

Bench Aids **for the** **diagnosis of** **intestinal** **parasites**



**World Health
Organization**

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Cultured trophozoites of an isolate of *Giardia* spp. stained with Giemsa.
Courtesy of Nicolette Binz, WHO Collaborating Centre for the Molecular Epidemiology of Parasitic
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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.

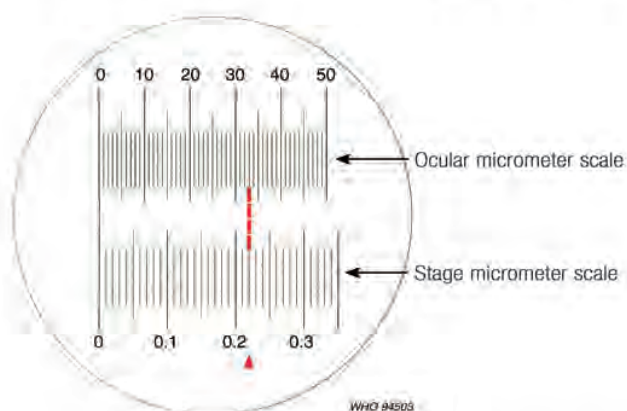
Ash LR, Orihel TC. *Atlas of human parasitology*, 3rd ed. Chicago, ASCP Press, 1990.

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.



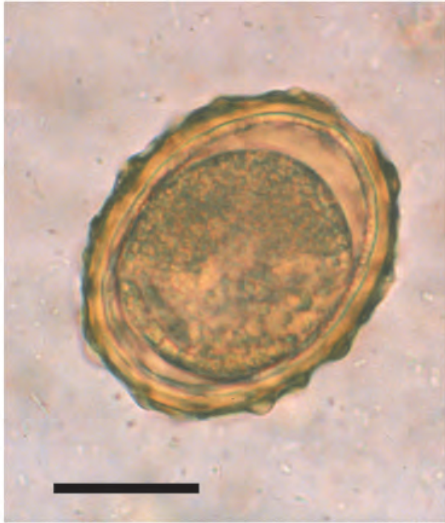
4. Without moving the stage micrometer, find another point at the extreme right where two other lines are exactly superimposed. This second set of superimposed lines should be as far to the right as possible from the 0 lines. This distance will vary with the objective used. At higher magnifications, 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:

$$33 \text{ ocular units} = 0.22 \text{ mm}$$

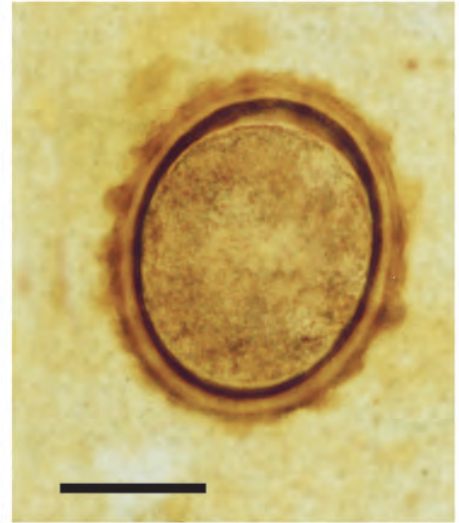
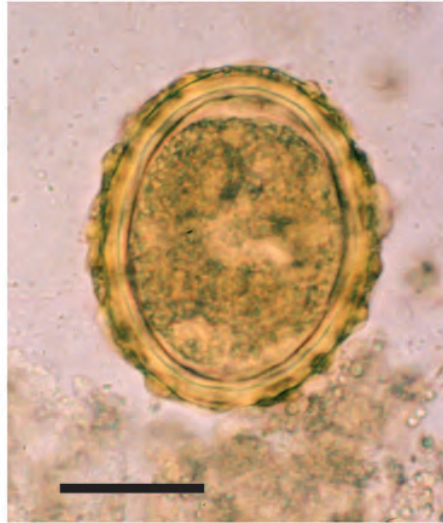
$$1 \text{ ocular unit} = \frac{0.22 \text{ mm}}{33} = 0.0066 \text{ mm} = 6.6 \mu\text{m}$$

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

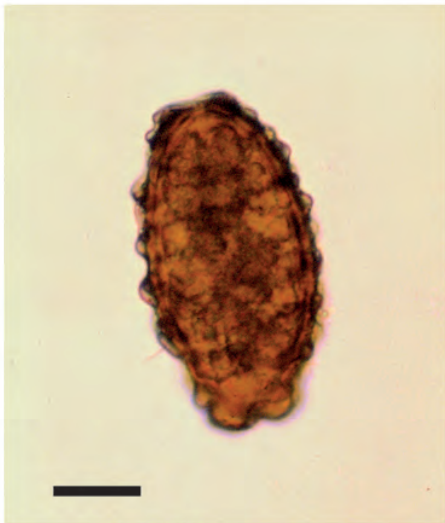
8. When all objectives have been calibrated, prepare a simple chart that displays the calibration factor for each one.



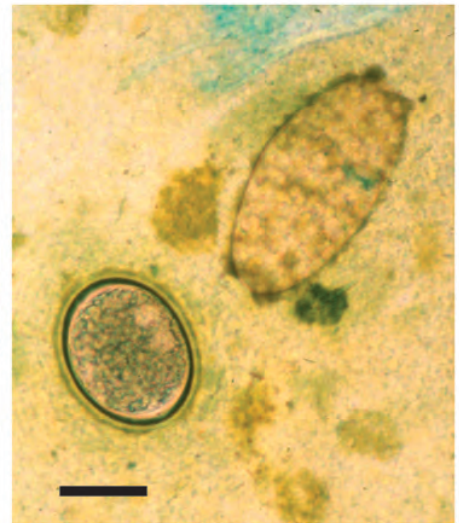
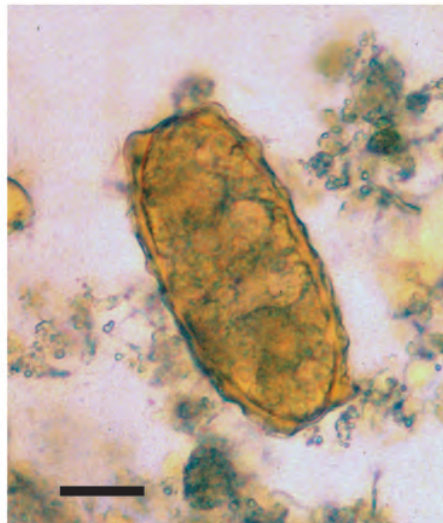
Normal fertile *Ascaris lumbricoides* eggs measure 55-75 µm by 35-50 µm, are golden yellow to brown in colour and are in the single cell stage when passed in the faeces. The egg has conspicuous mammillations on its surface.



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



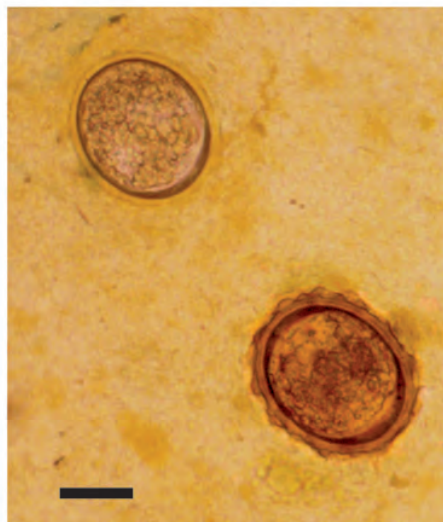
Typical infertile *A. lumbricoides* eggs in faeces. These eggs are elongated and much larger in size (85-95 µm by 43-47 µm), have thin shells and a grossly irregular mammillated layer. The content of the egg is usually granular and lacks any organization.



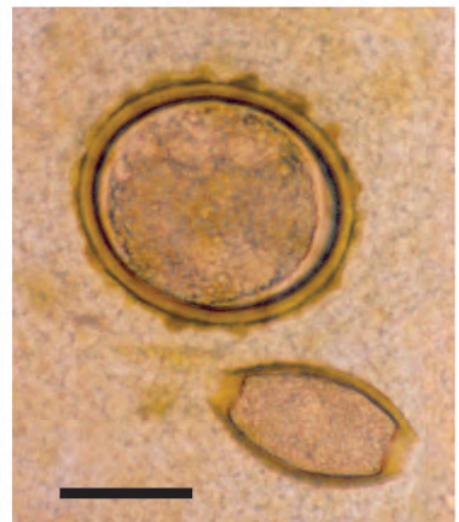
Fertile (lower left) and infertile eggs of *A. lumbricoides* in a Kato-Katz preparation.



A. lumbricoides. Sometimes, normal fertile eggs lack the mammillated 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

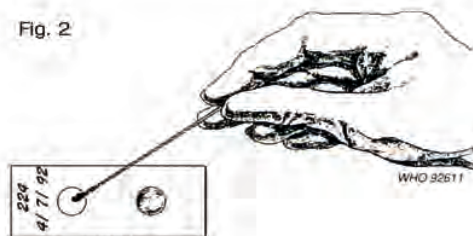
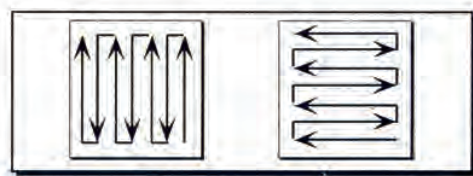
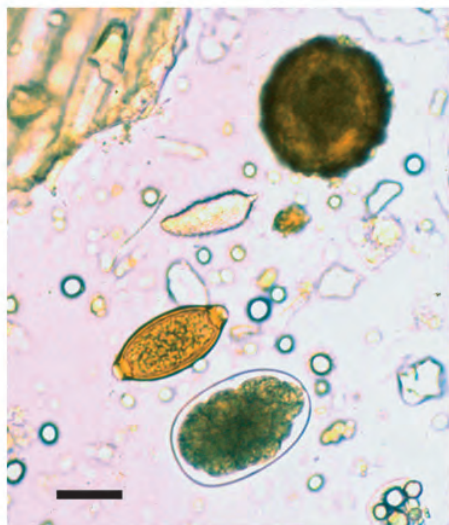


Fig. 3



Fig. 4

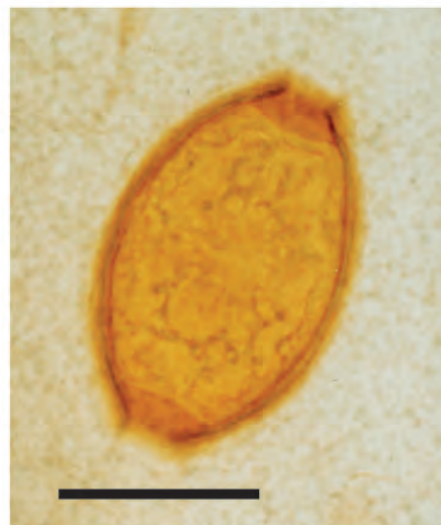




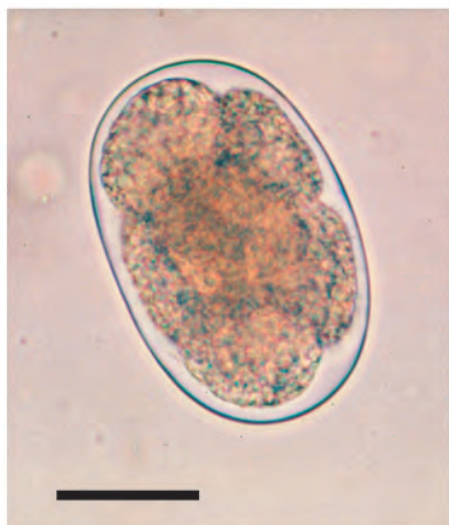
A. lumbricoides (upper), *Trichuris trichiura* (middle) and hookworm (lower) eggs in the same microscopic field, illustrating their relative sizes.



