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**World Health Organization**
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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

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**Division of Control of Tropical Diseases**

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## TABLE OF CONTENTS

## 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)**<br>• Medically relevant helminths **(90 min)**<br>**Break (30 min)**<br>• Identification of medically relevant helminths **(90 min)**<br>**Lunch break (60 min)** | • Medically relevant protozoa **(90 min)**<br>• Techniques of staining and diagnosis of protozoa **(45 min)**<br>**Break (30 min)**<br>• Practical session **(90 min)**<br>**Lunch break (60 min)** | • **POST-TEST** Helminths and Protozoa **(45 min)**<br>**Break (30 min)**<br>• General discussion and evaluation of the workshop **(90 min)** |
| • Laboratory methods for diagnosis of intestinal helminths and demonstration of selected diagnostic procedures **(90 min)**<br>**Break (30 min)**<br>• Practical session **(90 min)** | • Practical session **(90 min)**<br>**Break (30 min)**<br>• 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*.

[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. Ciliates**

*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

number of dark granules. However, oocysts of *C. parvum* are most readily detected in faecal smears stained by acid-fast stains (Ziehl-Neelsen or Kinyoun stains). In acid-fast stained preparations, the red-staining oocysts can be readily differentiated from green-staining yeasts which are of similar size and shape. In these preparations, the oocysts stand out as pink (Slide 118) or red-staining cysts which contain numerous dark staining granules (Slide 119). *C. parvum* oocysts generally are not well stained in routine trichrome-stained faecal smears but occasionally do demonstrate well the 4 sporozoites present in the infective oocysts (Slide 120). Oocysts of *C. parvum* are not stained by iodine.

*C. cayetanensis* oocysts must be differentiated from those of *C. parvum*. *C. cayetanensis* oocysts are larger, 8-10 µm in diameter, and are not sporulated when excreted in faeces (Slides 121 and 122). In acid-fast stained faecal smears, the oocysts of *C. cayetanensis* are variable in their staining reaction (Slides 123, 124 and 125). Oocysts may appear as unstained, wrinkled, round spheres, or stain from a light pink to darker red. The oocysts of *C. cayetanensis* can be detected in direct smears from fresh faeces on the basis of their size and refractility.

Infection with *I. belli* occurs both in immunocompetent and immunocompromized individuals but the disease is more prolonged and severe in the latter group. Diagnosis is made by demonstrating typical large (20-33 µm long by 10-19 µm wide), thin-walled, unsporulated oocysts in faeces (Slide 126). Infections can be diagnosed by direct wet mount examination or by flotation or sedimentation concentration procedures. In acid-ast stained faecal smears, the sporoblast within the oocyst stains red and frequently red stain is deposited on the outside of the thin oocyst wall (Slides 127 and 128).

### e. Microsporidian infections

Various genera of the phylum microspora have been shown to cause human disease, most frequently in individuals infected with AIDS.

Two microsporidians, *Enterocytozoon bieneusi* and *Encephalitozoon intestinalis*, infect the intestinal tract and produce diarrhoea. Unlike the amoebae and flagellates which produce cysts and the intestinal coccidians which produce oocysts, characteristic spores are produced by microsporidians. Spores are typically oval, small (1-3 µm long) and difficult to find in faecal examination. The best means of diagnosis is staining faecal smears by a modification of the standard trichrome stain; in this modification, one of the stain components, chromotrope 2R, is increased in concentration. The spore wall stains a bright pink-red colour and many of the spores demonstrate a red, belt-like stripe that encircles the spore equatorially. Spores may also occur in urine and other bodily secretions (Slide 129). Slide 120 shows the spores in a calcofluor white-KOH preparation with ultraviolet illumination.

## 2. Laboratory diagnosis of protozoan infections

Trophozoites and cysts of the intestinal amoebae, flagellates and ciliates can be found and identified best in permanently stained faecal smears. Trophozoite stages are most often found in watery or diarrhoeic faecal specimens and usually cysts are not seen in such specimens. On the other hand, cysts are the stage typically found in formed faecal specimens. A mixture of trophozoites and cysts may occur in softer and semi-formed faeces.

In direct smears of faeces in saline, motile trophozoites may be found. A motile amoebic trophozoite containing red blood cells is immediately identifiable as *E. histolytica*. Motile amoebic trophozoites that do not contain ingested erythrocytes are more difficult to classify and are best diagnosed in permanently stained smears as all cysts are observed. Although experienced technologists can often identify species accurately in wet mount faecal preparations, permanently stained smears are superior to saline- or iodine-stained wet mounts.

