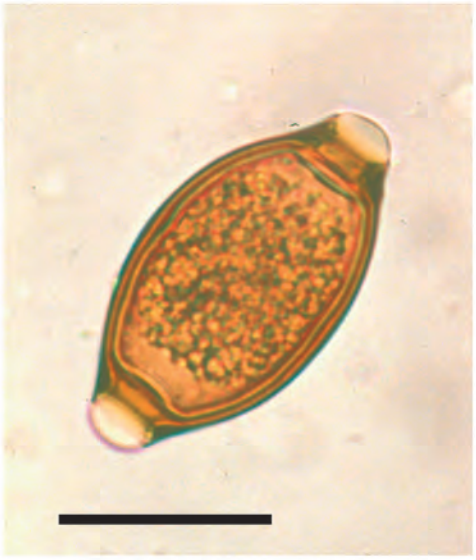
Plate 2 - Helminths

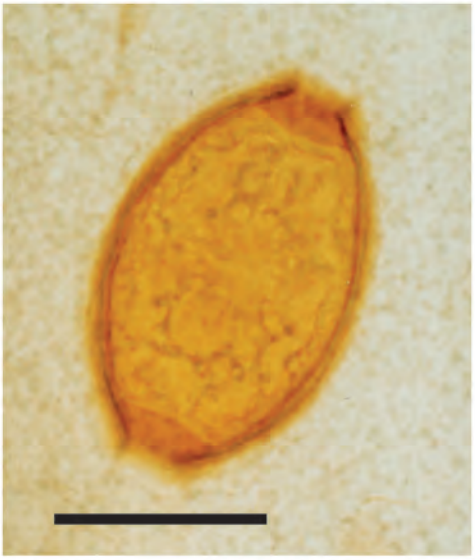
Note: all measuring bars = 25 µm



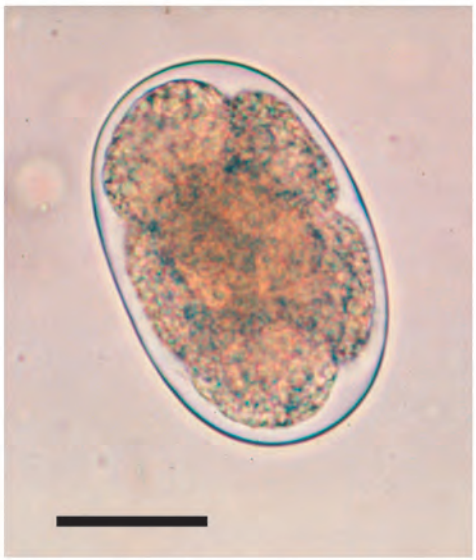
A. lumbricoides (upper), ***Trichuris trichiura*** (middle) and **hookworm** (lower) eggs in the same microscopic field, illustrating their relative sizes.



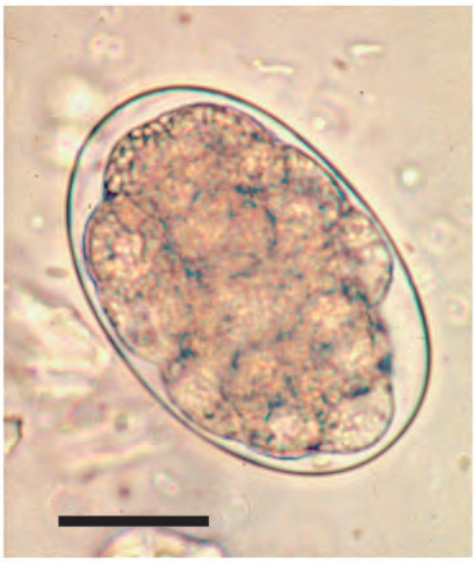
Typical ***Trichuris trichiura*** eggs measure 50-55 µm by 22-24 µm, have a brown, smooth shell, bipolar prominences (plugs) and contain a single-cell ovum.