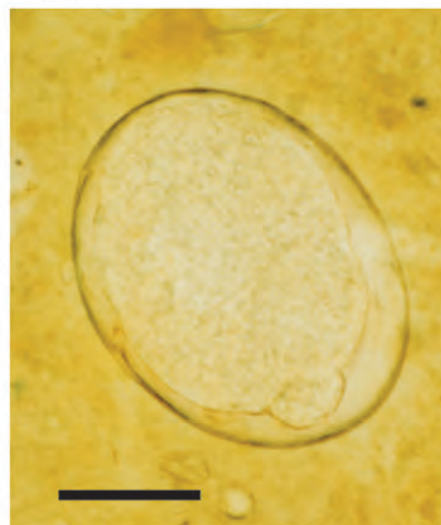
Typical *Trichuris trichiura* eggs measure 50-55 µm by 22-24 µm, have a brown, smooth shell, bipolar prominences (plugs) and contain a single-cell ovum.



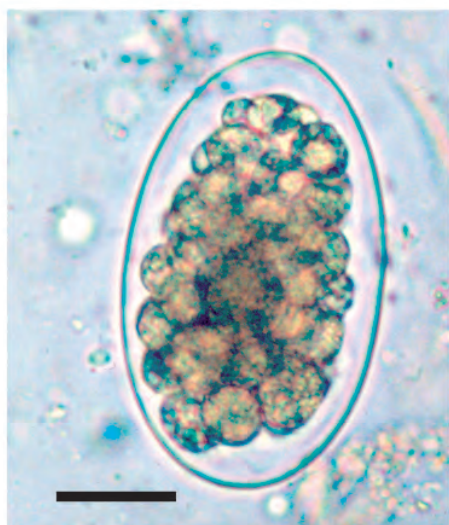
In a Kato-Katz preparation, *T. trichiura* eggs may appear larger and swollen with degenerated contents. The bipolar prominences and the layers of the shell are not sharply defined.



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

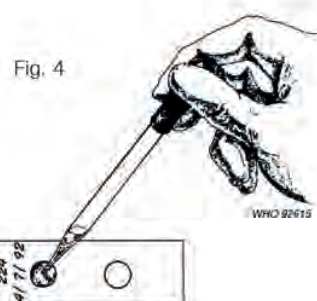
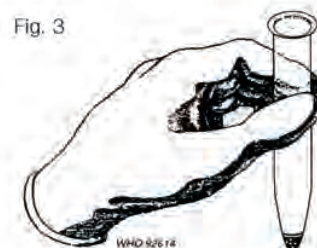
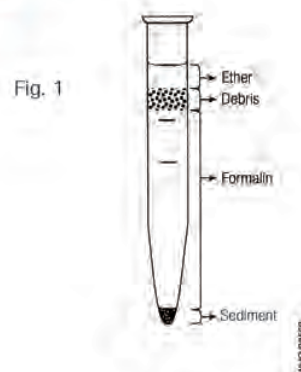
Materials and reagents

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).

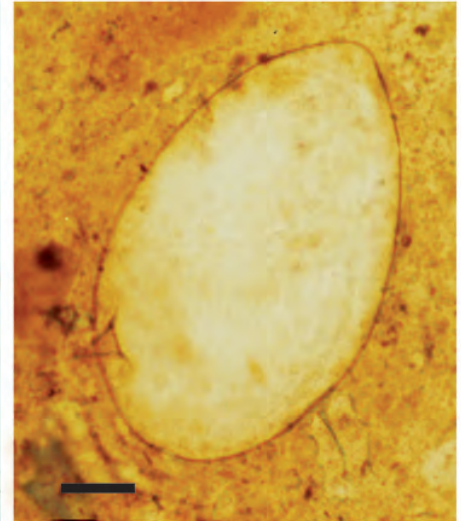
Procedure

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 400-500g 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 off the top 3 layers in a single movement, allowing the tube to drain inverted for at least five seconds. When done properly a small amount of residual fluid from the walls of the tube will flow back onto the sediment (Fig. 2, 3).
8. Mix the fluid with the sediment (sometimes it is necessary to add a drop of saline to have sufficient fluid to suspend the sediment) with a disposable glass pipette. Transfer a drop of the suspension to a slide for examination under a coverslip; an iodine-stained preparation can also be made (Fig. 4).
9. Examine the preparations with the 10X objective or, if needed for identification, higher power objectives of the microscope in a systematic manner so that the entire coverslip area is observed (see Plate 1, Fig. 4). When organisms or suspicious objects are seen, switch to higher magnification to see more detailed morphology of the object in question.





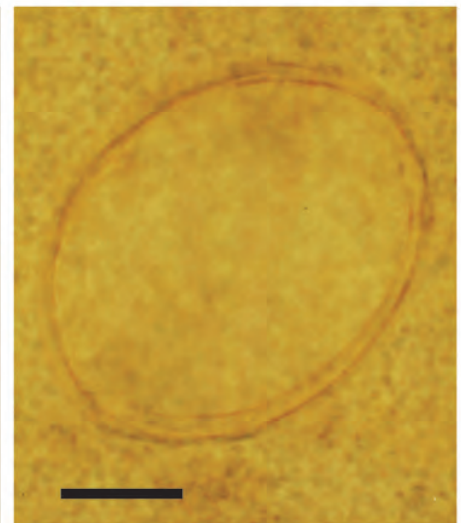
Schistosoma mansoni eggs are large, measuring 114-175 µm by 45-70 µm, have a thin, transparent shell and a prominent lateral spine, and contain a miracidium. If the spine is hidden from view, gently tapping the coverslip may expose it.



S. mansoni eggs in Kato-Katz preparations are easily identified on the basis of size, shape and presence of the lateral spine.



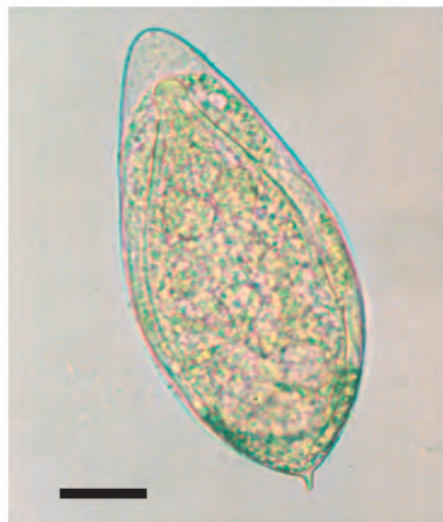
S. japonicum eggs are smaller than those of *S. mansoni* and *S. haematobium*. They measure 70-100 µm by 55-65 µm and tend to be round to oval in shape, have a thin shell and a small, inconspicuous, lateral spine. The eggs contain a miracidium. Frequently, faecal debris adherent to the egg surface or orientation may obscure the spine.



In Kato-Katz preparations, the spine of the egg of *S. japonicum* is rarely seen and the miracidium quickly becomes inapparent. Size and thin shell help identify the species.



The eggs of *S. haematobium* have a terminal spine and contain a miracidium. They measure 112-170 µm by 50-70 µm. These eggs are usually found in the urine but occasionally they may also be found in faeces.



The eggs of *S. intercalatum* are usually larger than those of *S. haematobium*, measure about 140-240 µm, are typically found in faeces and have an equatorial bulge.



Kato-Katz technique - cellophane faecal thick smear

Materials and reagents

1. Applicator sticks, wooden.
2. Screen, stainless steel, nylon or plastic; 60-105 mesh (Fig. 1).
3. Template, stainless steel, plastic, or cardboard (Fig. 1). Templates of different sizes have been produced in different countries. A hole of 9 mm on a 1 mm thick template will deliver 50 mg of faeces; a hole of 6 mm on a 1.5 mm thick template, 41.7 mg; and a hole of 6.5 mm on a 0.5 mm thick template, 20 mg. The templates should be standardized in the country and the same size of templates should always be used to ensure repeatability and comparability of prevalence and intensity data.
4. Spatula, plastic (Fig. 1).
5. Microscope slides (75 x 25 mm).
6. Hydrophilic cellophane, 40-50 µm thick, strips 25 x 30 or 25 x 35 mm in size (Fig. 2).
7. Flat-bottom jar with lid (Fig. 2).
8. Forceps.
9. Toilet paper or absorbent tissue.
10. Newspaper.
11. Glycerol-malachite green or glycerol-methylene blue solution (1 ml of 3% aqueous malachite green or 3% methylene blue is added to 100 ml of glycerol and 100 ml of distilled water and mixed well). This solution is poured onto the cellophane strips in a jar and left for at least 24 h prior to use.

Procedure

1. Place a small mound of faecal material on newspaper or scrap paper and press the small screen on top so that some of the faeces are sieved through the screen and accumulate on top (Fig. 3).
2. Scrape the flat-sided spatula across the upper surface of the screen to collect the sieved faeces (Fig. 4).
3. Place template with hole on the centre of a microscope slide and add faeces from the spatula so that the hole is completely filled (Fig. 5). Using the side of the spatula pass over the template to remove excess faeces from the edge of the hole (the spatula and screen may be discarded or, if carefully washed, may be reused).
4. Remove the template carefully so that the cylinder of faeces is left on the slide.
5. Cover the faecal material with the pre-soaked cellophane strip (Fig. 6). The strip must be very wet if the faeces are dry and less so if the faeces are soft (if excess glycerol solution is present on upper surface of cellophane, wipe with toilet paper). In dry climates, excess glycerol will retard but not prevent drying.
6. Invert the microscope slide and firmly press the faecal sample against the hydrophilic cellophane strip on another microscope slide or on a smooth hard surface such as a piece of tile or a flat stone. The faecal material will be spread evenly between the microscope slide and the cellophane strip (Fig. 7). It should be possible to read newspaper print through the smear after clarification (Fig. 8).
7. Carefully remove slide by gently sliding it sideways to avoid separating the cellophane strip or lifting it off. Place the slide on the bench with the cellophane upwards. Water evaporates while glycerol clears the faeces.
8. For all except hookworm eggs, keep slide for one or more hours at ambient temperature to clear the faecal material prior to examination under the microscope. To speed up clearing and examination, the slide can be placed in a 40 °C incubator or kept in direct sunlight for several minutes.
9. *Ascaris* and *Trichuris* eggs will remain visible and recognizable for many months in these preparations. Hookworm eggs clear rapidly and will no longer be visible after 30-60 minutes. Schistosome eggs may be recognizable for up to several months but it is preferable in a schistosomiasis endemic area to examine the slide preparations within 24 hours.
10. The smear should be examined in a systematic manner (see Plate 1, Fig. 4) and the number of eggs of each species reported. Later multiply by the appropriate number to give the number of eggs per gram of faeces (by 20 if using a 50 mg template; by 50 for a 20 mg template; and by 24 for a 41.7 mg template). With high egg counts, to maintain a rigorous approach while reducing reading time, the Stoll quantitative dilution technique with 0.1 mol/litre NaOH may be recommended (see *Basic laboratory methods in medical parasitology*, WHO, 1991).