Oocysts of the intestinal coccidians can be identified in wet mount faecal preparations, principally on the basis of size or shape. They typically do not stain well with trichrome or iron haematoxylin. The use of acid-fast staining of faecal smears is superior for demonstrating these oocysts. The extremely small size of microsporidian spores makes their identification difficult under any circumstances. Modification of the trichrome stain by increasing the amount of the stain components and increasing the time for staining aids in demonstrating the spores in faeces.

Flotation and sedimentation faecal concentration procedures are not useful for demonstrating trophozoite stages of the intestinal protozoa but can be highly efficient for demonstrating cyst stages.

Detailed instructions for performing the direct faecal smear, formalin-ether/ ethyl acetate/gasoline concentration technique, are given on the reverse side of Plates 1 and 2 of the *Bench Aids*. Slides 41 to 47 illustrate the techniques. The permanent stains for faecal smears are described as on the reverse side of Plate 5 of the *Bench Aids*.

**a. Permanent stains for faecal smears**

**Trichrome stain**

**Use:** Very good stain for fresh and PVA (polyvinyl alcohol fixative) preserved faecal smears: does not give good staining results with SAF (sodium acetate, acetic acid, formalin) 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.

**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 5min; 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.

**Modified Ziehl-Neelsen technique (acid-fast stain)**

**Use**: For detection of *C. parvum*, *C. cayetanensis*, 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.

## VIII Inventory of Slide Set[2]

Slide 1 *(Plate 1, Fig. 1)*
Slide 2 *(Plate 1, Fig. 2)*

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 faeces. The egg has conspicuous mamillations on its surface.

Slide 3 *(Plate 1, Fig. 4)*
Slide 4 *(Plate 1, Fig. 5)*

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

Slide 5 *(Plate 1, Fig. 6)*

Fertile and infertile eggs of *A. lumbricoides* in a Kato-Katz preparation. Note the conspicuous differences between the two.

Slide 6 *(Plate 1, Fig. 7)*

Sometimes normal fertile *A. lumbricoides* eggs lack the mamillated layer and are referred to as "decorticated" eggs. The microscopist must be careful not to confuse these eggs with those of other helminths of similar size.

[2] 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. 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.

Slide 7 *(Plate 2, Fig. 2)*
Slide 8
Slide 9

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 ( Slide 7 and 8). Note the differences in size and shape of *A. lumbricoides* compared to *T. trichiura* eggs ( Slide 9).

Slide 10 *(Plate 2, Fig. 4)*
Slide 11 *(Plate 2, Fig. 5)*
Slide 12

Hookworm eggs found in faeces characteristically are barrel-shaped with a thin, hyaline shell; they measure 60-75 µm by 36-40 µm. They usually are in the 4 or 8 cell stage in fresh faeces (Slides 10 and 12) or in a more advanced stage of cleavage (Slide 11) in faeces that have been kept at room temperature for a few hours.

Slide 13 *(Plate 2, Fig. 1)*

*A. lumbricoides*, *T. trichuria* and hookworm eggs in the same microscopic field illustrating size and morphological differences in the three species.

Slide 14 *(Plate 2, Fig. 7)*

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

Slide 15 (*Plate 2, Fig. 8)*

*Ternidens deminutus* is another strongyle parasite which infects humans, mostly in South 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 faeces.

Slide 16 *(Plate 2, Fig. 9)*
Slide 17

Infection with *Strongyloides stercoralis* is routinely diagnosed by the presence of first-stage rhabditoid larvae in faeces. Larvae measure 180-380 µm long by 14-20 µm in diameter. Larvae have a short buccal capsule, an attenuated tail, and a prominent genital primordium (Slide 16 stained, Slide 17 unstained).

Slide 18 *(Plate 3, Fig. 3)*

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

Slide 19 *(Plate 3, Fig. 1)*
Slide 20 *(Plate 3, Fig. 2)*
Slide 21

*S. mansoni* eggs are large, measuring 114-175 µm by 45-70 µm, have a thin, transparent shell, a prominent lateral spine (Slide 19 and 21) and contain a miracidium. Occasionally, the lateral spine may be hidden from view (Slide 20). Gentle tapping on the coverslip usually will reorient the egg exposing the spine to view. Viable eggs have the same appearance in faeces and in rectal biopsies.

