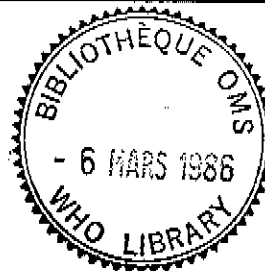


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PDP/85.2  
ENGLISH ~~ONLY~~

DIAGNOSTIC TECHNIQUES  
FOR INTESTINAL PARASITIC INFECTIONS (IPI)  
APPLICABLE TO PRIMARY HEALTH CARE (PHC) SERVICES<sup>1</sup>

*Intestinal Diseases Parasitic Diseases*

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ANNEXES:

1. Diagnosis of Intestinal Parasitic Infections at different PHC levels
2. Cellophane faecal thick smear examination technique (PDP/83.3)

<sup>1</sup> This document is one in a series of papers (PDP/85.1, 85.3, 85.4, 85.5) which have been prepared by the WHO Parasitic Diseases Programme and which are intended to provide up-to-date information on technical aspects of intestinal parasitic infections control. According to the advances in technology and as experience accumulates in national control programmes, these documents will be revised. Inquiries and comments may be directed to Director, Parasitic Diseases Programme, World Health Organization, 1211 Geneva 27, Switzerland.

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## 1. INTRODUCTION

The development of simple diagnostic techniques and their transfer to health workers involved in the Primary Health Care delivery system is one of the basic activities of the Parasitic Diseases Programme and an essential contribution towards Health for All by the year 2000. The general objective of this paper is to briefly review the simple techniques that are available for the microscopic diagnosis of human intestinal parasitoses in order to guide those responsible for primary health care to choose the most appropriate test(s) and procedures for specific circumstances.

Many of the simple techniques for the diagnosis of intestinal parasitic infections were developed at the beginning of this century (direct smear, brine flotation, acid-ether concentration). A few additions appeared in the 1950s to assist national control programmes (trichrome staining, cellophane thick smear, coproculture of helminthic larvae). Although several modifications have improved the practical usefulness of these techniques, in general there have been no original inventions during the past few decades.

The fact that most of the diagnoses of intestinal parasitic infections are carried out only by microscopical examination of stools considerably limits both the development of new technologies and their transfer into basic PHC practice. Conversely, the use of indirect diagnostic techniques (immunological, biochemical) is costly and requires sophisticated laboratory technology (e.g. tests for E. histolytica antigen in faecal material).

There are several other constraints on the development of simple diagnostic techniques: (1) it is impossible to utilize one single method to diagnose biological objects as diverse as protozoan trophozoites and cysts, helminthic eggs and larvae; they are identified by totally different morphological criteria (e.g. structure of nuclei in Entamoeba sp., size, shape and content of helminthic eggs etc.) and protozoa usually require the complicated fixation and staining procedures; (2) a technique in almost universal use for the diagnosis of common infections may be unsuitable for diagnosing infections which occur rarely (e.g. strongyloidiasis, opisthorchiasis); (3) different life cycles of different parasites (Enterobius, T. saginata, etc.) require the use of different material to be examined (e.g. proglottids, anal swabs).

There are several general rules related to the diagnosis of intestinal parasitoses that are worthy of mention. In medical laboratories, faeces are the most unpleasant material used for examination and therefore all manipulations should be minimized; possible exposure to faecal-transmitted bacteria and viruses should also be taken into account. Faeces are also the least uniform material, re-examination is frequently necessary and not all sophisticated quantitative studies are reproducible. A frequently-used semiquantitative clinical classification involves three categories of increasing intensity of infection, usually termed light, moderate, and heavy. There is an inverse relationship between the time used for the preparation of the specimen and the time needed for examining it (e.g. direct smear is easy to prepare but difficult to examine; zinc sulphate flotation has a complicated technology but the slides are easy to examine). Since the accuracy of coproscopic examinations depends greatly on skilled personnel and thorough technique, extensive training, reference services and periodic quality control are essential for maintaining appropriate standards of diagnosis in peripheral laboratories.

