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TRAINING MANUAL

ON DIAGNOSIS OF

INTESTINAL PARASITES

based on the WHO Bench Aids
for the diagnosis of intestinal parasites

Tutor's Guide

Schistosomiasis and Intestinal Parasites Unit
Division of Control of Tropical Diseases

World Health Organization
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I AIM OF THE MANUAL

This manual and the accompanying slide set have been developed to assist in the training of laboratory technicians and health workers in identifying medically relevant helminth eggs and larvae, and protozoal trophozoites and cysts. This manual complements the *Bench Aids for the Diagnosis of Intestinal Parasites* (produced by the World Health Organization in 1994) and should ideally be used together when training health personnel. The *Bench Aids* were developed to aid laboratory technicians and other health staff in diagnosing intestinal parasitic infections from faecal samples. They contain nine plates of photomicrographs of the diagnostic stages of the most common human intestinal parasites and descriptions of the laboratory procedures employed.

When teaching laboratory methods it is essential for the tutor to demonstrate how these techniques are applied in the examination of stool specimens. It is also important, that the learners do these procedures themselves to gain the necessary confidence and ease of dealing with the procedures as they will have to work without supervision after the training.

Objectives of the training

At the end of the training course, the learners should be able to:

- perform specific laboratory techniques:
  - direct faecal smear;
  - Kato-Katz cellophane thick faecal smear technique;
  - faecal formalin ether/ethyl-acetate/gasoline concentration procedures;
  - staining techniques for intestinal protozoa;
- identify intestinal parasites by *genus* and *species*;
- quantify helminth eggs in faeces by Kato-Katz procedure.

II USE OF THE MANUAL

This manual may serve as a guide to conducting a workshop utilizing the tutor's own expertise in the diagnosis of intestinal parasites with additional background information provided in the *Bench Aids*. Topical outlines of the various subjects to be covered, and a list of required equipment and laboratory supplies is provided.

While this manual covers most pathogenic intestinal parasites it is highly recommended that the tutor concentrates mainly on the locally relevant parasites.

The slide set used to illustrate the manual is based on the *Bench Aids*. However, the slides are not in the same order and additional slides have been included. In part **VIII, Inventory of Slide Set**, slides have been arranged and numbered according to the sequence proposed for presentation. Corresponding figures in the *Bench Aids* have also been identified and included. The numbering of the corresponding figures in the *Bench Aids* follows the same pattern in all plates, i.e. plate number, followed by the sequence on the plate (first figure is on the top left, last figure is on the bottom right). In figures presenting two different photomicrographs, *a* and *b* will refer to the left and right image respectively.

III SUGGESTED TIMETABLE

Day 1	Day 2	Day 3
• **PRE-TEST** Helminths and Protozoa **(45 min)** • Medically relevant helminths **(90 min)** **Break (30 min)** • Identification of medically relevant helminths **(90 min)** **Lunch break (60 min)**	• Medically relevant protozoa **(90 min)** • Techniques of staining and diagnosis of protozoa **(45 min)** **Break (30 min)** • Practical session **(90 min)** **Lunch break (60 min)**	• **POST-TEST** Helminths and Protozoa **(45 min)** **Break (30 min)** • General discussion and evaluation of the workshop **(90 min)**
• Laboratory methods for diagnosis of intestinal helminths and demonstration of selected diagnostic procedures **(90 min)** **Break (30 min)** • Practical session **(90 min)**	• Practical session **(90 min)** **Break (30 min)** • Practical session **(90 min)**	

IV LIST OF EQUIPMENT AND SUPPLIES

Slide projector
Microscopes (with a calibrated ocular micrometer)
Bench centrifuge
Staining jars
Kato-Katz kits
Saline and iodine solutions
Formalin or formaldehyde (or gasoline)
Glassware (centrifuge tubes)
Slides (glass) and coverslips (plastic)
Marking (grease) pencils
Pipettes
Containers for faecal samples
Trichrome stain solutions
Paper cups and applicator sticks
Distilled water
Immersion oil
Positive stool samples

V LIST OF ESSENTIAL PUBLICATIONS

Bench Aids for the diagnosis of intestinal parasites (WHO, 1994)
(one for each participant)
Basic Laboratory Methods in Medical Parasitology (WHO, 1991)
(one for each tutor and facilitator)

VI MEDICALLY RELEVANT HELMINTHS

Helminths include the nematodes (roundworms), trematodes (flukes) and the cestodes (tapeworms). Representatives of each of these groups are important human parasites. The adult worms, that inhabit the intestine, discharge the eggs or larvae they produce in faeces. Therefore, diagnosis is based on the detection of eggs or larvae in faecal samples.