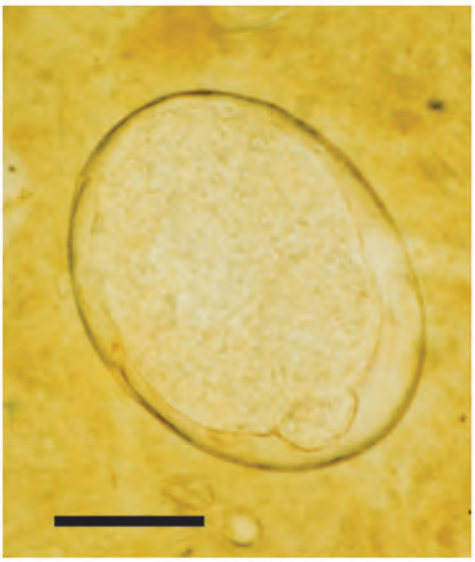


In a Kato-Katz preparation, ***T. trichiura*** eggs may appear larger and swollen with degenerated contents. The bipolar prominences and the layers of the shell are not sharply defined.

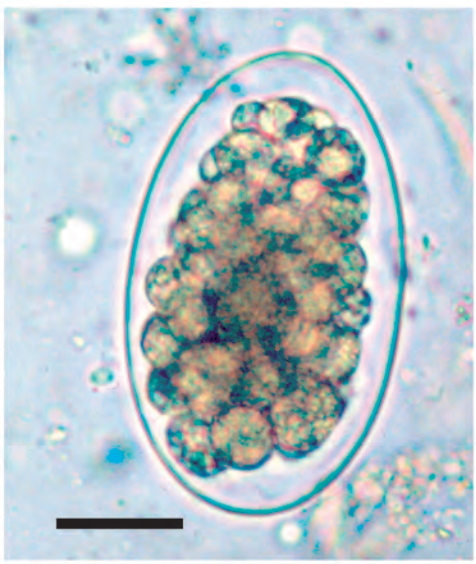


Hookworm eggs found in faeces are characteristically barrel-shaped with a thin, hyaline shell; they measure 60-75 µm by 36-40 µm. They are usually in the 4- or 8- cell stage in fresh faeces or in a more advanced stage of cleavage in faeces that have been kept at room temperature for even a few hours.

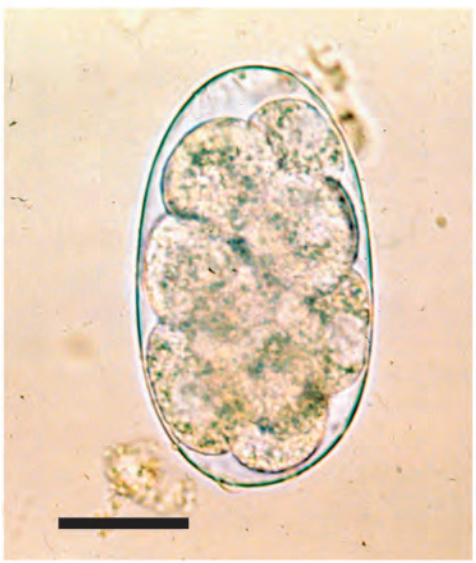




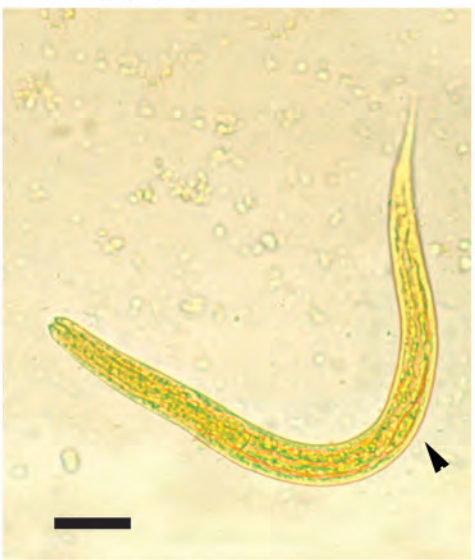
Hookworm eggs in Kato-Katz preparations are often almost round and the dividing ovum is increasingly difficult to see. In hot climates the glycerol will overclear the eggs and make them invisible 30-60 minutes after preparation.