## 2. DIAGNOSIS OF MAJOR INTESTINAL PARASITIC INFECTIONS AT DIFFERENT PHC LEVELS

Some intestinal parasitoses can be diagnosed macroscopically by community health workers or by people who have a very basic level of health education. The easiest to diagnose is Ascaris expelled in faeces or by mouth; treatment should follow in any case of Ascaris evacuation, since other Ascaris worms may be present, including pre-adults and males, which cannot be diagnosed by coproscopic examination. The macroscopical diagnosis of T. saginata taeniasis and enterobiasis is also relatively easy.

Some intestinal parasitoses may be suspected by the presence of characteristic symptoms and signs. For example, by examining faecal evacuations, an experienced person may suspect amoebiasis (dysenteric stool; blood and mucous stripes on the surface of evacuation in an individual with "walking" diarrhoea) or giardiasis (loose, yellow-gray, foamy, badly smelling stools). In areas with a high rate of symptomatic amoebiasis, if individual microscopic examinations of stools cannot be performed, the use of a chemotherapeutic test (with a nitroimidazole drug) is justified in suspected cases. Nowadays, it is easier to treat suspected amoebiasis than to diagnose it at PHC level.

Intensive hookworm infections usually manifest themselves in anaemia, which may be recognized by a health worker. In areas where intensive hookworm (especially *Ancylostoma duodenale*) infections are known to be common and individual microscopic diagnosis can not be performed, standard treatment with anthelmintics and iron at the PHC level is justified in those anaemic individuals in whom the other causes for anaemia are not evident (malaria, haemorrhage etc.).

In other words, in areas endemic for amoebiasis and hookworm infections an epidemiological survey may to some extent replace microscopic diagnosis in individuals, if this is not available.

An epidemiological survey should be carried out by a team trained in basic epidemiology and parasitological laboratory diagnosis. In the PHC organizational system the team should be organized somewhere above the first referral level (local hospital) possibly at a regional public health institution or even at an academic or professional school (school of nurses or health officers, etc.) The aim of an epidemiological survey is (i) data collection (from community based examinations and from dispensaries and rural hospitals); (ii) data analysis (bearing in mind the prevalences and distributions of infections as well as their public health impact); and (iii) elaboration of a system of response (organization of community-based mass or selective or target treatment; giving justification for "chemotherapeutic tests"; urging improvement of sanitary conditions; arrangements for stopping further transmission by contaminated water or food, etc.).

The specialized parasitological laboratories at public health or academic institutions should have good working relations with general medical laboratories in the region to which they serve as reference, quality control and training centres.

Routine microscopical diagnosis of all intestinal parasitic infections is likely to be available only in district or some peripheral hospitals. Some peripheral hospital and health centres may use simple parasitological laboratory techniques to diagnose most common infections provided they have a microscope and a trained laboratory assistant (WHO, 1979) (Annex 1).

### 3. REVIEW OF SIMPLE DIAGNOSTIC TECHNIQUES

Basic and simple diagnostic techniques have been selected for their practicability for PHC services, but this criterion should not exclude some local adaptation and modification if they are justified. The suggested techniques are described in detail in the WHO and CDC manuals (WHO, 1980; Melvin & Brooke, 1982).

#### 3.1 Direct thin smear

A direct thin smear is simple to prepare and requires the least equipment. It is good for the recognition of *E. histolytica* hematophagous trophozoites, amoebic and *Giardia* cysts and helminthic eggs and larvae (if more than 1000 eggs or larvae per gm of faeces are present). Examination of a direct smear is not easy and the diagnosis of most protozoan trophozoites and cysts as well as some helminthic eggs and larvae, requires a proper knowledge of the morphological diagnostic criteria and skill gained by experience.

Two direct thin smears should be prepared: one in physiological saline and the other with a temporary stain. The smear in physiological saline, made of fresh faecal material and/or mucous, is the best for finding hematophagous E. histolytica trophozoites. It is also useful for finding Giardia lamblia cysts (under a high magnification, i.e. 400x) and some helminthic eggs such as Ascaris, Trichuris, hookworm, and if abundant, those of liver flukes.