1. Overview of Nematodes

Ascaris lumbricoides

More than 1.3 billion people worldwide have ascariasis, and 250 million suffer from associated morbidity. Adult *Ascaris lumbricoides* live in the small intestine. Female worms measure as much as 35 cm in length, males are smaller. Female worms produce over 200,000 eggs per day. Eggs may be found easily in direct smears (2 mg) of faeces. The fertile unembryonated egg measures 55-75 µm by 35-50 µm. It is brown in colour and the surface of the shell has conspicuous "bumps" called mamillations. The egg contains a single-cell ovum (Slide 1). Eggs may vary slightly in their appearance (Slide 2) but key features (size, mamillations) are always evident.

Occasionally infertile female worms produce "infertile" eggs that are morphologically different from the typical fertile egg. The contents of the eggs are irregular and disorganized (Slides 3 and 4). Infertile eggs are larger (Slide 5). They measure 85-95 µm by 43-47 µm, mamillations may be irregular and sometimes are absent. In rare cases, eggs are produced which lack the surface, mamillated layer. These eggs are called "decorticated" eggs (Slide 6). They are about the same size as other fertile eggs and contain the single-cell ovum.

Trichuris trichiura

Trichuriasis afflicts about 1 billion people throughout the world. Adult *Trichuris trichiura* are small, females are about 50 mm in length and males slightly smaller. The anterior 2/3 of the body is slender and threaded into the mucosa of the caecum and colon. The posterior end is thick, giving a "whiplike" shape to the worm; hence the name whipworm. Female worms produce eggs which are excreted in faeces. They have distinct features which identify them. Eggs (Slides 7 and 8) measure 50-55 µm by 22-24 µm, have an oval shape and "plug-like" prominences at each pole. The shell is usually dark brown in colour and smooth. It contains a single-cell ovum. Note that the egg is smaller than that of *A. lumbricoides* (Slide 9). Because female worms produce smaller numbers of eggs, they are often present in faeces in smaller numbers than *A. lumbricoides* eggs.

Hookworms

Hookworms infect over 1.25 billion people throughout the world. The hookworms (*Necator americanus, Ancylostoma duodenale*) are medically important human parasites and cause serious morbidity in many parts of the world. The adult worms are small and live in the small intestine. They measure about 1.0 to 1.5 cm in length. Although the adult worms of these two species are easily identified on the basis of presence of cutting plates (*N. americanus*) or teeth (*A. duodenale*) around the mouth, the eggs they produce are nearly identical. The typical hookworm egg measures 60-75 µm by 36-40 µm (Slides 10, 11 and 12). It has a clear, thin shell and the ovum is usually in the 4 or 8 cell stage or sometimes more advanced (Slide 11). Note in this image (Slide 13) the relative sizes of *A. lumbricoides*, *T. trichiura* and hookworm eggs.

There are other strongyle parasites which produce hookworm-like eggs which must be dealt with in the course of this training as well.

Trichostrongyles

Species of *Trichostrongylus* are usually small worms, less than 1 cm in length that live in the small intestine and are of minor importance as human parasites. However, their eggs are hookworm-like. Typically, trichostrongyle eggs resemble the eggs of the hookworms but are larger, 70-95 µm by 40-50 µm (Slide 14). The thin shell is slightly pointed rather than flattened at one pole and the ovum is in a more advanced state of segmentation than is seen in hookworm eggs.

***Oesophagostomum* species and *Ternidens* species**

These two parasites produce hookworm-like eggs that tend to be larger than hookworm eggs. The adult worms are somewhat larger than those of either *N. americanus* or *A. duodenale* and both live primarily in the colon of people living in Africa. *Oesophagostomum bifurcum* eggs are only slightly larger than hookworm eggs whereas eggs of *Ternidens deminutus* measure 85 µm by 50 µm (Slide 15). In both, the ovum is in an advanced state of segmentation and nearly fills the shell.