Slide 22 *(Plate 3, Fig. 4)*
Slide 23 *(Plate 3, Fig. 5)*
Slide 24

*Schistosoma japonicum* eggs are smaller than those of *S. mansoni* and *Schistosoma 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 (Slides 22 and 24). The eggs contain a miracidium. Frequently, faecal debris adheres to the egg surface and may obscure the spine. The orientation of the egg may obscure the spine as well (Slide 23).

Slide 25

Eggs of *Schistosoma mekongi*, a human schistosome endemic in the Mekong River basin, are morphologically similar to those of *S. japonicum* but their size range is usually smaller, i.e. 51-78 µm by 39-66 µm.

Slide 26 *(Plate 3, Fig. 7)*

Slide 27 *(Plate 3, Fig. 8)*

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

Slide 28 *(Plate 3, Fig. 9)*

The eggs of *Schistosoma intercalatum*, a species occurring in humans in West and Central Africa, are usually larger than those of *S. haematobium* and are typically found in faeces. They measure about 140-240 µm in length and have an equatorial bulge, as illustrated here.

Slide 29 *(Plate 4, Fig. 1)*

*Clonorchis sinensis* eggs measure 27-35 µm long by 12-19 µm wide, have a seated operculum and usually a small protuberance on the end of the egg opposite to the operculum. Often the shell has minute debris adherent to it. Eggs contain a miracidium when passed in faeces.

Slide 30

Eggs of the related genus *Opisthorchis* are similar in size and morphology to those of *C. sinensis.*

Slide 31 *(Plate 4, Fig. 3)*

*Fasciola hepatica* eggs usually measure 130-150 µm long by 63-90 µm wide, have an inconspicuous operculum, are undeveloped when passed in faeces, and usually have an irregularity in the shell wall at the end opposite the operculum.

Slide 32

*Fasciolopsis buski* eggs are similar in size and morphology to those of *F. hepatica*, except that the irregularity in the shell wall is not present.

Slide 33 *(Plate 4, Fig. 4)*

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

Slide 34 *(Plate 4, Fig. 5)*

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

Slide 35 *(Plate 4, Fig. 7)*

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

Slide 36 *(Plate 4, Fig. 9)*

Slide 37

*Hymenolepis nana* eggs are typically spherical to subspherical in shape, measure about 30-47 µm in diameter, have a thin, hyaline shell and contain a 6-hooked onchosphere. There are two polar thickenings on the membrane around the onchosphere from which arise 4-8 filaments that extend into the space between the onchosphere and the shell.

Slide 38 *(Plate 4, Fig. 8)*

The eggs of *Hymenolepis diminuta* measure 70-85 µm by 60-80 µm, are spherical, yellowish brown in colour, and contain a 6-hooked embryo, the onchosphere. The filaments seen in *H. nana* extending into the space between the shell wall and the onchosphere are lacking.

Slide 39

Note the size difference and the absence of filaments in *H. diminuta* compared to *H. nana*.

Slide 40 *(Plate 4, Fig. 6)*

*Diphyllobothrium latum* eggs measure 58-75 µm by 40-50 µm, are unembryonated when passed in faeces and frequently have a knob or small protuberance on the abopercular end of the shell.

On Slides 41 to 43 the procedure to perform the direct smear is explained as on the *Bench Aids* (back of Plate 1).

Slide 41

With a wax pencil or other marker, write the patient's name or identification number and the date at the left-hand side of the slide. Place a drop of saline in the centre of the left half of the slide and place a drop of iodine in the centre of the right half of the slide. (Note: iodine wet mount preparations are most useful for protozoan organisms, less so for helminths.)

Slide 42

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: repeat and add it to the drop of iodine. Mix the faeces with the drops to form suspensions.

Slide 43

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.

(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.)

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. When organisms or suspicious objects are seen, one may switch to higher magnification to see the more detailed morphology of the object in question.

On Slides 44 to 47, the formalin-ether/ethyl-acetate/gasoline concentration procedure is explained as on the *Bench Aids* (back of Plate 2).

Slide 44

With an applicator stick, add 1.0 to 1.5 g faeces to 10 ml formalin in a centrifuge tube, stir, and bring into suspension. Strain 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. Add more 10% formalin to the suspension in the tube to bring the total volume to 10 ml. Add 3.0 ml of formalin-ether (or ethyl-acetate or gasoline) to the suspension in the tube and mix well by putting a rubber stopper in the tube and shake vigorously for 10 seconds. Place the tube with the stopper removed in centrifuge; balance the tubes and centrifuge at 400-500 x *g* for 2-3 minutes. Remove the tube from the centrifuge; the contents consist of 4 layers: (a) top layer of formalin-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.