Numerous procedures are used for temporary staining of direct smears. Iodine solution (Dobell & O'Connor, 1921) helps to determine glycogen as well as the basic structure and the number of nuclei in amoebic cysts; but it easily distorts the trophozoites. Buffered solution of methylene blue (Nair, 1953) stains well the nuclei of E. histolytica trophozoites. MIF (merthiolate-iodine-formaldehyde) solution (Sapero & Lawless, 1953) is good for staining both protozoan trophozoites and cysts. Chlorazol black E (Kohn, 1960) stains the chromatin in protozoan trophozoites and cysts almost as well as hematoxylin.

Sealing of the wet mounts with paraffin-vaseline (1:1) is recommended in order to prevent rapid drying and to make possible a re-examination possible after some hours.

### 3.2 The cellophane thick smear

The KATO cellophane thick smear in its original version (Kato & Miura, 1954) is a simple and cheap technique. A KATO-KATZ quantitative version (Katz et al., 1972) needs some care to be prepared correctly, but it is easier to examine or re-examine after a few days or even months. The cellophane thick smear needs very simple equipment, which is available in the forms of kits or bulk supply (Annex 2) and it is useful for the diagnosis of infections with Ascaris, Trichuris, hookworms (if examined within a few hours), Taenia and liver flukes. Additionally, skilled technicians are able to diagnose Giardia cysts, and Sarcocystis or Isospora oocysts and sporocysts. The technique is one of the best available for egg-counts, which are important both for epidemiological studies and for individual treatment (differentiation of light, moderate and heavy infections with hookworms and Trichuris).

### 3.3 Techniques for isolation of helminthic larvae

Helminthic larvae in faeces are best isolated in coprocultures or in a Baermann apparatus.

Coprocultures can be established in Petri dishes (Brumpt, 1949), test-tubes (Harada & Mori, 1955), polyethylene tubes (Sasa et al., 1965), or plastic boxes (Dancescu, 1968). Although the preparation of a coproculture is simple and cheap, cultures should be kept for 7 to 10 days in order to allow hookworm filariform larvae to develop (Strongyloides larvae need only a few days). This technique enables the differentiation of filariform larvae of Strongyloides, Ancylostoma, Necator and Trichostrongylus (WHO Technical Report Series, No. 666).

The Baermann (1917) technique does not necessarily need a screen, a funnel or a rubber tubing closed with a clamp. The examination for Strongyloides larvae present in stools can be carried out by simply immersing the stool sample on a screen in an ordinary glass filled with tepid saline (modification by Lumberras, 1962). After 30 minutes Strongyloides larvae can be collected from the bottom of the glass and examined under low magnification (100x). This technique is also useful for finding trophozoites of Balantidium coli.

### 3.4 Simple sedimentation technique

Simple sedimentation is cheap and easy, but time consuming (decantation of faecal material mixed with water repeated 3-7 times every 10 to 20 minutes). The examination of a deposit, being cleared-out of most of the debris, is similarly easy. The technique is good for all helminthic eggs (especially heavy ones such as unfertilized Ascaris and Fasciola) and larvae, and also for some protozoan cysts (Giardia, Entamoeba). The deposit can be preserved easily with saline-formalin or MIF, transported and re-examined after some months.

### 3.5 Concentration techniques

Among the various concentration techniques zinc flotation (Faust et al., 1938) and formalin-ether (Ritchie, 1948) or acid-ether (Telemann, 1908) sedimentations are most commonly used.

A modified zinc sulfate flotation technique requires sedimentation in water and flotation in 33% solution of Zn SO<sub>4</sub> by using a centrifuge. The end result of this laborious procedure is a thin film consisting of helminthic eggs (except heavy fluke eggs and unfertilized Ascaris eggs) and larvae as well as protozoan cysts. When a centrifuge is not available, a flotation in saturated brine may be used (Willis, 1921), but it is only good for light helminthic eggs (fertilized Ascaris eggs and Trichuris, hookworm, Hymenolepis spp., and Taenia spp.).

The acid-ether or formalin-ether techniques help to dissolve albuminous and fatty particles in faeces and hence obtain a concentrated, relatively clear deposit. Both techniques are laborious and require centrifugation. The acid or formalin component of the procedure can be replaced with MIF solution (Blagg et al., 1955). These techniques are very useful for examination of specimens preserved with MIF or formalin.