Strongyloides stercoralis

This is an important nematode parasite of humans because of its ability to autoinfect and disseminate throughout the organ systems of immunocompromized or immunosuppressed individuals or others with malignancies, e.g. lymphoma. This nematode parasite produces rhabditoid larvae (Slides 16 and 17) instead of eggs, which are found in faeces. The larva measures 180-380 µm in length by 14–20 µm in diameter. It has a short buccal capsule, a slender, pointed tail and a prominent genital primordium. The larva is easily recognized, either stained with iodine (Slide 16) or not (Slide 17).

2. Overview of Trematodes

The trematodes are usually referred to as flukes. They are solid-bodied worms, hermaphroditic (except schistosomes) and have a complicated life cycle that always involves a snail intermediate host as well as, in many instances, a second intermediate host. Depending on the species, trematodes inhabit the intestine, liver, lungs or blood vessels of their hosts.

Schistosoma species

The schistosomes cause the most important trematode infections. They infect over 200 million people. These worms live in the small blood vessels associated with the liver, intestine and bladder (depending on the species) and cause extreme pathology, morbidity and even death in individuals with heavy, chronic infections. They have a snail intermediate host and transmission is water-related. All of the schistosome species produce non-operculated eggs which are discharged in faeces or urine (depending on the species), and each egg has a spine on some part of the shell.

Schistosoma mansoni is most common in Africa but occurs in the Americas as well. The egg is discharged in faeces but typically in small numbers. The egg is large, has a relatively thin shell with a conspicuous lateral spine (Slides 18, 19, 20 and 21). *S. mansoni* are also easily identified in Kato-Katz preparations due to their size and presence of a lateral spine (Slide 18). The egg measures 114-175 µm by 45-70 µm and contains a larva called the miracidium. Occasionally, the egg may be oriented in a way that hides the spine (Slide 20); tapping the cover glass on the preparation will often reorient the egg and reveal the spine.

Schistosoma japonicum is transmitted in Asia. The egg which is found in faeces measures 70-100 µm by 55-65 µm, has a thin shell with an often inconspicuous, small lateral spine. The egg contains a miracidium (Slides 22 and 23). The shell is sticky, causing debris to adhere to the surface and making it more difficult to identify.

Schistosoma mekongi is closely related to *S. japonicum* and is transmitted in areas of Laos, Cambodia and Thailand. The egg is very similar to that of *S. japonicum* but is smaller, measuring 51-78 µm by 39-66 µm (Slide 25).

Schistosoma haematobium is transmitted in Africa and the Middle East. It differs from the others in that eggs are found in urine and sometimes in biopsies of the bladder and rectum. The egg is large (112-170 µm by 40-70 µm), thin shelled and has a terminal spine (Slides 26 and 27). It also contains a miracidium. In rare cases, eggs of *S. haematobium* are also found in faeces.

Schistosoma intercalatum is restricted in its geographic distribution to West and Central Africa. The egg resembles that of *S. haematobium* in that it has a terminal spine (Slide 28) and is found in faeces rather than urine. It is very large, measuring 140-240 µm long, has an equatorial bulge and contains a miracidium.

Foodborne Trematodes

There are a number of species of foodborne trematodes or flukes that live in the liver and produce operculate eggs that are shed in faeces.

Clonorchis sinensis is transmitted in Asia. The parasite is easily recognized by its small egg (Slide 29) that measures 27-35 µm by 12-19 µm. The operculum is seated on a prominent rim or shoulder. At the opposite pole there may be a small protuberance. The egg contains a miracidium.

The related liver fluke, ***Opisthorchis viverrini***, is transmitted in Southeast Asia and parts of Europe and produces an egg almost indistinguishable from that of *C. sinensis* (Slide 30).

Fasciola hepatica

This liver fluke is transmitted throughout the world, especially in sheep-raising countries. The adult fluke is large (about 3 cm), lives in the biliary ducts and produces eggs which are discharged in faeces. The egg is large, measuring 130-150 µm by 63-90 µm, has a thin shell

that is brown in colour, a small operculum and is unembryonated when passed in faeces (Slide 31).

The related intestinal fluke, ***Fasciolopsis buski***, which occurs in Asia has an egg almost indistinguishable from that of *F. hepatica*. The *F. buski* egg (Slide 32) lacks the thickening at the abopercular end of the shell characteristic of *F. hepatica*.