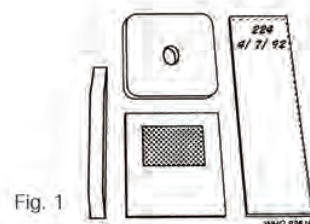


Fig. 1

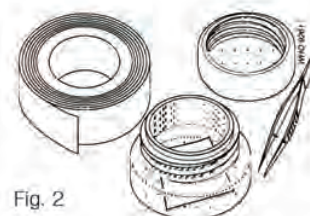


Fig. 2



Fig. 3

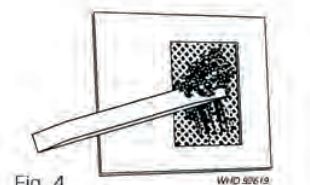


Fig. 4



Fig. 5

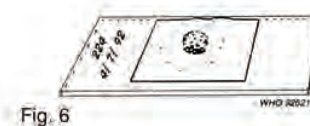


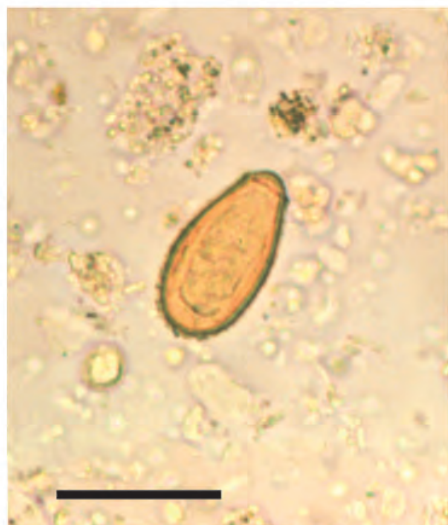
Fig. 6



Fig. 7



Fig. 8



Clonorchis sinensis eggs are 27-35 µm by 12-19 µm, have a seated operculum and usually a small protuberance at the opposite end. The shell may have minute adherent debris. Eggs in faeces contain a miracidium. ***Opisthorchis*** eggs are similar.



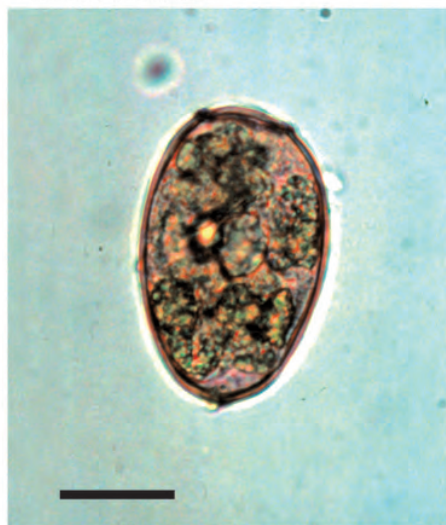
Metagonimus yokogawai eggs measure 20-30 µm by 15-17 µm, have an inconspicuous operculum and lack a knob or protuberance at the abopercular end; the shell is usually devoid of adherent debris. Eggs in faeces contain a miracidium.



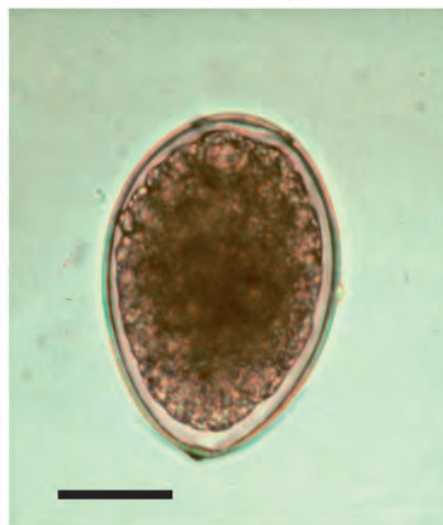
Fasciola hepatica eggs are usually 130-150 µm by 63-90 µm, have an inconspicuous operculum, are unembryonated, and often have a shell irregularity at the abopercular end (the latter is not seen in the similar ***Fasciolopsis buski*** egg).



Paragonimus westermani eggs measure 80-120 µm by 45-70 µm, are golden brown in colour, thick shelled, unembryonated in faeces or in sputum and have a prominent operculum. The shell is thickened at the abopercular end.



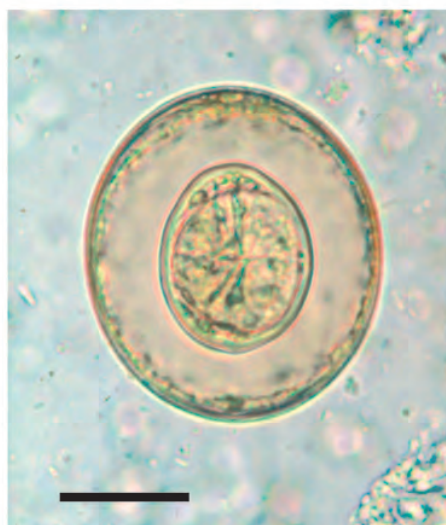
P. uterobilateralis eggs, an African species, are usually smaller than those of ***P. westermani***, i.e. 50-95 µm by 35-55 µm, and the operculum is less prominent.



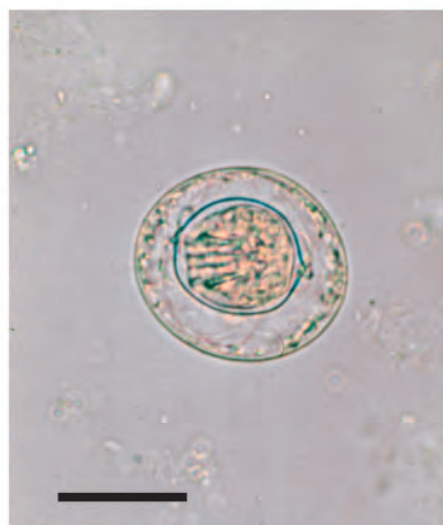
Diphylobothrium latum. These operculated cestode eggs usually measure 58-75 µm by 40-50 µm, are unembryonated in faeces and may have a knob or small protuberance at the abopercular end.



***Taenia* spp.** eggs are all virtually identical in size and morphology, i.e. 31-43 µm in diameter, with a thick prismatic-appearing shell wall, and contain a 6-hooked embryo, the oncosphere. Occasionally a thin, hyaline primary embryonic membrane may be retained around these eggs.

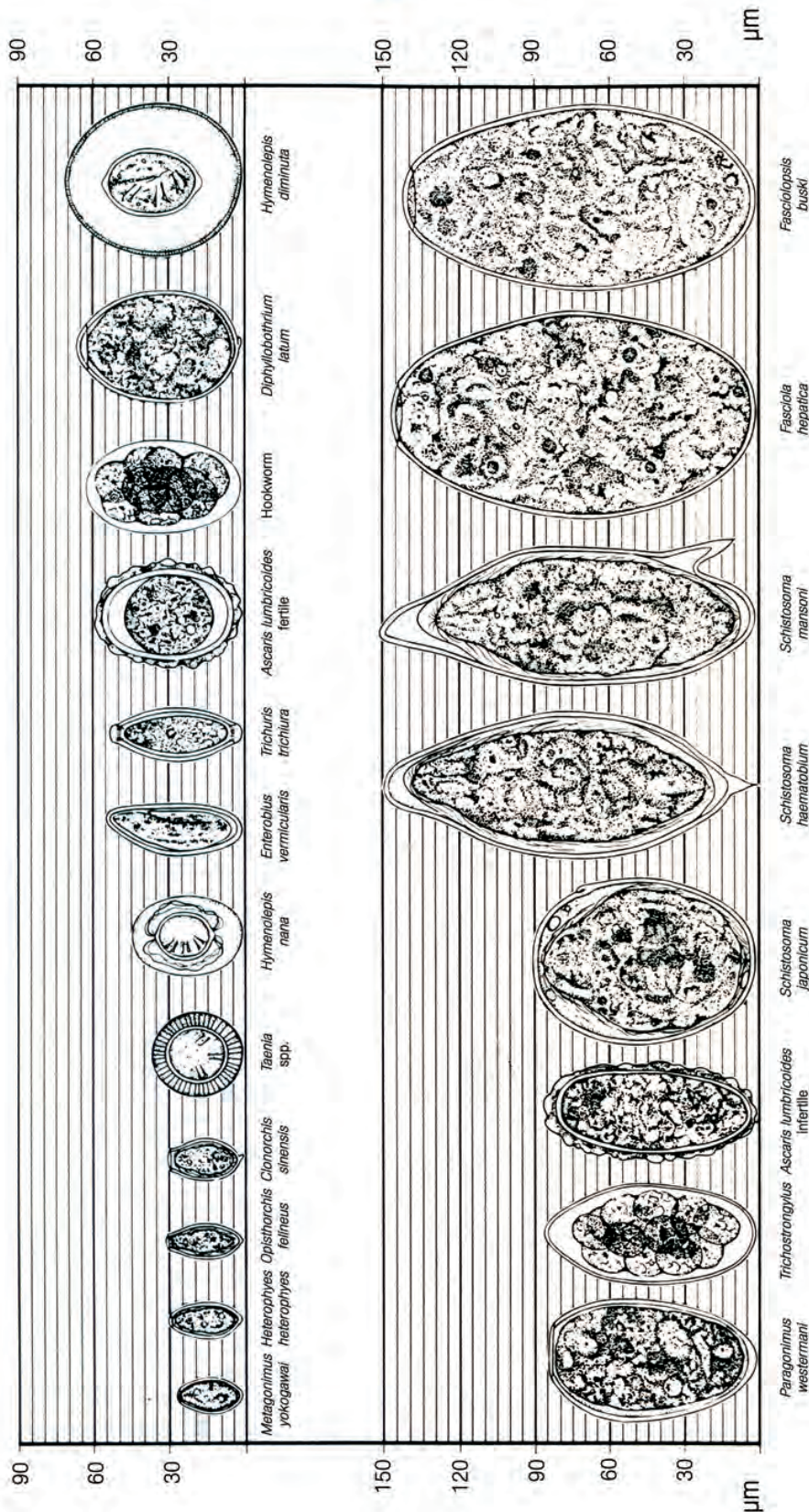


Hymenolepis diminuta eggs measure 70-85 µm by 60-80 µm, are spherical, yellowish brown, and contain a 6-hooked embryo, the oncosphere. There are no filaments, as in ***H. nana***.



Hymenolepis nana eggs are usually spherical, 30-47 µm in diameter, have a thin hyaline shell and contain a 6-hooked oncosphere. There are two polar thickenings on the membrane around the oncosphere from which arise 4-8 filaments extending into the space between the oncosphere and the outer shell.

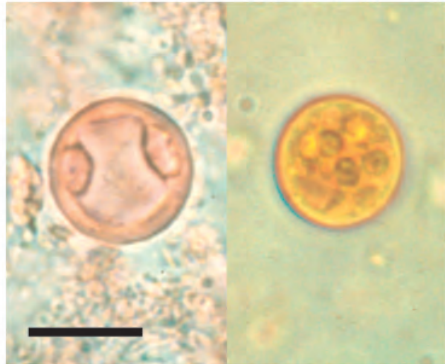
Relative size of helminth eggs*



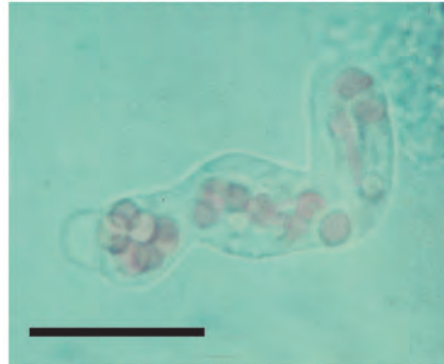
WHO 90580

* *Schistosoma mekongi* and *Schistosoma intercalatum* have been omitted. Eggs of *S. mekongi* measure 51-78 µm by 39-66 µm; eggs of *S. intercalatum* measure 140-240 µm long.