### 3.6 Trichrome permanent staining

The staining techniques using iron-hematoxylin are the best for the identification of intestinal protozoa but because they need a high skill in the preparation and examination of specimens they are usually reserved for protozoological laboratories. The colour staining of amoebic and cysts is easy to obtain by a standard trichrome procedure (Wheatley, 1951) and is relatively easy to examine by a technician, without the necessity of a parasitologist. Trichrome staining can be easily carried out with PVA preserved material.

### 3.7 Other simple techniques

An anal swab using a piece of Scotch adhesive tape is a simple technique to find Enterobius or T. saginata eggs (Melvin & Brooke, 1982).

Finding adult worms of Enterobius, Trichuris and hookworms or Taenia scoleces and proglottids and intestinal flukes after treatment can be facilitated by simple staining or repeated sedimentation of faecal material mixed with water.

### 3.8 Preservation of specimens

Where a parasitological examination has to be performed in a reference parasitological laboratory, the material (faeces, deposits from sedimentation or concentration techniques, tapeworm proglottids) should be preserved before transportation.

Faecal material for protozoological examination is best preserved in PVA (polyvinyl alcohol) fixative, both in small bottles and on slides. The PVA preserved faecal material can easily be stained with hematoxylin or trichrome techniques.

Faecal material for helminthological examination should be fixed in 10% formalin (5% formalin is suggested if protozoan cysts are going to be looked for as well). MIF solution, containing about 5% of formalin, can be used for fixation of both helminthic eggs and protozoan cysts. A formalin-ether concentration technique may be used for both formalin and MIF fixed material.

The appropriate proportion of faecal material and fixatives to be mixed is 1:3.

Adult helminths should be fixed with 5% formalin; for tapeworm proglottids or nematodes (planned to be stored for a longer time), fixation in 70% alcohol with glycerin (10:1) is preferred.

#### 4. LABORATORY METHODOLOGY FOR AN EPIDEMIOLOGICAL SURVEY

##### 4.1 Background

There are three major purposes of a laboratory examination:

(a) research, (b) an epidemiological survey and (c) clinical diagnosis (not discussed in this document).

For field research purposes the material and methods of examination should be carefully chosen according to the objectives of studies. Proper selection of the population examined is essential (see WHO document PDP/85.4). A constellation of several methods is needed if the research should cover all intestinal parasitoses (e.g. direct smear, Kato-Katz technique, sedimentation and/or flotation concentration, trichrome staining, nematode larvae coproculture). In case the research is focused on a specific infection only, e.g. ascariasis, a single, most specific, sensitive and practical method has to be chosen, e.g. Kato-Katz technique. The research should be done by skilled personnel under the direct supervision of a parasitologist. The highest possible standard of accuracy is required, e.g. counting all Ascaris eggs in Kato-Katz technique. Research is usually time-consuming and expensive.

In an epidemiological survey which usually covers the larger samples of population, a compromise has to be made between the expected standard of accuracy and the practical aims of the studies. The techniques used should be simple, inexpensive, but appropriate for a given purpose. For example in a hookworm infection survey Kato-Katz technique plus coprocultures of 10% of positive samples are most appropriate. A combination of a direct thin smear and Kato-Katz technique is the most useful for a general survey on intestinal parasitoses.

##### 4.2 Direct thin smear and Kato-Katz technique: advantages and limitations.

Advantages. A direct thin smear and the Kato-Katz technique are both simple and low cost. When used together they are able to detect major protozoan and helminthic infections and are useful in estimating the worm burden (Ascaris, hookworm, Trichuris) and in evaluating the effectiveness of control activities. In addition, the Kato-Katz technique provides comparable results between different members of a laboratory team, between different teams and between different endemic areas; it also facilitates quality control and re-examination of the slides after a longer time without additional preservation.

Limitations. The direct thin smear may not be useful for identification of protozoan trophozoites, if the faecal sample is not fresh, which is the case in many epidemiological surveys (for this reason a thin smear in physiological saline may be omitted if the faecal sample is kept unpreserved for more than 30 minutes).