Paragonimus westermani

Paragonimiasis occurs in Asia, Africa and the western hemisphere. The adult worms are encapsulated in the tissues of the lungs and eggs are discharged in sputum and in faeces. The egg is moderately large, ovoid in shape, has a thick, brown shell and prominent operculum (Slide 33). It is unembryonated when found in sputum or faeces. It measures 80-120 µm by 45-70 µm. Other species of *Paragonimus* infect people in various parts of the world. The eggs are all very similar in appearance but differ in size. This egg (Slide 34) of ***Paragonimus uterobilateralis*** which occurs in Africa serves as an example.

3. Overview of Cestodes

The adult cestodes live in the human intestine and those of interest here produce eggs which are found in faeces.

Taenia solium* and *Taenia saginata

The adults, as indicated above, live in the intestine and are very large worms, i.e. several meters in length. Proglottids as well as eggs appear in faeces. The eggs of the two species are identical (Slide 35); they are round to oval in shape, measuring 35-43 µm in diameter and have a thick, radially-striated shell. The egg contains a 6-hooked embryo called an oncosphere. These eggs must be handled with extreme care because the egg of *Taenia solium* is infective to humans and produces cysticercosis.

Hymenolepis nana

The adult parasite, found in the intestine, is very small, only a few centimeters long. The egg is unique in its appearance (Slide 36). It is small, measuring 30-47 µm in diameter with a thin, colourless shell. The membrane surrounding the hexacanth embryo has 4-8 filaments arising from each pole that fill much of the space between the embryo and the shell (Slide 37).

Hymenolepis diminuta

This tapeworm is a natural parasite of rats but also infects humans, especially children. The adult which measures up to 60 cm in length, produces eggs which pass in faeces. The egg, which resembles that of *Hymenolepis nana,* is larger measuring 70-85 µm by 60-80 µm and contains a hexacanth embryo (Slide 38). The shell is thick and usually brown in colour. There are no polar filaments emanating from the surface of the hexacanth embryo. In this illustration (Slide 39), the two species of *Hymenolepis* are shown together to illustrate their differences.

Diphyllobothrium latum

This tapeworm, which lives in the intestine, measures several meters in length. It is found in temperate climates rather than the tropics. Its egg is passed in faeces. The egg of *Diphyllobothrium latum* differs from other tapeworms in that it has an operculum (Slide 40). It is ovoid in shape, measures 58-75 µm by 45-50 µm and is unembryonated when found in faeces. There is a small knob on the abopercular end. Sometimes this egg is confused with that of *Paragonimus* spp. (Slides 33 and 34).

4. Laboratory diagnosis of helminth parasites

A calibrated ocular micrometer, a measuring device, for the microscope is an essential tool for the microscopist. It provides a mean of accurately measuring objects such as eggs, larvae or protozoan cysts. Detailed instructions for calibration of the ocular micrometer are provided in the *Bench Aids.* Read it carefully and follow the instructions closely. The learners will have an opportunity

to measure eggs, etc. in the faecal samples they will examine. The relative sizes of helminth eggs are shown on the back of Plate 4 of the *Bench Aids*. Frequently eggs and/or larvae are present in sufficient numbers to be directly observed in a faecal smear of one or two mg volume. The procedure for performing faecal smears is outlined and illustrated on the back of Plate 1 of the *Bench Aids* (Slides 41, 42 and 43). Whilst direct smears will often detect helminth eggs, it is usually more efficient to do a simple concentration procedure 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 detection of schistosome or soil-transmitted nematode infections.

A modification of the direct smear procedure, the Kato-Katz technique, is especially useful for field surveys for these infections because it also gives an estimation of the intensity of infection. Detailed instructions for performing the concentration technique is found on the reverse side of Plate 2 of the *Bench Aids*, and Slides 44, 45, 46 and 47 illustrate the formalin-ether/ethyl-acetate/gasoline concentration procedure. The Kato-Katz procedure is outlined on the back of Plate 3 of the *Bench Aids* and Slides 48, 49, 50, 51, 52 ,53, 54 and 55 show how to perform the Kato-Katz technique.

In part **VIII - Inventory of Slide Set** - there is a detailed explanation of the diagnostic technique following the texts on the backs of Plates 1 to 3 of the *Bench Aids*.