Trichostrongyle eggs resemble hookworm eggs but are larger (75-95 µm by 40-50 µm) and more elongated in shape. The ovum is in an advanced state of cleavage when passed in the faeces.



Ternidens deminutus is another strongyle parasite which infects humans, mostly in southern Africa. The egg resembles the hookworm egg and measures about 85 x 50 µm. It tends to be in an advanced stage of cleavage when passed in the faeces.



Strongyloides stercoralis infection is routinely diagnosed by the presence in faeces of first-stage rhabditoid larvae of 180-380 µm by 14-20 µm. Larvae have a short buccal capsule, an attenuated tail and a prominent genital primordium (arrow).

Faecal concentration procedure - formalin-ether/ethyl acetate/gasoline

Materials and reagents

1. Centrifuge, with head and cups to hold 15 ml conical tubes. Sealed buckets must be used.
2. Centrifuge tubes, 15 ml, conical (make a graduation at 10 ml with a grease pencil).
3. Bottles, dispensing or plastic "squeeze", 250 or 500 ml.
4. Wooden applicator sticks, 145 x 2.0 mm.
5. Small beaker - 25, 50 or 100 ml.
6. 400 µm plastic or metal sieve or surgical gauze.
7. Microscope slides (75 x 25 mm).
8. Coverslips.
9. Pipettes, disposable Pasteur, with rubber bulbs.
10. Rubber stoppers for centrifuge tubes.
11. Rack or support for tubes.
12. Formalin, 10%*.
13. Ether, ethyl acetate or, if these solvents are unavailable, gasoline. (***Caution***: Ether is highly volatile and will ignite and explode quickly if there is an open flame or spark nearby. Store open cans or bottles on an open shelf in the coolest part of the laboratory. Do not put opened containers of ether in a refrigerator as fumes escape, build up and may cause an explosion when the door is opened.)
14. Dropping bottles containing:
    isotonic saline solution (0.85%, 8.5 g/l).
    Lugol's iodine (1% solution).

* For reagent preparation, consult the WHO publication, *Basic laboratory methods in medical parasitology*, 1991 (ISBN 92 4 154410 4).

Fig. 1

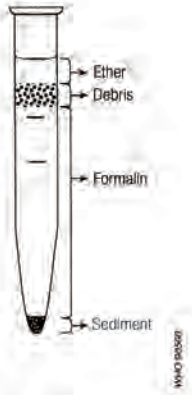


Fig. 2

Procedure

1. With an applicator stick add 1.0-1.5 g of faeces to 10 ml of formalin in a centrifuge tube and stir to form a suspension.
2. Strain the suspension through the 400 µm mesh sieve or 2 layers of wet surgical gauze directly into a different centrifuge tube or into a small beaker. Discard the gauze.
3. Add more 10% formalin to the suspension in the tube to bring the total volume to 10 ml.
4. Add 3.0 ml of ether (or ethyl acetate or gasoline) to the suspension in the tube and mix well by putting a rubber stopper in the tube and shaking vigorously for 10 seconds.
5. Remove the stopper and place the tube in the centrifuge; balance the tubes and centrifuge at 400-500*g* for 2-3 minutes.
6. Remove the tube from the centrifuge; the contents consist of 4 layers: (a) top layer of ether (or ethyl acetate or gasoline), (b) a plug of fatty debris that is adherent to the wall of the tube, (c) a layer of formalin, and (d) sediment (Fig.1).
7. Gently loosen the plug of debris with an applicator stick by a spiral movement and pour

Fig. 3