In the Kato-Katz technique, delicate hookworm and Hymenolepis sp. eggs disappear during the clearing process in a short time of 15-120 minutes. It is suggested to check (by examining every 15 minutes the few first positive smears kept in the particular local conditions) when hookworm or Hymenolepis eggs become invisible or undiagnosable and examine all the other slides well before this critical time. Some of the hookworm and Hymenolepis eggs may still be found by examining the direct thin smear in case the infection is intensive.

#### 4.3 Value of quantitative examinations

In an epidemiological survey a quantitative examination is useful in some helminthic infections for measuring the intensity of infection and related morbidity and in evaluating the effectiveness of control activities.

The most objective method for measuring the intensity of nematode infections is by counting the number of worms expelled after a successful therapy but this is usually done only for research purposes.

Direct smear made of about 2 mg of faeces is rather a poor quantitative technique: in direct smear one egg (being found or not) equals 500 eggs per gram. However, direct smear can well distinguish light from highly intensive infections. Kato-Katz technique based on 41.7 mg of faeces is theoretically 21 times more accurate in measuring the number of eggs per g of faeces. Several parasite and host factors may influence the actual number of eggs per g, however, for practical purposes of a survey what is needed are not definite numbers of eggs but rather some categories of intensity of infection, e.g. light, moderate and heavy.

Heavy infection may be related to morbidity in the following helminthiases:

- ascariasis (impairment of nutritional status, frequency of intestinal obstruction but not other surgical complications);
- hookworm infection (hookworm anaemia);
- trichuriasis (intensive trichuriasis in children);
- hymenolepiasis (symptomatic infection).

The worm load most likely to cause morbidity depends much on age, sex, nutritional status and concomitant infections. For hookworm infections the critical number of worms which cause anaemia vary widely from area to area and must be locally estimated.

At present the boundaries between light and moderate as well as moderate and heavy infections can only be made arbitrarily and in cooperation with local clinicians.

In ascariasis the infection is of a light intensity if any unfertilized eggs are present; the U-ratio, i.e. number of slides with unfertilized eggs versus all positive slides has been used for evaluating the intensity of infection in some control programmes. Unless more quantitative studies are undertaken on intestinal obstruction and impaired nutrition due to Ascaris, there is no other than an arbitrary distinction between moderate and heavy infections.

#### 4.4 Preparation of a laboratory survey

The preparation of a simple laboratory examination as part of a survey needs the following questions to be answered:

(1) What is expected from the laboratory examination?

- objectives (see document PDP/85.1)

(ii) How to do it?

- material (see PDP/85.4)
- methods (discussed above)
- joint action with other programmes (see PDP/85.1 & 85.3)

(iii) Who can do it?

- local cadres available (including a reference centre)
- local training and quality control system necessary
- consultant from outside needed both for survey and training

(iv) What equipment and materials are needed?

- microscopes
- kits, chemicals, glass (see Annex 2)
- transportation
- drugs against intestinal parasitic diseases being diagnosed

(v) How much would it cost?

- in terms of money
- in terms of labour
- any support from outside (see PDP/85.1 & 85.3)

(vi) How to organize a laboratory survey?

- preparing laboratory facilities
- collection of faecal material
- preservation of material, if needed
- laboratory examination
- reporting
- cleaning laboratory facilities

(vii) What to do with the results?

- treat those infected who need treatment
- decide upon activities at community level (improvement of sanitation, health education, community-oriented chemotherapy)
- decide upon activities at regional/district levels (antiepidemic interventions, general control and preventive measures, standard
- use the results for supporting other major health programmes (see PDP/85.1 & 85.3)
- publish the results

5. RECOMMENDATIONS FOR FUTURE RESEARCH AND ACTION

- (i) There is a great need to further develop simple, cheap, and more effective techniques for the diagnosis of intestinal parasitoses (staining of amoebae, tests for antigens in faeces etc.)
- (ii) Effective transfer of existing technologies into the PHC system will need a network of parasitological reference laboratories (or institutes) involved actively in surveillance, training, reference services and quality control.
- (iii) Although most of the activities related to prevention and control of intestinal parasitoses can be carried out within the PHC system, there may still be a need for vertically oriented control programmes in epidemic situations or in highly endemic areas.