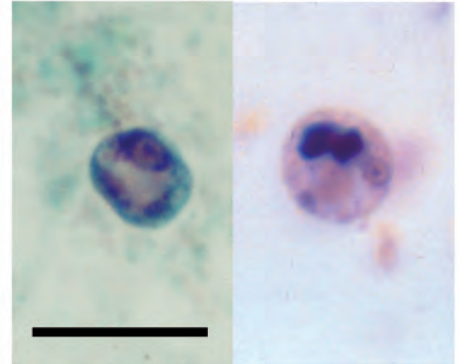
Entamoeba histolytica and *Entamoeba hartmanni*



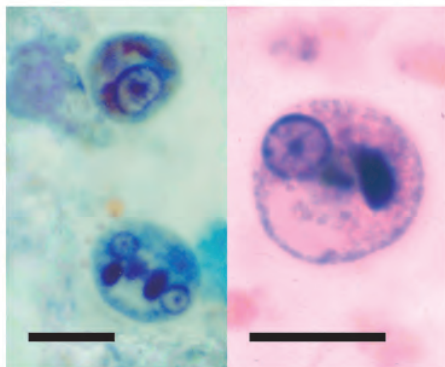
Left: *E. histolytica* binucleate cyst in MIF wet mount; large glycogen vacuole lies between nuclei. Right: *E. histolytica* mature cyst in iodine wet mount; 3 of the 4 nuclei are seen.



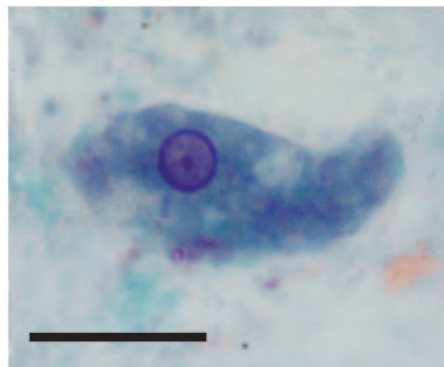
E. histolytica living trophozoite containing many red blood cells; unstained wet mount.



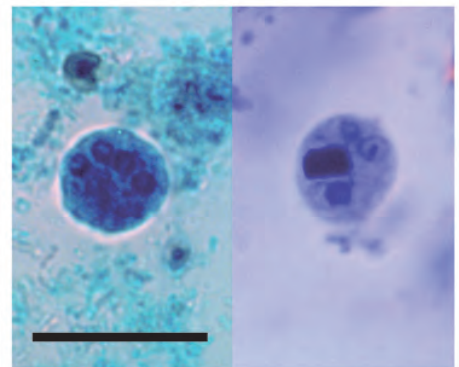
E. hartmanni uninnucleate cysts. Left: glycogen vacuole and chromatoid bodies present; trichrome. Right: chromatoid bodies present; iron haematoxylin.



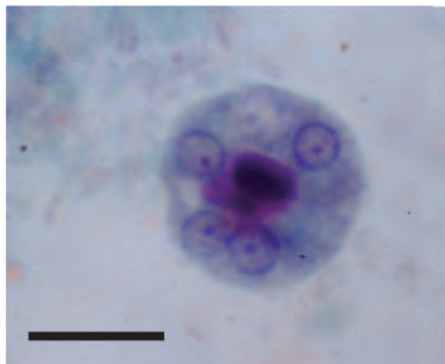
Left: *E. histolytica* uninnucleate cyst (top) and binucleate cyst, each with glycogen vacuole and chromatoid bodies; trichrome. Right: *E. histolytica* uninnucleate cyst with chromatoid bodies present; iron haematoxylin.



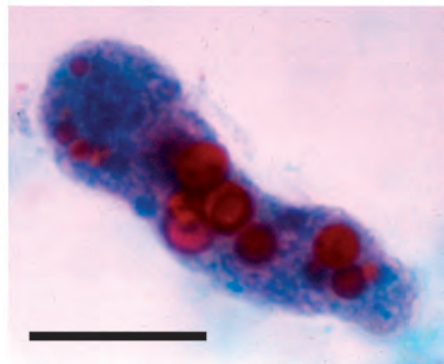
E. histolytica trophozoite; trichrome.



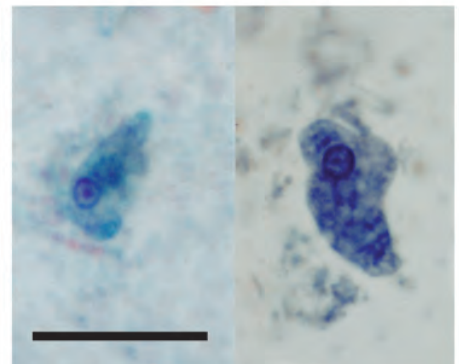
Left: *E. hartmanni* mature cyst with 4 nuclei; trichrome. Right: binucleate cyst with 1 nucleus in sharp focus and chromatoid bodies present; iron haematoxylin.



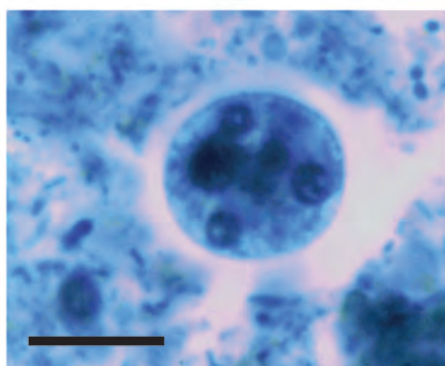
E. histolytica mature cyst with 4 nuclei and chromatoid bodies; trichrome.



E. histolytica trophozoite with ingested, red-staining erythrocytes; nucleus visible along lower margin of organism; trichrome.



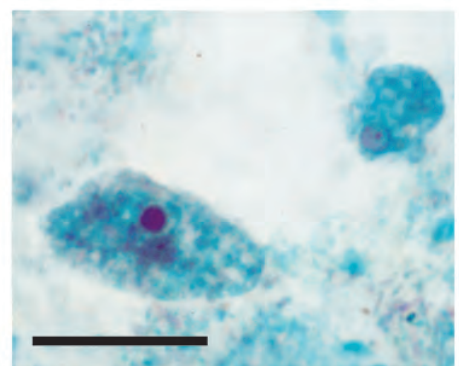
E. hartmanni trophozoites. Left: trichrome. Right: iron haematoxylin.



E. histolytica mature cyst showing 3 of the 4 nuclei and chromatoid bodies which are not in focus; iron haematoxylin.



E. histolytica trophozoite; iron haematoxylin.



E. hartmanni (right) and *Iodamoeba butschlii* (left) trophozoites; trichrome. Note size difference.



Staining procedures for protozoa in faeces

The use of Lugol's iodine for staining wet mount preparations from fresh or formalin-preserved faecal specimens is described on Plate 1. Here are presented some procedures for permanent staining of smears prepared from fresh, PVA- or SAF-preserved faecal material. Many details of preparation of faecal smears and the application of various staining procedures are also presented in the references listed in the Introduction.

Permanent stains for faecal smears

A. Trichrome stain

Use. Very good stain for fresh and PVA-preserved faecal smears: does not give good staining results with SAF preservation.

Preparation. Add 10 ml of glacial acetic acid to 6 g of chromotrope 2R, 3 g of light green SF and 7 g of phosphotungstic acid in a clean flask. Swirl to mix and let stand for 30 min. Add 1000 ml of distilled water and mix thoroughly; the stain should be a deep purple. Store in a glass-stoppered bottle; the stain is stable and is used undiluted.

Staining procedure. Place slides, fixed in either Schaudinn's fixative or PVA, into 70% alcohol for 2 min. Add Lugol's diluted iodine solution to 70% ethanol to produce a colour of strong tea: place slides in the solution for 5 min. Place slides in two changes of 70% alcohol. Stain slides in undiluted trichrome stain for 10 min. Remove slides, drain thoroughly, and place them in 90% acidified alcohol (prepared by adding 4.5 ml of glacial acetic acid to 1 litre of 90% ethanol) for 2-3 seconds. Dip slides in 95% alcohol to rinse and then dehydrate through 100% ethanol and xylene or through carbol-xylene mixture. Using resinous mounting medium, place a coverslip on the smear.

B. Iron haematoxylin stain

Use. Very good stain for fresh, PVA- or SAF-preserved faecal smears.

Preparation.

Stock solution A: dissolve 1 g of haematoxylin crystals in 100 ml of 95% alcohol; allow solution to stand in light for 1 week and then filter.

Stock solution B: mix 1 g of ferrous ammonium sulfate, 1 g of ferric ammonium sulfate and 1 ml of hydrochloric acid in 97 ml of distilled water.

Prepare a working solution by combining 25 ml each of stock solutions A and B; prepare at least 3-4 h prior to staining. Prepare picric acid solution for destaining by adding 25 ml of saturated aqueous picric acid to 25 ml of distilled water.

Staining procedure. Place slides into 70% alcohol for 5 min; into 50% alcohol for 2 min; into tapwater for 5 min; into working haematoxylin stain solution for 10 min; into distilled water for 1 min; into picric acid solution for 1 min; into running tapwater for 10 min; into 70% alcohol containing 1 drop of ammonia for 5 min; and into 95% alcohol for 5 min. Dehydrate through 100% ethanol and xylene or through carbol-xylene mixture. Using resinous mounting medium, place a coverslip on the smear.

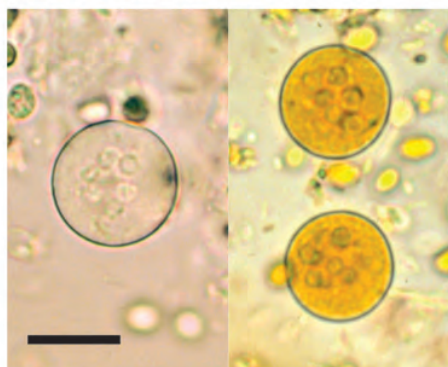
C. Modified Ziehl-Neelsen technique (acid-fast stain)

Use. For detection of *Cryptosporidium*, *Cyclospora*, and other coccidian infections.

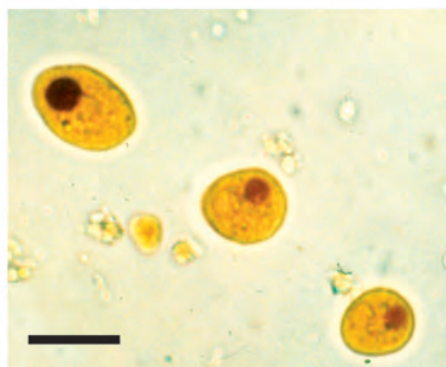
Reagents. Carbol-fuchsin, formalin, HCl-ethanol solution, glycerol-malachite green (or methylene blue) solution, HCl-methanol solution. For preparation of reagents see the WHO publication *Basic laboratory methods in medical parasitology*, 1991.

Staining procedure. Prepare a thin smear of faeces; air-dry and fix in methanol for 2-3 min. Stain with cold carbol-fuchsin for 5-10 min. Differentiate in 1% HCl-ethanol until colour ceases to flow out of smear. Rinse in tapwater. Counterstain with 0.25% malachite green (or methylene blue) for 30 sec. Rinse in tapwater. Blot or drain dry.