Slide 45

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 to drain inverted for at least five seconds.

Slide 46

When done properly, a small amount of residual fluid from the walls of the tube will flow back onto the sediment.

Slide 47

Mix the fluid with the sediment (sometime 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. 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. When organisms or suspicious objects are seen, one may switch to higher magnification to see more detailed morphology of the material in question.

Slides 48 and 49 show the material needed to perform the Kato-Katz cellophane faecal thick smear technique as in the *Bench Aids* (back of Plate 3).

Slide 48

Applicator sticks (wooden or plastic); screen (stainless steel, nylon or plastic) 60-105 mesh; template (stainless steel, plastic, or cardboard); templates of different sizes have been produced in different countries. A 50 mg template will have a hole of 9 mm on a 1 mm thick template; a 41.7 mg a hole of 6 mm on a 1.5 mm thick template; a 20 mg a hole of 6.5 mm on a 0.5 mm thick template. The templates should be standardized in the country and the same size of templates should be used to ensure repeatability and comparability of prevalence and intensity data; spatula, plastic; microscope slides (75 x 25 mm).

Slide 49

Hydrophilic cellophane, 40-50 µm thick, strips 25 x 30 mm in size; flat bottom jar with lid.

Slides 50 to 55 show the procedure to perform the Kato-Katz technique as in the *Bench Aids* (back of Plate 3).

Slide 50

Place a small mount of faecal material on newspaper or scrap paper and press the small screen on top of the faecal material so that some of the faeces will be sieved through the screen and accumulate on top of the screen.

Slide 51

Scrape the flat-sided spatula across the upper surface of the screen so that the sieved faeces accumulate on the spatula.

Slide 52

Place template with hole on the centre of a microscope slide and add faeces from the spatula so that the hole is completely filled. 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 again).

Slide 53

Remove the template carefully from the slide so that the cylinder of faeces is left completely on the slide. Cover the faecal material with the pre-soaked cellophane strip. The strip must be very wet if faeces are dry and less so with soft faeces (if excess glycerol solution is present on upper surface of cellophane, wipe the excess with toilet paper). In dry climates, excess glycerol will retard but not prevent drying.

Slide 54

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. With this pressure, the faecal material will be spread evenly between the microscope slide and the cellophane strip.

Slide 55

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. Newspaper print can be read through the smear after clarification. Water evaporates while glycerol clears the faeces.

Slides 56 to 60 show appearance of *A. lumbricoides*, *T. trichuria* and hookworm eggs in Kato-Katz preparations.

Slide 56

*A. lumbricoides*, fertile and infertile eggs with a *T. trichuria* egg in the middle.

Slide 57 *(Plate 1, Fig. 8)*

Normal and decorticated *A. lumbricoides* egg in a Kato-Katz preparation. Decorticated eggs may be slightly smaller than normal eggs due to the absence of the mamillated layer.

Slide 58

*T. trichuria* egg.

Slide 59

*A. lumbricoides* and hookworm egg.

Slide 60 *(Plate 1, Fig. 9)*

*A. lumbricoides* and *T. trichuria* eggs. Characteristic features of both are preserved in this preparation.

Slide 61 *(Plate 5, Fig. 2)*

An elongated trophozoite of *Entamoeba histolytica* in an unstained saline wet mount preparation demonstrates numerous red blood cells in its cytoplasm. In diarrhoeic and dysenteric stool specimens of individuals with amoebiasis, it is often possible to find amoebae with ingested erythrocytes. 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.

Slide 62 *(Plate 5, Fig. 8)*

In this trichrome-stained faecal smear, a trophozoite of *E. histolytica* contains numerous red-staining erythrocytes in its cytoplasm. The nucleus, with its centric karyosome and fine peripheral chromatin, is partially obscured by the ingested red blood cells but it is located centrally along the lower margin of the amoeboid organism. There is a large glycogen vacuole which compresses the nucleus up against the cyst membrane; several rounded chromatoid bodies are seen around the vacuole.

Slide 63 *(Plate 5, Fig. 5)*

Slide 64 *(Plate 5, Fig.11)*

*E. histolytica/E. dispar* trichrome- and iron haematoxylin-stained trophozoites in a faecal smear. Note the typical nucleus of this species containing a small central karyosome and fine, evenly distributed peripheral chromatin on the nuclear membrane. The cytoplasm is vacuolated.