6. REFERENCES

- BAERMANN, G. (1917) Eine einfache Methode zur Auffindung von Ankylostomum (Nematoden) Larven in Erdproben. Mededeel. Geneesk. Lab. Weltevreden, Feestbundel, Batavia, pp 41-47
- BLAGG, W., SCHLOEGEL, E.L., MANSOUR, N.S. & KHALAF, G.I. (1955) A new concentration technic for the demonstration of protozoa and helminth eggs in feces. American Journal of Tropical Medicine and Hygiene, 4:23-28
- BRUMPT, E. Précis de Parasitologie, (1949) Paris, Masson et Cie, Ed., Vol. 1, pp 882-916
- DANCESCU, P. (1968) Investigations on the intensity of the infection in a strongyloidiasis focus: the coal culture method. Transactions of the Royal Society of Tropical Medicine and Hygiene, 62, 4:490-495
- DOBELL, C. & O'CONNOR, F.W. (1921) Intestinal protozoa of man. William Wood, New York
- FAUST, E.C., D'ANTONI, J.S., ODOM, V., MILLER, M.J., PERES, C., SAWITZ, W., THOMEN, L.F., TOBIE, J.E. & WALKER, J.H. (1938) A critical study of clinical laboratory technics for the diagnosis of protozoan cysts and helminth eggs in feces. American Journal of Tropical Medicine and Hygiene, 18:169-183
- HARADA, Y. & MORI, O. (1955) A new method for culturing hookworm. Yonago acta medica, 1:177-179
- KATO, K. & MIURA, M. (1954) Comparative examinations. Japanese Journal of Parasitology. (Japanese text) 3:25
- KATZ, N., CHAVES, A. & PELLEGRINO, J. (1972) A simple device for quantitative stool thick smear technique in schistosomiasis mansoni. Revista do Instituto de Medicina Tropical de Sao Paulo, 14:397-400
- KOHN, J. (1960) A one stage permanent staining method for fecal protozoa. Dapim Refuim Med. Quart. Israel, 19 (2/3):160-161
- LUMBRERAS, H. (1963) Strongyloidosis: I. Evaluacion de la "Tecnica de Baermann modificada en copa" en el estudio de la strongyloidosis. Revista Medica Peruana, Vol. 32, 334:119-133
- MELVIN, D.M. & BROOKE, M.M. (1982) Laboratory procedures for the diagnosis of intestinal parasites. US HHS Publication (CDC) No. 82.8282
- NAIR, C.P. (1953) Rapid staining of intestinal amoebae on wet mounts. Nature, 172:1051
- RITCHIE, L.S. (1948) An ether sedimentation technique for routine stool examinations. Bulletin of the US Army Medical Department, 8:326
- SAPERO, J.J. & LAWLESS, D.K. (1953) The "MIF" stain-preservation technique for the identification of intestinal protozoa. American Journal of Tropical Medicine and Hygiene, 2:613-619

- SASA, M., MITSUI, G., HARINASUTA, C. & VAJRASTHIRA, S. (1965) A polyethylene-tube culture method for diagnosis of parasitic infections by hookworms and related nematodes. Japanese Journal of Experimental Medicine, 35, 4:277-289
- TELEMANN, W. (1908) Eine Methode zur Erleichterung der Auffindung von Parasiteneiern in den Faeces. Dtsch. Med. Wochenschr., 34:1510-1511
- WHEATLEY, W.B. (1951) A rapid staining procedure for intestinal amoebae and flagellates. American Journal of Clinical Pathology, 21:990-991
- WILLIS, H.H. (1921) A simple levitation method for the detection of hookworm ova. Medical Journal Australia, 8:375-376
- WORLD HEALTH ORGANIZATION. (1979) Laboratory services at Primary Health Care Level. Informal Document (LAB/79.1)
- WORLD HEALTH ORGANIZATION. (1980) Manual of Basic Techniques for a Health Laboratory.
- WORLD HEALTH ORGANIZATION. (1981) Intestinal protozoan and helminthic infections. Report of a WHO Scientific Group, Geneva. Technical Report Series, 666, Geneva
- WORLD HEALTH ORGANIZATION. (1985) General strategies for prevention and control of intestinal parasitic infections within Primary Health Care (PHC) (PDP/85.1)
- WORLD HEALTH ORGANIZATION. (1985) Planning, implementation, monitoring and evaluation of the control of intestinal parasitic infections (IPI) programmes (PDP/85.3)
- WORLD HEALTH ORGANIZATION. (1985) Surveillance and survey methodology for intestinal parasitic infections (IPI) (PDP/85.4)