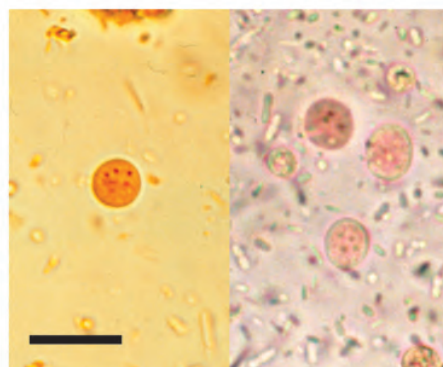
Commensal amoebae



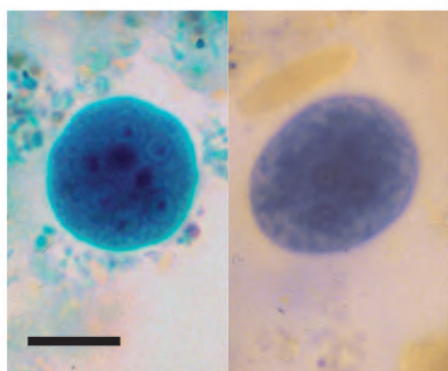
Entamoeba coli mature cysts. Left: unstained formalin wet mount. Right: iodine-stained wet mount.



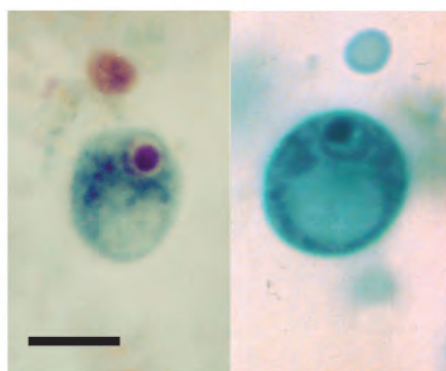
Iodamoeba bütschlii cysts in iodine wet mount. Note brown-staining glycogen vacuoles in each; the nucleus is typically not visible in such preparations.



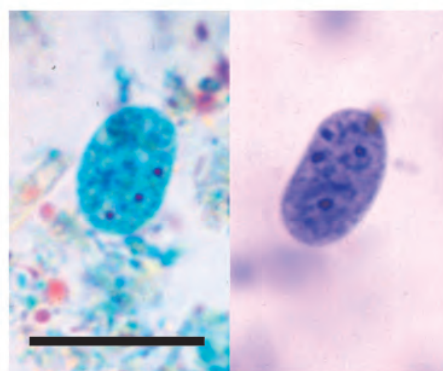
Endolimax nana cysts in wet mounts. Left: a cyst, stained in iodine, shows 3 of the 4 nuclei. Right: 3 cysts in MIF, with the top one showing 3 of the 4 nuclei.



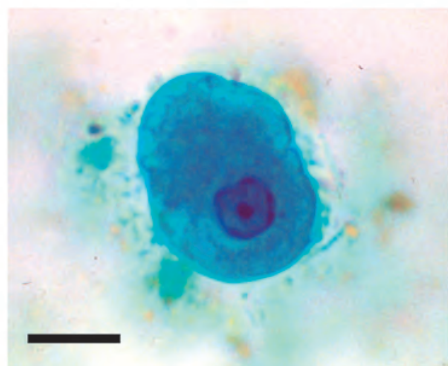
E. coli mature cysts stained in trichrome (left) and in iron haematoxylin (right).



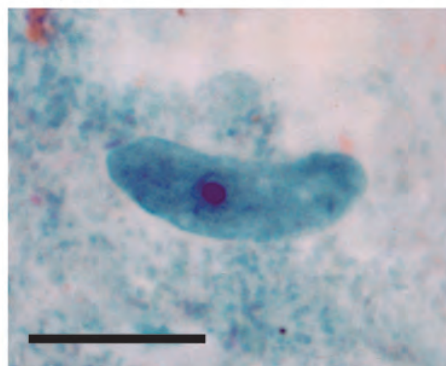
Cysts of *I. bütschlii*. On the left, stained with trichrome, vacuole is not as clearly seen as it is on the right, stained with iron haematoxylin; with these stains the single nucleus with the large karyosome is readily seen.



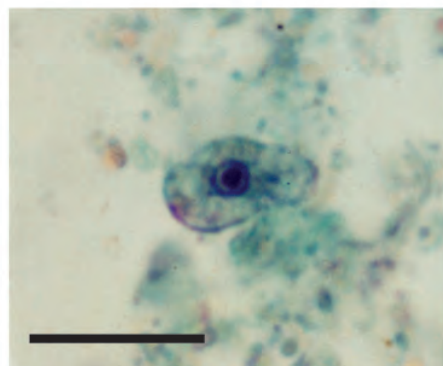
E. nana cysts. Left: 3 of 4 nuclei visible; trichrome. Right: all 4 nuclei seen; iron haematoxylin.



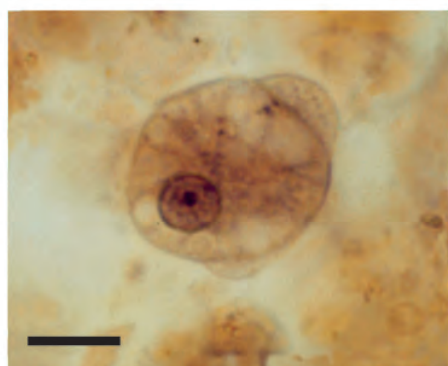
E. coli trophozoite, trichrome. Note irregular peripheral chromatin on nuclear membrane.



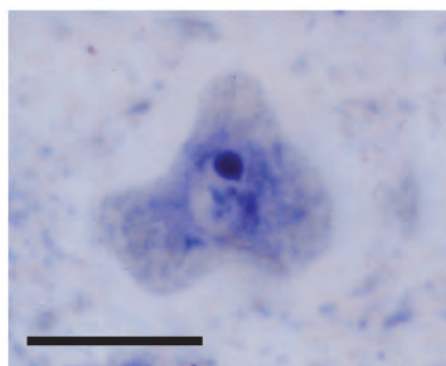
I. bütschlii trophozoite, trichrome.



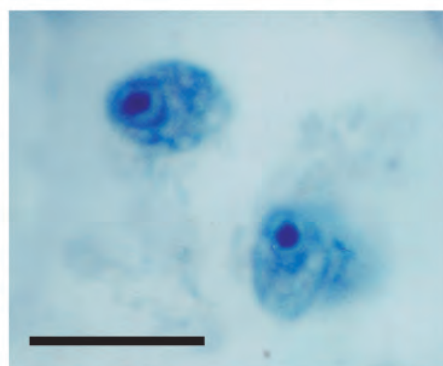
E. nana trophozoite, trichrome. Small size of organism and the large karyosome nearly filling the nucleus which lacks peripheral chromatin are diagnostic.



E. coli trophozoite, iron haematoxylin. Note large, off-center karyosome in nucleus.

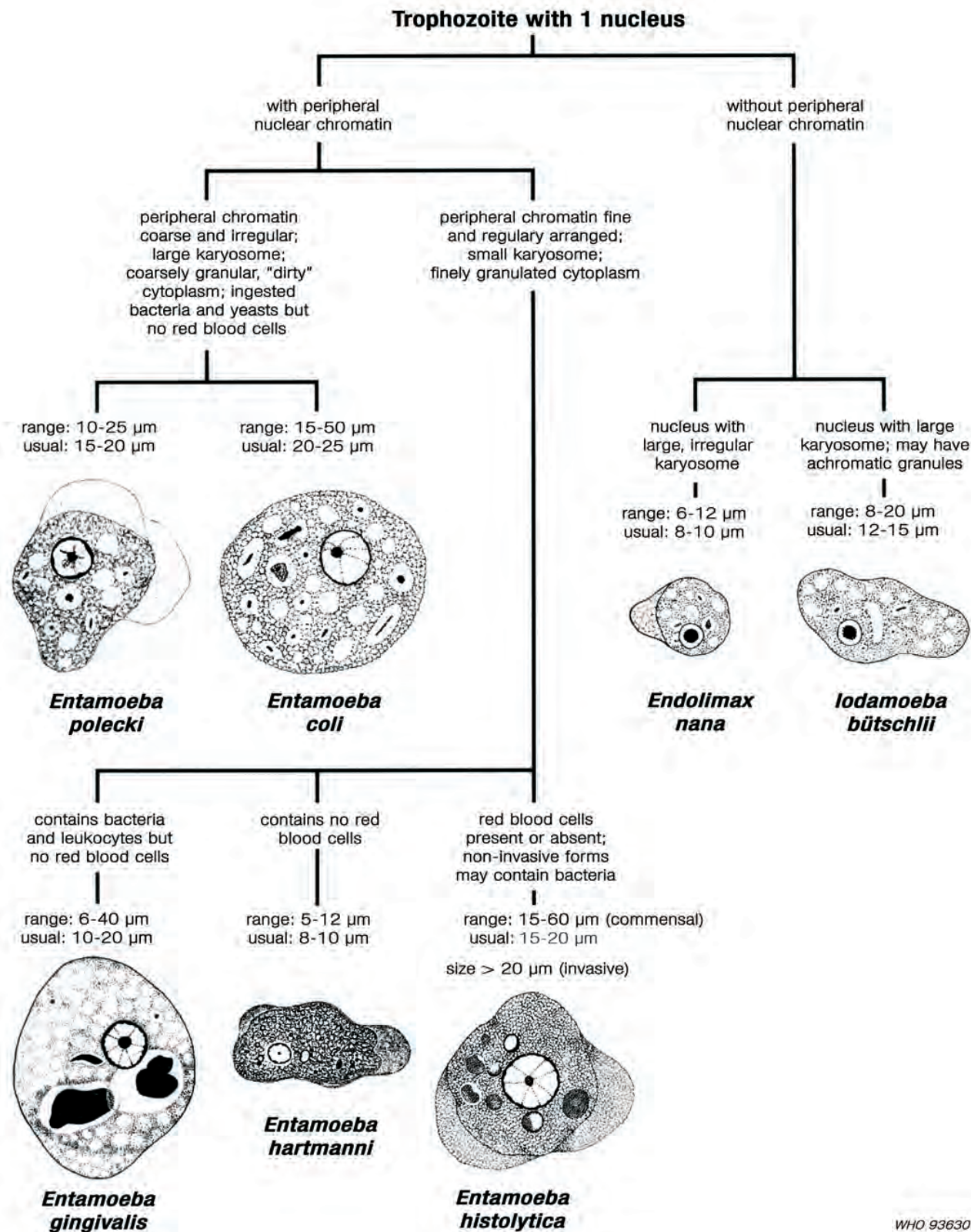


I. bütschlii trophozoite; iron haematoxylin.

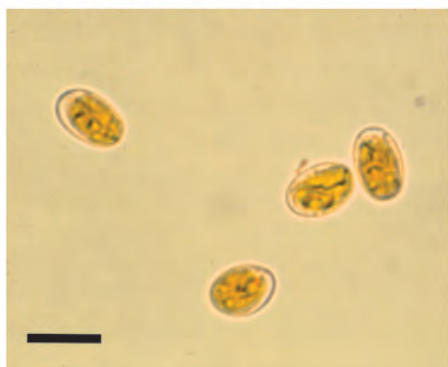


E. nana trophozoites, iron haematoxylin.

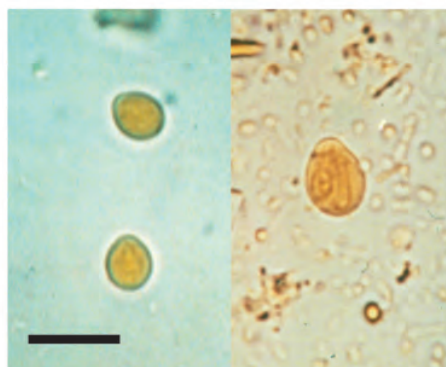
Key for the identification of amoebic trophozoites in stained smears



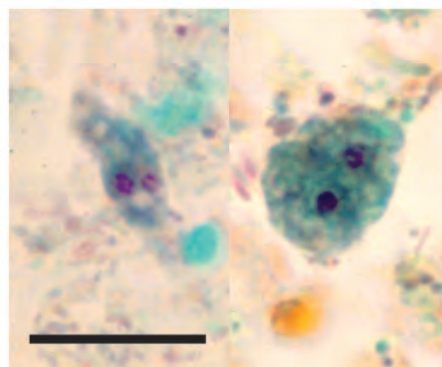
Intestinal flagellates



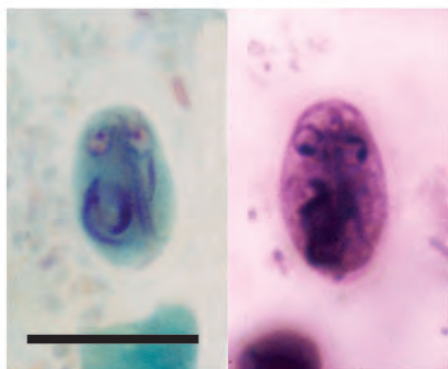
Giardia spp. cysts, iodine wet mount.