Slide 65 *(Plate 5, Fig. 1a)*

*E. histolytica/E. dispar* binucleate cyst in MIF (merthiolate-iodine-formalin) wet mount preparation. In immature cysts such as this one, the nuclei are larger than those seen in mature cysts. The large glycogen vacuole lying between the two nuclei often compresses the nuclei against the cyst membrane so that it may be difficult to recognize the karyosomes.

Slide 66 *(Plate 5, Fig. 4a)*

Both uninucleate and binucleate cysts of *E. histolytica/E. dispar* are seen in the same field of this trichrome-stained faecal smear. In the uninucleate cyst, the large nucleus lies on top of and partially obscures the glycogen vacuole, numerous red-staining chromatoid bodies encircle the vacuole. In the binucleate cyst, the nuclei are smaller and at opposite poles with the glycogen vacuole in the center of this immature cyst. A number of red-staining chromatoid bodies are overlying the vacuole.

Slide 67 *(Plate 5, Fig. 4b)*

An uninucleate cyst of *E. histolytica/E. dispar*, stained with iron haematoxylin, demonstrates a large glycogen vacuole and dark-staining chromatoid bodies. Note the large size of the nucleus.

Slide 68 *(Plate 5, Fig. 1b)*

In this iodine-stained mature cyst of *E. histolytica/E. dispar*, 3 of the 4 nuclei are seen in this plane of focus. In amoebic and other protozoan cysts it is often difficult to demonstrate all nuclei in the same plane of focus. It is recommended that permanently stained slides be prepared on faecal specimens to confirm the diagnosis.

Slide 69 *(Plate 5, Fig. 7)*

*E. histolytica/E. dispar* trichrome-stained mature cyst demonstrating all 4 nuclei and red-staining chromatoid bodies in the center.

Slide 70 *(Plate 5, Fig. 10)*

*E. histolytica/E. dispar* iron haematoxylin-stained mature cyst showing 3 of the 4 nuclei and chromatoid bodies which are not in the same plane of focus.

Slide 71 *(Plate 6, Fig. 1a)*

A mature cyst of *Entamoeba coli* is seen in this formalin-preserved, unstained faecal smear. The nuclei of *E. coli* cysts are readily visible in wet preparations, whether unstained, as here, or in MIF or iodine. All 8 nuclei of the mature cyst usually are not visible in the same plane of focus. Immature, binucleate cysts of *E. coli* are often of similar size and morphology to those of *E. histolytica*/*E. dispar* so that it may be difficult to specify such organisms unless other identifiable cysts or trophozoites are present in the same faecal smear.

Slide 72 *(Plate 6, Fig. 1b)*

Two mature cysts of *E. coli* demonstrate multiple nuclei in this iodine-stained faecal smear.

Slide 73 *(Plate 6, Fig. 4a)*
Slide 74 *(Plate 6, Fig. 4b)*

A mature cyst of *E. coli* is present in this trichrome-stained faecal smear. Five nuclei are easily seen in this plane of focus (Slide 73), whereas in the iron haematoxylin staining only four nuclei can be identified (Slide 74).

Slide 75 *(Plate 6, Fig. 7)*
Slide 76 *(Plate 6, Fig. 10)*

*E. coli* trophozoite in trichrome- and iron haematoxylin-stained faecal smear. The nucleus has a large, centrally located karyosome and the peripheral chromatin is irregular and prominent on the nuclear membrane. The cytoplasm appears highly vacuolated.

Slide 77 *(Plate 8, Fig. 1)*

An uninucleate cyst of *Entamoeba polecki* in a trichrome-stained faecal smear; cysts of this species typically are uninucleate and may contain a dense inclusion mass (as seen here) and/or a large number of chromatoid bodies of varying sizes and shapes. The inclusion mass is not glycogen and in iodine wet mounts stains light brown as compared to the dark brown seen with glycogen vacuoles. In this organism, the nucleus is large and has a small karyosome and somewhat

peripheral chromatin on the nuclear membrane. The inclusion mass is larger than the nucleus and is situated to the left of it.

Slide 78 *(Plate 8, Fig. 4)*

In this trichrome-stained, mature cyst of *E. polecki*, there are many chromatoid bodies of various sizes and shapes scattered throughout the cytoplasm. The single nucleus is situated on the left side and is partially obscured by the chromatoid bodies. It has a minute central karyosome and irregular peripheral chromatin. The presence of large numbers of chromatoid bodies of various shapes and sizes and the presence of a single nucleus is highly suggestive of *E. polecki*.