The following slides demonstrate the appearance of common helminth eggs in Kato-Katz preparations:

Slide 56 *A. lumbricoides*, fertile and infertile eggs, with a *T. trichiura* egg in the middle
Slide 57 *A. lumbricoides,* normal and decorticated eggs
Slide 58 *T. trichiura* egg
Slide 59 *A. lumbricoides* and hookworm eggs
Slide 60 *A. lumbricoides* and *T. trichiura* eggs.

A. lumbricoides and *T. trichiura* eggs will remain visible and recognizable for many months in these preparations. Hookworm eggs clear rapidly, and if slides are not examined within 30-60 minutes, the eggs no longer will be visible.

The smear should be examined in a systematic manner and the number of eggs of each species reported. Later multiply by the appropriate multiplication factor to give the number of eggs per gram of faeces (when using a 50 mg template by 20; for a 20 mg template by 50; for a 41.7 mg template by 24). With high egg counts, to maintain a rigorous approach while reducing reading time, the Stoll quantitative dilution technique with 0.1 N NaOH may be recommended.

VII MEDICALLY RELEVANT PROTOZOA

1. Overview of intestinal protozoa

The human intestinal protozoa include non-pathogenic (***Entamoeba dispar***, ***Entamoeba coli***, ***Entamoeba hartmanni***, ***Entamoeba polecki***, ***Endolimax nana*** and ***Iodamoeba bütschlii***) and pathogenic (***Entamoeba histolytica***) amoebae, non-pathogenic (***Chilomastix mesnili*** and ***Pentatrichomonas hominis***) and pathogenic (***Giardia lamblia*** [***intestinalis***] and ***Dientamoeba fragilis***) flagellates and the pathogenic ciliate parasite, ***Balantidium coli***. In addition, human intestinal coccidian parasites producing human disease include ***Cryptosporidium parvum***, ***Cyclospora cayetanensis*** and ***Isospora belli***. Representatives of a separate phylum, the microspora, include various genera and species of microsporidian organisms that cause disease principally in immunocompromized individuals, such as ***Enterocytozoon bieneusi***, ***Encephalitozoon intestinalis***, ***Encephalitozoon hellem*** and others.

The importance of the recognition of non-pathogenic (commensal) amoebae and flagellates lies in the fact that these organisms are indicative of faecal-oral transmission having occurred. When these organisms are found in stool samples, it is important to be on the lookout for the possible presence of pathogenic species. All of the major amoebae found in the intestinal tract have both trophozoite and cyst stages in their life cycles. Of the intestinal flagellates, *G. lamblia* (*intestinalis*) and *C. mesnili* have trophozoites and cysts, whereas

D. fragilis and *P. hominis* have only a trophozoite stage and lack a cyst stage. The intestinal coccidians (*C. parvum, C. cayetanensis* and *I. belli)* all produce cyst stages (known as oocysts) which are excreted in faeces. Microsporidian organisms produce resistant spores which are excreted in faeces, urine or other bodily secretions.

a. Intestinal amoebae

A 1997 WHO/PAHO/UNESCO Expert Consultation on Amoebiasis[1] made several conclusions including:

- Biochemical, immunological and genetic data now indicate that there are two species with the same morphological characteristics - *E. histolytica* and *E. dispar* - previously known as pathogenic and nonpathogenic *E. histolytica* respectively. Only *E. histolytica* is capable of causing invasive disease. In future, the name *E. histolytica* should only be used in this sense.
- When diagnosis is made by light microscopy, the cysts of the two species (10-16 µm in diameter) are indistinguishable and should be reported as *E. histolytica*/*E. dispar*.
- Trophozoites with ingested red blood cells in fresh stool or other specimens and trophozoites in tissue biopsies are both strongly correlated with the presence of *E. histolytica* and invasive disease.

E. histolytica is invasive and may cause disease within the wall of the colon resulting in ulcer formation. Trophozoites of *E. histolytica* can phagocytize erythrocytes and they are the only intestinal amoeba to do so (Slides 61 and 62). Trophozoites without erythrocytes in their cytoplasm can also be found (Slides 63 and 64) and should be reported as *E. histolytica*/*E. dispar*.