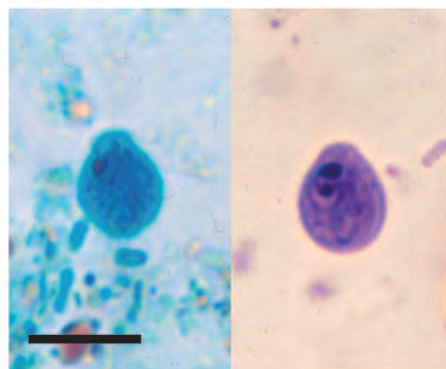
Chilomastix mesnili cysts, iodine wet mounts. Two cysts at left are at lower magnification and show typical lemon-shaped appearance; at higher magnification (right), nucleus and cytostome are faintly visible.



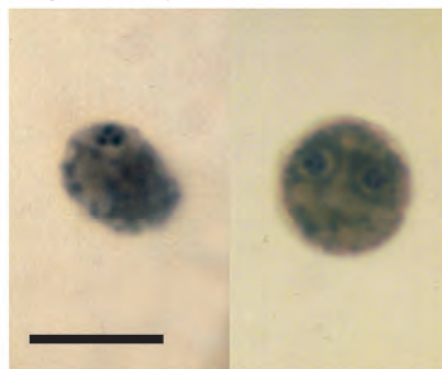
Dientamoeba fragilis binucleate trophozoites, trichrome. On the left, only one of two nuclei is clearly seen. With trichrome it is characteristic for trophozoites to take a pale stain. No cyst stage occurs in this species.



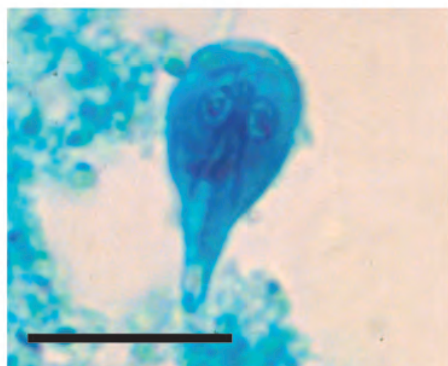
Giardia spp. cysts stained in trichrome (left) and iron haematoxylin (right).



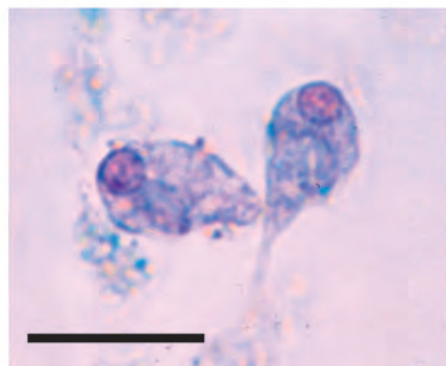
C. mesnili cysts in trichrome (left) and iron haematoxylin (right). Both show typical lemon-shaped appearance and in the figure on the right the cytostome is faintly visible.



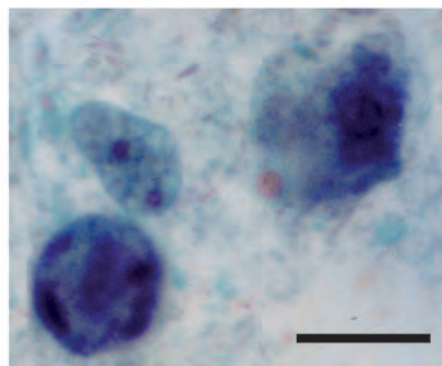
D. fragilis trophozoites, iron haematoxylin. Left: a uninucleate form with karyosome fragmented into 3 pieces; right: two nuclei, both showing fragmentation of the karyosomes, are present.



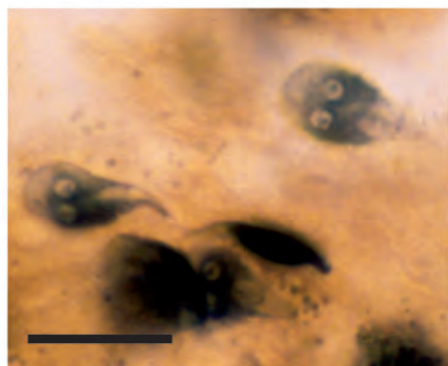
Giardia spp. trophozoite, trichrome.



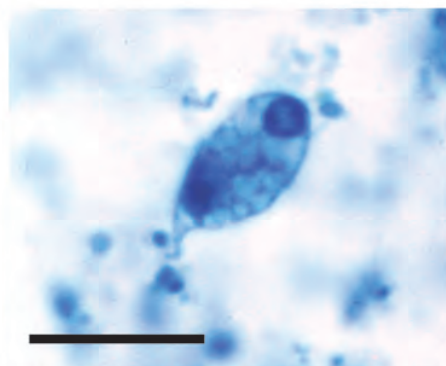
C. mesnili trophozoites, trichrome. With trichrome, trophozoites often take a pale stain as seen here; one organism shows finely pointed posterior end and faintly staining cytostome.



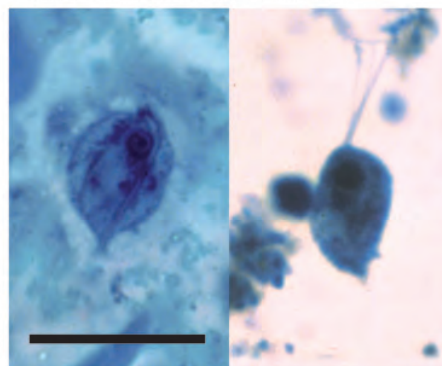
A binucleate trophozoite of *D. fragilis* which is more delicately stained, is seen between an *E. histolytica* trophozoite (upper right), and a smaller, uninucleate cyst of *E. histolytica* containing chromatoid bodies. Note size differences. Trichrome.



Giardia spp. trophozoites, iron haematoxylin. Three are ventral views of the organisms and two are seen in lateral aspect.

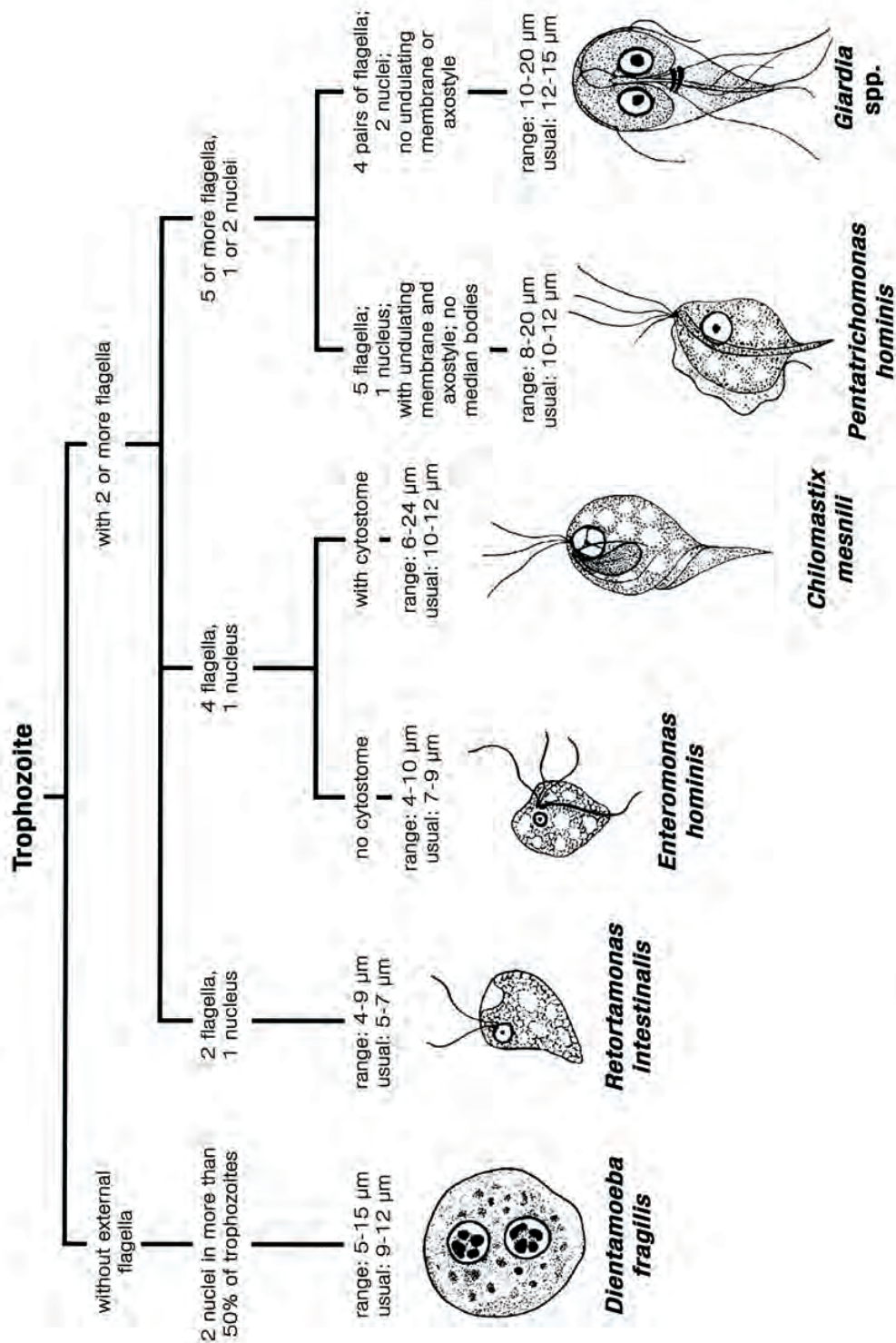


C. mesnili trophozoite, iron haematoxylin. Note nucleus at anterior end and finely pointed posterior end.



Pentatrichomonas hominis trophozoites stained in trichrome (left) and iron haematoxylin (right). Note nucleus and axostyle in organism on left, and anteriorly directed, faintly staining flagella in organism on right.