Slide 79 *(Plate 8, Fig. 7)*

A trichrome-stained trophozoite of *E. polecki* is seen here. The nucleus of this species is similar in morphology to that of *E. histolytica/E. dispar* trophozoites in that the karyosome is often small and centrally located and there is fine peripheral chromatin on the nuclear membrane. However, in *E. polecki* the nucleus may frequently be irregularly shaped, the karyosome may appear to be lacking, and the peripheral chromatin may be irregular and unevenly distributed on the nuclear membrane.

Slide 80 *(Plate 5, Fig. 3a)*
Slide 81 *(Plate 5, Fig. 3b)*

*Entamoeba hartmanni* uninucleate cysts are shown in these trichrome- (Slide 80) and iron haematoxylin-stained (Slide 81) faecal smears. Glycogen (Slide 80) and chromatoid bodies are present (Slides 80 and 81).

Slide 82 *(Plate 5, Fig. 6a)*

A mature cyst of *E. hartmanni* demonstrates all 4 nuclei in this trichrome-stained faecal smear.

Slide 83 *(Plate 5, Fig. 6b)*

A binucleate cyst of *E. hartmanni* with one nucleus in sharp focus and chromatoid bodies is seen in iron haematoxylin staining.

Slide 84 *(Plate 5, Fig. 9a)*

A delicately stained trophozoite of *E. hartmanni* is seen in this trichrome-stained faecal smear. The small nucleus contains a punctuate karyosome and the nuclear membrane has fine peripheral chromatin. Trophozoites of this species usually take a pale stain in permanently stained faecal smears and they can be easily overlooked if the microscopist scans the slide too rapidly.

Slide 85 *(Plate 5, Fig. 9b)*

*E. hartmanni* trophozoite in iron haematoxylin staining.

Slide 86 *(Plate 5, Fig. 12)*

Note the size difference of *E. hartmanni* and *Iodamoeba bütschlii* trophozoites in this trichrome staining.

Slide 87 *(Plate 6, Fig. 3a)*

*Endolimax nana* mature cyst in an iodine wet mount. Three of the 4 nuclei are seen, each with a large, brown-staining karyosome.

Slide 88 *(Plate 6, Fig. 3 b)*

*E. nana* mature cysts are stained in MIF, with the top one showing 3 of the 4 nuclei.

Slide 89 *(Plate 6, Fig. 6a)*
Slide 90 *(Plate 6, Fig. 6b)*

In the trichrome-stained faecal smear, a mature cyst of *E. nana* demonstrates 3 of 4 nuclei (Slide 89), whereas in the iron haematoxylin staining all 4 nuclei are seen (Slide 90).

Slide 91 *(Plate 6, Fig. 2)*

*I. bütschlii* cysts in an iodine wet mount. As is typical for this species, the glycogen vacuoles have a prominent brown colour in such preparations. The nucleus typically is not visible in wet mounts.

Slide 92 *(Plate 6, Fig. 5a)*
Slide 93 *(Plate 6, Fig. 5b)*

The typical appearance of *I. bütschlii* cysts in trichrome- (Slide 92) and iron haematoxylin-stained (Slide 93) faecal smear. The karyosome is large, stains red, and there is no peripheral chromatin on the nuclear membrane. With both trichrome stains (Slide 92) and iron haematoxylin (Slide 93), the vacuole remains unstained.

Slide 94 *(Plate 6, Fig. 8)*

An elongated *I. bütschlii* trophozoite in a trichrome-stained faecal smear. The nucleus shows the typical large, red-staining karyosome and the nuclear membrane lacks peripheral chromatin.

Slide 95 *(Plate 8, Fig. 2)*

*Entamoeba gingivalis* trophozoite in an iron haematoxylin-stained smear made from material taken from the parodontal space of the oral cavity. Typically, these non-pathogenic amoebae occur in the mouth of patients who have parodontitis. As in this specimen, the nucleus is *E. histolytica/E. dispar*-like, in having a small, central karyosome and fine peripheral chromatin on the nuclear membrane. The cytoplasm of these trophozoites typically contains leukocytes in varying stages of digestion (as seen here) as well as bacteria and other detritus. Trophozoites of this species have also been reported from smears made from the cervix and vagina of women utilizing intrauterine-contraceptive devices. *E. gingivalis* has no cyst stage.

Slide 96 *(Plate 7, Fig. 7)*

*Giardia lamblia* (*intestinalis*) trophozoite in trichrome-stained faecal smear. The characteristic pyriform shape and two anteriorly positioned nuclei on either side of the paired, longitudinally oriented axonemes readily identify this organism.