POSSIBLE DIAGNOSTIC ACTIVITIES AT DIFFERENT PHC LEVELSPOSSIBLE DIAGNOSIS OR ACTIVITIES RELATED TO DIAGNOSISLEVEL  
(AND RESPONSIBLE PERSON)

LEVEL (AND RESPONSIBLE PERSON)	POSSIBLE DIAGNOSIS OR ACTIVITIES RELATED TO DIAGNOSIS
HOME (mother/father, older children, relatives)	Macroscopic diagnosis of ascariasis
COMMUNITY (community health worker)	As above plus - macroscopic diagnosis of enterobiasis and taeniasis, observations for some signs of amoebiasis, giardiasis and hookworm disease.
FIRST LEVEL HEALTH FACILITIES - health centre, dispensary (medical assistant, nurse, laboratory assistant)	As above plus - simple Kato thick smear and other simple microscopic techniques (direct smear, Harada-Mori coproculture, modified Baermann technique) for Giardia cysts and hemato-phagous E. histolytica trophozoites as well as for major helminthic infections, such as ascariasis, trichuriasis, hookworm disease, and strongyloidiasis. Preservation of faecal material for further examination in parasitological laboratories.
FIRST REFERRAL LEVEL - rural or district hospital (physician, laboratory technician)	As above plus - more complicated microscopic techniques (simple sedimentation, zinc-sulphate and/or formalin-ether sedimentation techniques, trichrome staining) for major intestinal protozoan and helminthic infections.
- district health office (public health medical officer, laboratory technician)	Simple techniques for community diagnosis purposes.
REGIONAL PUBLIC HEALTH INSTITUTIONS (epidemiologist, parasitologist, staff from medical professional schools for health officers, nurses, etc.)	Community survey in intestinal parasitoses (prevalence, intensity of infections, age distribution, public health importance). Training in parasitology.
NATIONAL REFERENCE CENTRE(S) FOR PARASITOLOGY AT A MEDICAL FACULTY OR A PUBLIC HEALTH INSTITUTE	Surveillance at a national level. Transfer of modern diagnostic technologies and strategies to PHC level. Reference services and quality control of peripheral laboratories. Training in parasitology.



WORLD HEALTH ORGANIZATION

ORGANISATION MONDIALE DE LA SANTÉ

ORIGINAL: ENGLISH

CELLOPHANE FAECAL THICK SMEAR EXAMINATION TECHNIQUE (KATO) FOR DIAGNOSIS  
OF INTESTINAL SCHISTOSOMIASIS AND GASTROINTESTINAL HELMINTH INFECTIONS

Photograph 1 Different materials are available. This slide shows the plastic spatula, plastic template and nylon screen in a commercially available Kato-Katz kit. The first two items and the microscope slides may be re-used. The nylon screen is disposable. A few kits may be ordered to provide standard re-usable templates.

Photograph 2 The nylon screen and the cellophane required for the thick smear technique may be purchased in bulk. From the roll, cellophane is cut into 25-30 mm sections and placed in a wide-mouth, flat-bottom jar containing a 50% (or greater) glycerine solution with malachite-green or methylene blue stain (100 ml water, 100 ml glycerine, 1 ml 3% aqueous malachite green or methylene blue).

Photograph 3 The procedure for this technique is the same no matter which material is used. The faecal specimen is forced through the screen by a spatula to separate faecal material from the large debris.

Photograph 4 The screened faecal material is transferred to the template which is laid flat centrally on a microscopic slide. The template hole is completely filled with screened faecal material and levelled to the surface of the template. The Kato-Katz template shown delivers 41.7 mg of faeces. The number of eggs observed is multiplied by 24 to obtain the number of eggs per gram of faeces.

Photograph 5 The cellophane square soaked in glycerine for at least 24 hours is placed over the faecal specimen.