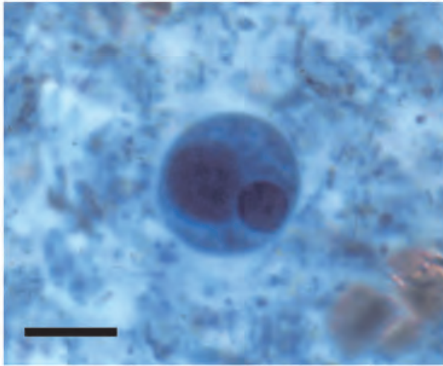
Key for the identification of trophozoites of intestinal flagellates in stained smears



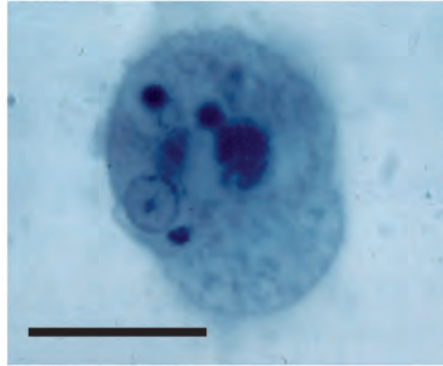
Note: *Dientamoeba fragilis* and *Pentatrichomonas hominis* do not have a cyst stage

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Uncommon protozoa and artefacts



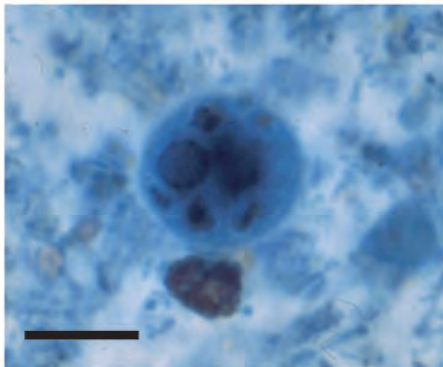
Entamoeba polecki uninnucleate cyst, trichrome. Note rounded, dense inclusion mass at left side of cyst and nucleus to its right. Cysts are typically uninnucleate and may or may not contain an inclusion mass.



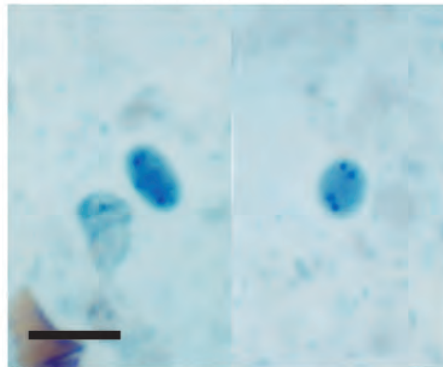
Entamoeba gingivalis trophozoite, iron haematoxylin. This amoeba has no cyst stage and is usually found in smears made from material taken from between teeth and gums. Trophozoites have *E. histolytica*-like nucleus and usually contain ingested leukocytes and bacteria.



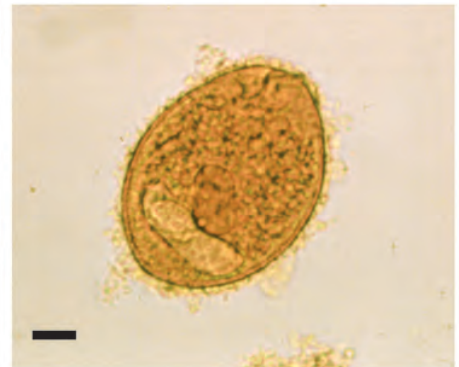
Balantidium coli cyst in unstained wet mount. Large macronucleus is visible as a clear area at right side of cyst.



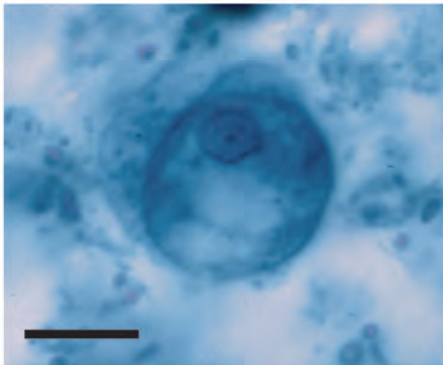
E. polecki uninnucleate cyst, trichrome. Nucleus at left side is considerably obscured by large number of chromatoid bodies of varying sizes. Cysts often have many chromatoid bodies, with or without an inclusion mass.



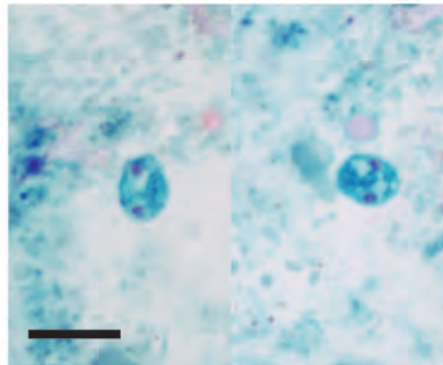
Enteromonas hominis trophozoite and binucleate cyst (left), iron haematoxylin. The trophozoite is to the left and the nuclei are at both ends of the cyst. The mature cyst (right) typically has 4 nuclei, 2 at each end. Iron haematoxylin.



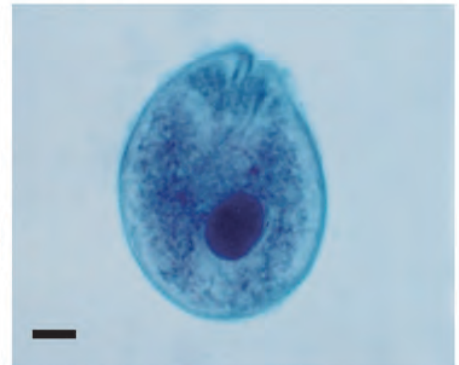
B. coli trophozoite, MIF wet mount. The cytostome is visible at top of organism and the large clear area at the bottom is the macronucleus. Cilia are visible on surface.



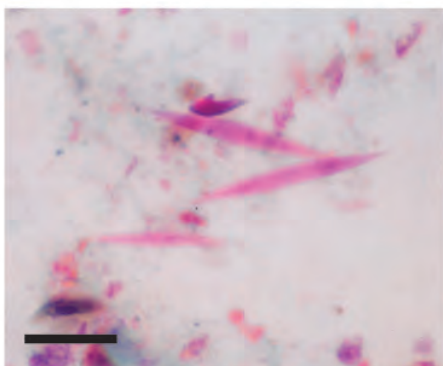
E. polecki trophozoite, trichrome. Nucleus is similar in morphology to that of *E. histolytica* trophozoites, i.e. with a small karyosome and fine peripheral chromatin on nuclear membrane.



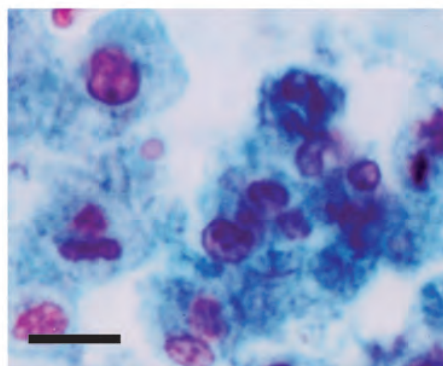
E. hominis binucleate cysts, trichrome.



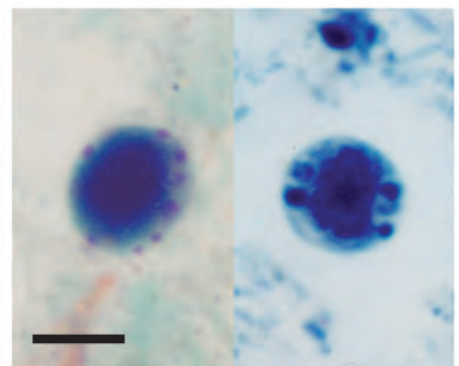
B. coli trophozoite, trichrome. The cytostome is visible at top of organism; the macronucleus is the dark-staining structure in mid-body. Cilia are visible on surface.



Charcot-Leyden crystals, trichrome. These pink-staining, elongated, pointed bodies are breakdown products of eosinophils and may often be found in faeces and sputum of patients with various types of infection.

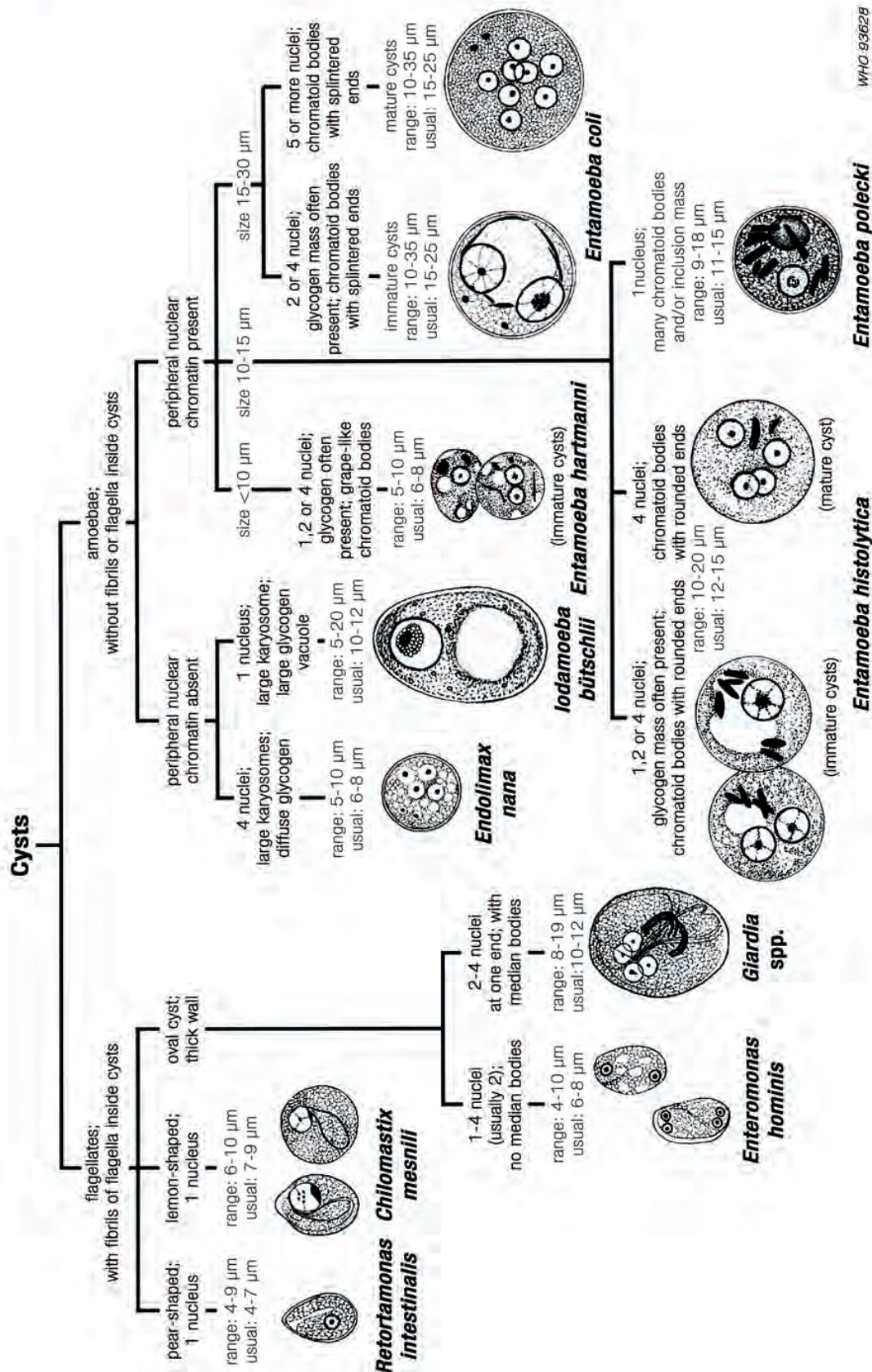


Polymorphonuclear leukocytes are seen clustered in trichrome-stained smear. Although these may be mistaken for amoebae, the large size of nuclei in relation to the cytoplasm of the cell and their structure indicate that these are inflammatory cells.