Slide 97 *(Plate 7, Fig. 10)*

Three *G. lamblia* trophozoites are seen in ventral views and two in lateral aspect (iron haematoxylin staining).

Slide 98 *(Plate 7, Fig. 1)*

Several *G. lamblia* cysts are seen in this iodine wet mount prepared from a flotation concentration procedure. The characteristic shape and appearance of these cysts demonstrating nuclei, axonemes and median bodies is diagnostic.

Slide 99 *(Plate 7, Fig. 4a)*
Slide 100 *(Plate 7, Fig 4b)*

Mature cyst of *G. lamblia,* two of the 4 nuclei, axonemes and the median body are evident (Slide 99 trichrome, Slide 100 iron haematoxylin). A halo around the organism is due to the shrinkage of the organism during fixation (Slide 99).

Slide 101 *(Plate 7, Fig. 3a)*
Slide 102 *(Plate 7, Fig. 3b)*

In these trophozoites of *Dientamoeba fragilis* in a trichrome-stained faecal smear, two nuclei are seen, with one of them being more prominent than the other. Trophozoites of this species may have one or two nuclei, with the binucleate forms being more frequent. The karyosomes within the nuclei are variable in appearance; often they are fragmented into 3 to 8 pieces but in other instances they may appear as a single mass. There is no peripheral chromatin on the nuclear membrane. This organism has no cyst stage and its exact manner of transmission has yet to be determined. With both trichrome and iron haematoxylin stains, it is characteristic for these trophozoites to take a pale stain which results in their being overlooked when smears are scanned too rapidly.

Slide 103 *(Plate 7, Fig. 6a)*

This iron haematoxylin-stained *D. fragilis* trophozoite has a single nucleus and a karyosome which is clearly fragmented into 3 pieces.

Slide 104 *(Plate 7, Fig. 6b)*

A binucleate trophozoite of *D. fragilis* in an iron haematoxylin-stained faecal smear. Both nuclei demonstrate fragmentation of their karyosomes into 3 or 4 pieces.

Slide 105 *(Plate 7, Fig. 9)*

A binucleate trophozoite of *D. fragilis* which is more delicately stained (trichrome) is seen between an *E. histolytica/E. dispar* trophozoite, and a smaller, uninucleate cyst of *E. histolytica/E. dispar* containing chromatoid bodies.

Slide 106 *(Plate 7, Fig. 8)*

Two pale-staining trophozoites of *Chilomastix mesnili* are seen in this trichrome-stained faecal smear. They have a pyriform shape which tapers to a point at the posterior end and is most clearly seen in the organism on the right. The single nucleus is located at the rounded anterior end of the trophozoites.

Slide 107 *(Plate 7, Fig. 11)*

*C. mesnili* trophozoite iron haematoxylin-stained shows finely pointed posterior end. Note the nucleus at the anterior end.

Slide 108 *(Plate 7, Fig. 2a)*
Slide 109 *(Plate 7, Fig. 2b)*

In this iodine wet mount preparation, the typical lemon-shaped appearance of *C. mesnili* cysts are seen. At higher magnification (Slide 109), there is a clear, nipple-like prominence at the anterior end of the cyst, a single nucleus on the left side, and a faint outline of the cytostome visible to the right of the nucleus.

Slide 110 *(Plate 7, Fig. 5a)*
Slide 111 *(Plate 7, Fig. 5b)*

*C. mesnili* cysts in trichrome- (Slide 110) and iron haematoxylin-staining (Slide 111) demonstrate the lemon-shaped appearance, the nucleus and the outline of the cytostome to the right of the nucleus.

Slide 112 *(Plate 7, Fig. 12a)*
Slide 113 *(Plate 7, Fig. 12b)*

In this trichrome-and iron haematoxylin-stained faecal smear a trophozoite of *Pentatrichomonas hominis* (formerly identified as *Trichomonas hominis*) is seen. The organisms demonstrate the single, anteriorly placed nucleus and the prominent axostyle which is directed posteriorly and extends beyond the posterior end. In stained specimens it is often possible to see a series of longitudinally oriented granules (hydrogenosomes) adjacent to the axostyle. This flagellate has no cyst stage. Trophozoites do not always stain well with permanent stains and diagnosis is often best made by finding the motile trophozoites in freshly passed diarrhoeic infection stools.