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[1] WHO, 1997. WHO News and Activities. Entamoeba taxonomy. *Bulletin of the World Health Organization*, 1997, 75, pp 271-292

E. histolytica trophozoites can disseminate via the bloodstream or direct tissue spread to other organs and tissues, including the liver, lung, kidney, brain, skin and diaphragm. Cysts of *E. histolytica/dispar* excreted in faeces may contain 1 or 2 nuclei (immature cysts – Slides 65, 66 and 67) or 4 nuclei (mature cysts – Slides 68, 69 and 70). Cysts of *E. histolytica*/*E. dispar* often contain chromatoid bodies with rounded ends (Slides 67, 69 and 70).

There are a number of non-pathogenic intestinal amoebae including *E. coli* (Slides 71, 72, 73, 74, 75 and 76), *E. polecki* (Slides 77, 78 and 79), *E. hartmanni* (Slides 81, 82, 83, 84, 85 and 86), *Endolimax nana* (Slides 88, 89 and 90) and *I. bütschlii* (Slides 91, 92, 93 and 94).

All of the these amoebae can be morphologically distinguished from each other on the basis of morphological features such as size, morphology of the nucleus, granularity and inclusions in the cytoplasm, and a number of nuclei in cysts.

There is an amoeba that lives in the oral cavity of humans, ***Entamoeba gingivalis***, which has only a trophozoite stage (Slide 95); this organism appears to be non-pathogenic and is rarely found.

b. Intestinal flagellates

G. lamblia (*intestinalis*) and *D. fragilis* are the two medically important flagellates. *G. lamblia* (*intestinalis*) parasitizes the small intestine where its clinical manifestations may range from asymptomatic to acute and chronic diarrhoeal conditions. The pear-shaped trophozoite measures 10-20 µm long, has two nuclei and eight flagella, four of which are lateral, two vertical and two that trail posteriorly. The trophozoite has a concavity or bowl-shaped depression (the “sucking disk”) which occupies the ventral surface of the anterior part of the body (Slides 96 and 97). Living trophozoites have a characteristic tumbling kind of motility when viewed in direct smears in saline prepared from fresh faeces. The cysts of *G. lamblia* (*intestinalis*) are oval and measure 8-19 µm. Mature cysts have 4 nuclei and the cytoplasm contains numerous fibrils (Slides 98, 99 and 100). *D. fragilis* appears to occasionally cause diarrhoea in some individuals. This amoeba-like organism has no visible flagella and may be confused with amoebae. It only exists in the trophozoite stage;

a cyst stage has not been described. Trophozoites contain one or more often two nuclei (Slides 101, 102, 103, and 104). In Slide 105, *E. histolytica*/*E. dispar* is seen together with a trophozoite of *D. fragilis.* The nuclei often have fragmented karyosomes.

The most common non-pathogenic intestinal flagellates include *C. mesnili* and *P. hominis*. *C. mesnili* trophozoites are 10-15 µm long, have 3 anteriorly directed flagella, a cytostomal groove at the broader, anterior part of the organism, and a single nucleus (Slides 106 and 107).

Cysts of *C. mesnili* are lemon-shaped, uninucleate and measure 6-10 µm long (Slides 108, 109, 110 and 111). *P. hominis* lacks a cyst stage and the uninucleate trophozoites are from 7-23 µm long (Slides 112 and 113).

c. Ciliate*s*

B. coli is the only human ciliate parasite, living in the colon and appendix. Trophozoites are large (50-200 µm long), move with a rotary, boring movement and have two nuclei, one of which is prominent and large and the other, small and infrequently visible (Slides 114 and 115). Balantidiasis can be a severe and fatal disease due to trophozoite colonization of the bowel wall. Cysts are 50-70 µm in diameter (Slide 116).

d. Intestinal coccidia

Intestinal coccidian infections of humans are caused by *C. parvum*, *C. cayetanensis* and *I. belli*. All of these have faecal-oral transmission and can be found in individuals who are immunocompetent as well as in those who are immunocompromized (AIDS or other immunodeficiency syndromes). Cryptosporidiosis produces a more severe and prolonged disease in patients with AIDS. *C. cayetanensis* infection is also an important cause of diarrhoeal disease in immunocompetent individuals. *C. parvum* infection is diagnosed by demonstrating round, sporulated oocysts, 4-6 µm in diameter, in faeces. These oocysts are difficult to recognize, especially when present in small numbers in wet mounts prepared from fresh faeces (Slide 117). In fresh smears, the oocysts are retractile and contain a