Photograph 6 The slide is inverted against a piece of glass or another glass slide and the faecal specimen spread evenly under the cellophane as shown here in the field. The properly prepared slide is shown. After the slide is prepared an additional drop of glycerine may be placed on the cellophane and the edges of the cellophane pressed smooth to ensure conservation of the slide. If air bubbles form under the cellophane during storage, a couple of drops of glycerine on the cellophane allowed to stand overnight will eliminate the bubbles. Cellophane thick smear slides can be prepared in the field, stored in microscopic slide boxes and shipped great distances, which permits examination at a central laboratory if required within days or weeks after preparation.

Photograph 7 Ascaris (left) and Trichuris eggs (right) are visible at any time. Hookworm eggs (not shown) are visible for up to 30 minutes after preparation.

Photograph 8 The ideal time for observing S. mansoni, S. intercalatum or S. japonicum eggs is 24 hours after preparation. In bright sunlight the slides clear rapidly and a 24-hour delay may not be necessary.

The major complaint about the thick smear technique from most microscopists has been that it is impossible to visualize the helminth ova in some hard (constipated) stool specimens. In such cases:

1. After preparation by the standard method, be sure to wait 24 or 48 hours before counting eggs on these slides. The slide may clear slowly.
2. Re-make another pair of samples on a large (2 x 3 inches) microscope slide and use a slightly larger piece of cellophane (35 x 35 mm) and press very hard to flatten the specimen as much as possible.
3. When the large slide is used, the stool may be softened with saline or glycerine to the stool specimen on the microscope slide before applying the cellophane and pressing will be easier.

Suppliers

1. Wettable cellophane

1.1 Description: No. 124PD, thickness 33 microns, weighs approximately 50 g/m<sup>2</sup>  
Bulk supplier: E. I. Dupont Nemours Plastic Products and Resins Department  
Wilmington, Delaware 19898, USA

1.2 Description: Rhone Poulenc 500 P 601  
Bulk supplier: Rhone Poulenc S.A., France

Product supplier (in rolls of 50 metres x 22 mm):

1.2.1 (for 1.2 above) Société Normande de Coupage (in lots of 1000 rolls only)  
72, rue des Chênaux  
Ymare, 76520 Boos, France

2. Screen

2.1 Stainless steel

Item: 105 mesh, stainless steel, bolting cloth  
Supplier: W. S. Tyler, Inc.  
8200 Tyler Boulevard  
Mentor, OH 44040, USA

2.2 Nylon screen

Item: T1250, HD 16243 A  
Supplier: L'Union Gazes à Bluter SA, Place de la Liberté, BP 2,  
42360 Panissières, France

3. Complete kit including all necessary material

3.1 Japanese Association of Parasite Control c/o Hokenkaikan  
1-1 Ichigaya-Sadohara  
Shinjuku-ku, Tokyo, Japan (contains cardboard templates)

3.2 OVO-FEC kits (Kato-Katz) for 100 or 500 examinations  
Boehringer Mannheim Bioquímica  
Rua Nair 170, Olaria,  
CEP 21021 Rio de Janeiro, RJ, Brazil (contains re-usable plastic templates)

(Note: The glycerine/malachite green solutions of the kits may be defective.  
Preparation of fresh glycerine/malachite green or methylene blue is recommended.)

REFERENCES

Komiya, Y. & Kobayashi, A. Evaluation of Kato's thick smear technique with a cellophane cover for helminth eggs in faeces. Japanese Journal of Medicine, Science and Biology, 19: 59-64 (1966)

Katz, N., Chaves, A. & Pellegrino J. A simple device for quantitative stool thick smear technique in schistosomiasis mansoni. Revista do Instituto de Medicina Tropical de Sao Paulo, 14: 397-400 (1972)

Peters, P. A. et al. Quick Kato smear for field quantification of Schistosoma mansoni eggs. American Journal of Tropical Medicine and Hygiene, 29: 217-219 (1980)

WHO Workshop. Quantitative aspects of the epidemiology of S. japonicum infection in a rural community of Luzon, Philippines. Bulletin of the World Health Organization, 58(4): 629-638 (1980)

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