Slide 114 *(Plate 8, Fig. 6)*

The large trophozoite of *Balantidium coli* is seen in this MIF wet mount. The cytostome is visible at the top of the organism and the large clear area at the bottom is the macronucleus. Cilia are see on the surface.

Slide 115 *(Plate 8, Fig. 9)*

A trichrome-stained trophozoite of *B. coli*. The cytostome is seen at the top of the organism and the macronucleus is the dark-staining structure located in midbody. Cilia are visible on the surface as hair-like projections.

Slide 116 *(Plate 8, Fig. 3)*

A cyst of *B. coli*, the only ciliate parasite of humans, in an unstained wet mount of formalin-preserved faeces. The large macronucleus is visible as a clear area at the right side of the cyst.

Slide 117 *(Plate 9, Fig. 1)*

Four oocysts of *Cryptosporidium parvum* are seen in this wet mount preparation from formalin-preserved faeces. The small size of these oocysts (4-6 µm) and the presence of black granules within them are diagnostic for these organisms. However, confirmation of the diagnosis is best accomplished by acid-fast stains of faecal smears. Although not visible here,

these infective oocysts contain four sporozoites when excreted in faeces.

Slide 118 *(Plate 9, Fig. 7)*
Slide 119 *(Plate 9, Fig. 4)*

Various modifications of acid-fast stains can be used for demonstrating *C. parvum* oocysts; organisms will vary in colour from dark red to light pink but the black granules are usually readily visible with all of the stain modifications used (Slide 118). In this acid-fast stained faecal smear, 3 oocysts of *C. parvum* take an intense red colour and show the presence of the black granules (Slide 119).

Slide 120 *(Plate 9, Fig. 10)*

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

Slide 121 *(Plate 9, Fig. 2a)*
Slide 122 *(Plate 9, Fig. 2b)*

*Cyclospora cayetanensis* unsporulated oocysts are visible in this wet mount preparation from formalin-preserved faeces. These oocysts must be differentiated from the similarly appearing oocysts of *C. parvum*. *C. cayetanensis* oocysts are approximately twice the size (8 to 10 µm) of the oocysts of *C. parvum*. It takes approximately one week for the oocysts of *C. cayetanensis* to undergo sporulation and develop two sporocysts containing two sporozoites each within the oocyst which is then the infective stage (Slide 122).

Slide 123 *(Plate 9, Fig. 5)*

A group of unsporulated oocysts of *C. cayetanensis* are seen in this acid-fast stained faecal smear. The oocysts of this species stain variably with acid-fast stains, as seen here, and may be pink to red, bluish or not stained at all.

Slide 124 *(Plate 9, Fig. 8a)*
Slide 125 *(Plate 9, Fig. 8b)*

*C. cayetanensis* oocyst in acid-fast staining. The organism can remain unstained (Slide 124) or typically becomes red-stained (Slide 125).

Slide 126 *(Plate 9, Fig. 3)*

An unsporulated oocyst of *Isospora belli* in a wet mount of formalin-preserved faeces. These oocysts are much larger (20-33 µm long) than the oocysts of either *C. parvum* or *C. cayetanensis*. They have a very thin cyst wall and they can be easily overlooked when examining wet mount preparations. Typically, oocysts of *I. belli* will sporulate outside of the human host to become the infective stage.

Slide 127 *(Plate 9, Fig. 6a)*
Slide 128 *(Plate 9, Fig. 6b)*

An atypical oocyst of *I. belli* appearing empty as often seen in patients undergoing treatment (Slide 127). In Slide 128, an unsporulated oocyst of *I. belli* is seen in this acid-fast stained faecal smear. The sporoblast inside the oocyst stains intensely red and there is also pink stain deposition on the outside of the oocyst wall.

Slide 129 *(Plate 9, Fig. 9)*

Oval-shaped spores of the microsporidian parasite, *Encephalitozoon hellem*, are seen in Gram-stained urinary sediment from an HIV-positive patient with disseminated infection. Microsporidiosis is an opportunistic infection increasingly associated with patients having the acquired immunodeficiency syndrome (AIDS) or other immunocompromized conditions. These spores are approximately 1 to 2 µm in length and not readily found or recognized unless specialized staining techniques are utilized.

Slide 130 *(Plate 9, Fig. 12)*

Spores of one of the intestinal microsporidians, *Enterocytozoon bieneusi* or *Septata intestinalis*, are readily seen by using oil immersion microscopy with ultraviolet illumination on methanol-fixed faecal smears stained with 0.5% Calcofluor white in 10% KOH. The bright white fluorescence of the oval spores makes them readily visible.