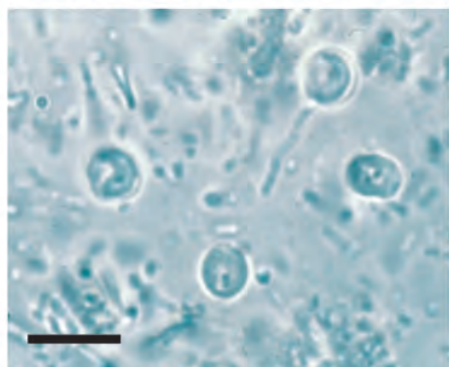


Blastocystis hominis, trichrome (left) and iron haematoxylin (right). Rounded, nucleus-like bodies, surrounding a central vacuole, are seen at periphery in both organisms.

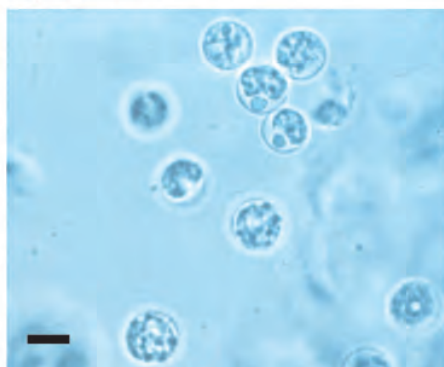
Key to identification of cysts of amoebae and flagellates in stained smears



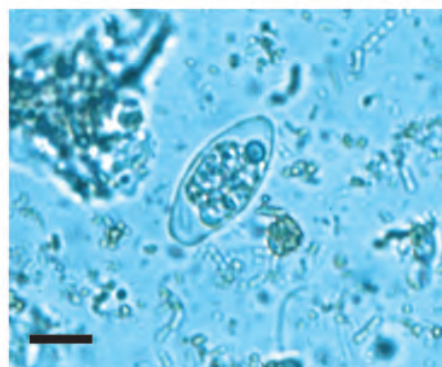
Intestinal coccidians and microsporidians



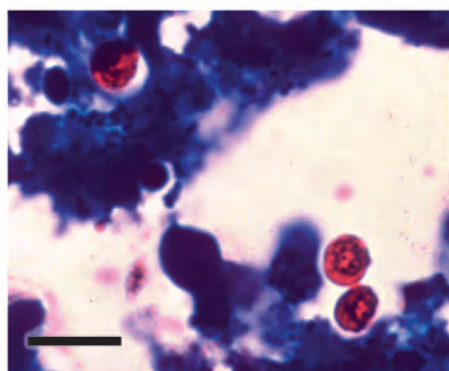
Cryptosporidium parvum oocysts, formalin wet mount. Small size (4-6 µm) and presence of black granules within oocysts are diagnostic for these organisms.



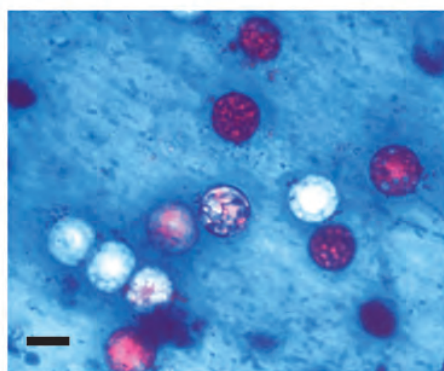
Cyclospora cayetanensis unsporulated oocysts, formalin wet mount, contain numerous spherical bodies.



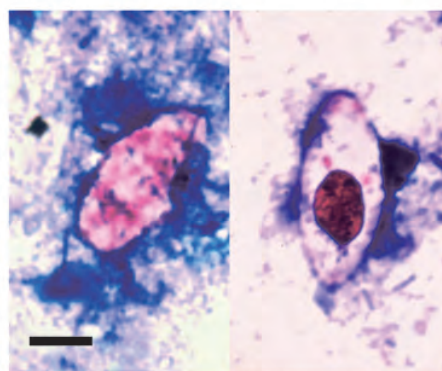
Isospora belli oocyst, formalin wet mount. Oocysts are not sporulated when excreted in faeces and are much larger (20-33 µm long) than either *Cryptosporidium* or *Cyclospora*.



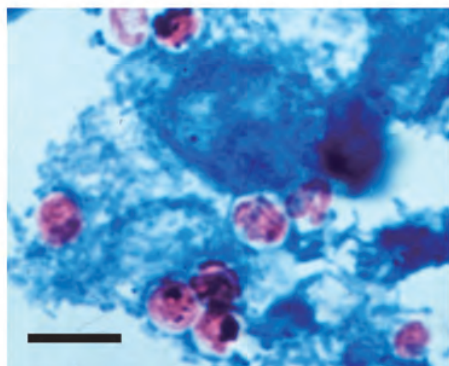
C. parvum oocysts, acid-fast. Small size, intense red coloration and presence of black granules are diagnostic for these organisms.



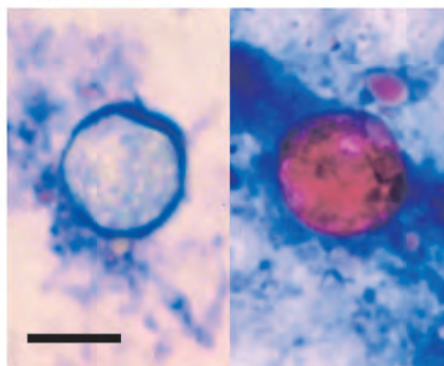
C. cayetanensis unsporulated oocysts, acid-fast. With this stain, oocysts stain variably, red, bluish, or not at all. This feature and their larger size (8-10 µm) help distinguish them from *Cryptosporidium* oocysts.



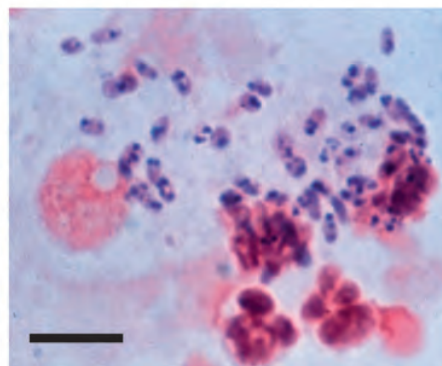
I. belli oocysts, acid-fast. On the left, atypical oocyst appears empty: these are often seen in patients undergoing treatment. On the right, typical unsporulated oocyst contains red-staining sporoplasm.



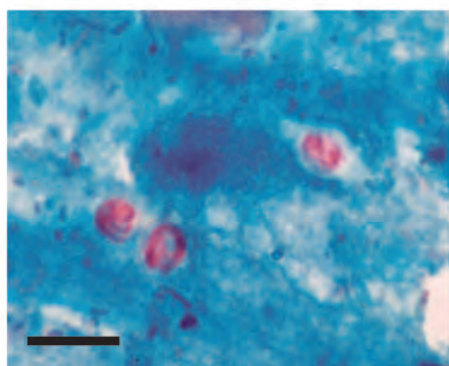
C. parvum oocysts, acid-fast. With various modifications of acid-fast stain, oocysts may stain from red to pink (as here); black granules are also seen.



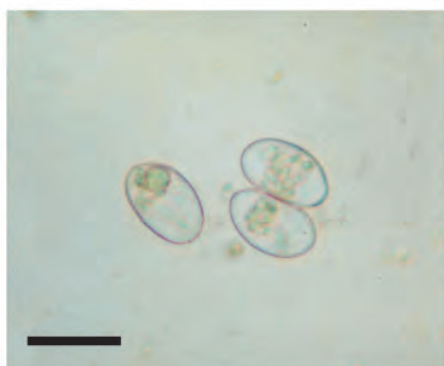
C. cayetanensis oocysts, acid-fast. The organism on the left remains unstained whereas on the right, a typical red-staining oocyst is seen.



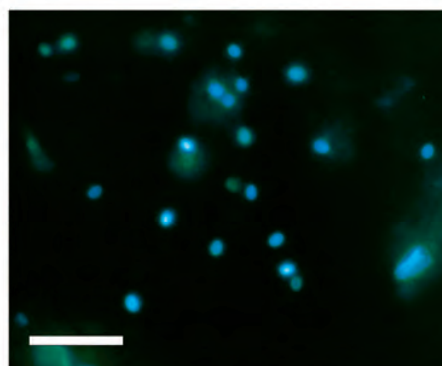
Encephalitozoon hellem spores, Gram stain. These microsporidian spores are in urinary sediment; their small size is well demonstrated.



C. parvum oocysts, trichrome. Oocysts do not always stain with trichrome, but when they do, the four sporozoites within them can be seen, as illustrated here.



Sarcocystis spp. sporulated oocyst and sporocyst, formalin wet mount. The thin-walled oocyst contains two sporocysts but is easily ruptured so that free sporocysts are often found in faeces, as here. Size of all *Sarcocystis* species oocysts are very similar.



Spores of intestinal microsporidian, *Enterocytozoon bienewsi* or *Septata intestinalis*, in a calcofluor white-KOH preparation with ultraviolet illumination. Small size of both species precludes specific identification in this preparation.



Diagnostic features of human intestinal coccidian parasites and microsporidians

Organism	Stage in faeces	Size range	Useful stains	Other tissue sites
<i>Cryptosporidium</i> species	Sporulated oocyst	4-6 µm	Acid-fast; rhodamine-auramine O	Liver and gallbladder, respiratory epithelium; possibly other tissues
<i>Cyclospora cayetanensis</i>	Unsporulated oocyst	8-10 µm	Acid-fast	Not reported from other tissues
<i>Isospora belli</i>	Unsporulated oocyst	20-33 µm X 10-19 µm	Acid-fast	Usually not; has been reported from lung
<i>Sarcocystis hominis</i> and <i>S. suihominis</i>	Sporulated oocysts and sporocysts	Oocysts: 15-19 µm X 15-20 µm; Sporocysts: 15-19 µm X 8-10 µm	None	Not in humans; tissue cyst stages in other animals which are intermediate hosts
<i>Enterocytozoon bienersi</i>	Microsporidian spores	1.5 µm X 1.0 µm	Modified or "super" trichrome; calcofluor white; Warthin-Starry stain	Probably widely disseminated in body
<i>Septata intestinalis</i>	Microsporidian spores	2.2 µm X 1.2 µm	Modified or "super" trichrome; calcofluor white; Warthin-Starry stain	Probably widely disseminated in body
<i>Encephalitozoon hellem</i>	Microsporidian spores*	2.2-2.5 µm X 1.5 µm	Tissue Gram stains	Probably widely disseminated in body

* *E. hellem* spores can be found in urine but have not been reported from faeces.



Bench Aids for the diagnosis of intestinal parasites

The bench aids for the diagnosis of intestinal parasites are intended both as a guide for laboratory and field workers in endemic countries and as a teaching aid for students and trainees. They provide guidance on the preparation of direct and stained faecal smears for the diagnosis of intestinal helminths and protozoan trophozoites and cysts. Photomicrographs demonstrate the appearance and diagnostic features of the various parasites in the different preparations.

The bench aids have been produced in a weatherproof plastic sealed format that is robust and easy to use at the bench. They are recommended for use by all health workers engaged in the routine diagnosis of intestinal parasitic infections.



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