E. Helminths

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Russell LJ. The pinworm, *Enterobius vermicularis*. Primary Care 1991;18(1):13-24

Sirisinha S, Chawengkirttikul R, Haswell-Elkins MR, Elkins DB, Kaewkes S, Sithithaworn P. Evaluation of a monoclonal antibody-based enzyme-linked immunosorbent assay for the diagnosis of *Opisthorchis viverrini* infection in an endemic area. American Journal of Tropical Medicine and Hygiene 1995;52(6):521-4

Stoltzfus R, Chwaya H, Tielsch J, Schulze K, Albonico M, Savioli L. Epidemiology of iron deficiency anemia in Zanzibari schoolchildren: the importance of hookworms. American Journal of Clinical Nutrition 1997;65:153-9

Tietze PE, Tietze PH. The roundworm, *Ascaris lumbricoides*. Primary Care 1991;18(1):25-41

Wahlquist SP, Eberhard M. Sodium azide: ineffective as a faecal preservative for parasitological diagnosis. Annals of Tropical Medicine and Parasitology 1991;85(3):365-8

Walden J. Other roundworms: trichuris, hookworm and strongyloides. Primary Care 1991;18(1):53-74

Ware B, Jones J. The office diagnosis of common intestinal parasitic diseases. Primary Care 1991;18(1):25-41

Author:	**Char S, Farthing MJG**
Title:	**DNA probes for diagnosis of intestinal infection.**
Source:	**Gut 1991;32(1):1-3**

In vitro amplification of DNA by polymerase chain reaction (PCR) and the identification of strain or species-specific oligonucleotides, is an attractive alternative to conventional culture techniques or microscopy for identifying bacteria, protozoa, viruses and helminths. AAfter a simple lytic processing of faecal suspension in buffer, pathogen DNA is drawn through manifold with shaped dots (dot blot) or slots (slot blot) on to nitrocellulose or nylon membranes, denatured in mild alkali-salt solution and fixed. . . Pathogens in paraffin wax embedded tissue sections (intestinal biopsy specimens) on microscopic slides can be detected by *in situ* hybridization with DNA probes.@

An alternative is to purify DNA from faeces and amplify target DNA by PCR before immobilization on a membrane. PCR includes: denaturation of target DNA, annealing (pairing complimentary strands of DNA, in this case it is two synthetic oligonucleotide primers) and extension of primers using denatured strands of DNA as templates. Success depends on correct selection of species-specific probes which are selected from muliticopied DNA. Recently, an rRNA-based diagnostic system has been used, which employs RNA rather than DNA.

As more cycles are performed, the copy number increases exponentially. Rapidity is increased, as Ait is possible to amplify specific DNA sequences more than a million-fold in a few hours. Using such amplified systems means signals are quantifiable, which may be useful clinically and epidemiologically.

Hybridization used to rely on radioactive labeling for detection whose cost, short half-life and safety requirements made it impractical for field application. Now there are a variety of non-radioactive labeling methods including biotin, alkaline phosphatase and dioxigenin.

The conventional steps of collection, transportation and growing organism cultures may compromise viability, while ADNA probes@ detect the pathogen directly and can differentiate between morphologically similar pathogens (i.e., DNA probes differentiate between two similar tapeworms, *Taenia solium* and *T. saginata*). DNA probes can also detect a small number of pathogens. Rapidity, sensitivity and specificity are increased, though specific increase is not quantified here. Costs are not given.

Author:	**Croese J, Loukas A, Opdebeeck J, Fairley S, Prociv P**
Title:	**Human enteric infection with canine hookworms**
Source:	**Annals of Internal Medicine 1994;120:369-74**

Study methods: An 8-year, retrospective case study was performed on nine patients with enteric hookworm infection from domestic pets who had been diagnosed by finding a single organism *in situ*. Demographic and clinical data were collected. At least one serum sample was collected within four weeks of diagnosis, and formalin- or alcohol-fixed worms were available for all but one patient. Sera were stored at -15^{o}C and tested among numerous control sera for IgG and IgE antibodies. ELISA was performed, which incorporated excretory-secretory antigens from adult *Ancylostoma caninum.* Cut-off absorbence levels for IgG and IgE ELISAs were 0.147 and 0.155 optical density units, respectively. In Western blots, these antigens were separated electrophoretically on polyacrylamide gels and transferred to nitrocellulose paper, which was then incubated with patient sera diluted. Both IgG and IgE Western blots were reported as positive

when a protein band with a molecular weight of 68 kd was recognized. Controls were from Tasmania, where *A. caninum* does not exist.

Results: Hookworm detection can be difficult, especially when infection is caused by a single organism. Classic helminthiases associated with eosinophilic infiltration of the gut, such as ancylostomiasis, schistosomiasis, trichinelliasis, etc. can be easily diagnosed by fecal microscopy, routine endoscopy or serological testing. *A. caninum* does not fall into this category; fecal micrsocopy is likely to be negative, the worm is usually beyond reach of the endoscopy, and serological tests are not fully developed.

The positive rate for the ELISA in this series was surprisingly low: seventy percent of patients with suspected infection had positive ELISA values compared with 8% of healthy controls. Falling values in the ELISA coincided with chronic symptoms during convalescence. The positivity rate of combined Western blots was 87.5% in diseased patients compared with 10% in patients who had nonparasitic gastrointestinal disease, higher than the ELISA. The ELISA did not consistently correlate with band intensities on the Western blots.

AIt is not clear how these detectable antibodies relate to gut inflammation and why a variation in the dominance of the different classes if immunoglobin should exist among patients. In its current form, serological testing has only limited diagnostic application.@

Author: **Genta R**
Title: **Predictive value of an enzyme-linked immunosorbent assay (ELISA) for the serodiagnosis of strongyloidiasis**
Source: **American Journal of Clinical Pathology 1988;89:391-4**

The predictive value of ELISA for the detection of IgG antibodies is evaluated on 268 patients, 571 non-infected controls, and 78 individuals with other parasitic infections. Conventional parasitological methods (direct fecal smear and formalin-ether concentration) have low sensitivity for the diagnosis of stronglyoidiasis, even when repeated several times. Baermanization and fecal culture are more sensitive, but they are time-consuming and have to be performed on fresh stools, a disadvantage when screening asymptomatic patients. Duodenal intubation or the Enterotest are reportedly more sensitive, but they are invasive and cumbersome, making them inappropriate for the initial evaluation of patients.

Antigen for the ELISA was prepared with larvae from dogs infected with *S. stercoralis* and the absorbency of each serum was obtained by an authorized ELISA reader who subtracted the absorbency of the well without antigen from that of the sensitized well. An absorbency index (AI) was created which is the ratio between the net absorbency of the test serum to the net absorbency of a pool of high positive control. The AI values of the 571 control sera varied between 0.001 and 0.313, with 95% of the values being less than 0.150, and were not normally distributed. The 78 parasitized controls represented a heterogeneous population in whom 83% of the AI values were less than 0.150. Using the AI of 0.300 or higher as a positive test, 236 of 268 patients infected with *S. stercoralis* (88%) were positive and 32 (12%) were negative. Of the 571 non-parasitized persons, 570 were negative and 1 had a slightly positive AI value (0.313). Seventy-two of the 78 patients with other parasites were negative, and six had positive values ranging from 0.398 to 0.854.

The sensitivity of the ELISA test was 0.88 and the specificity was 0.99. Because sensitivity and specificity are retrospective values, an index of positive accuracy was calculated to denote how

often the test was correct when its results were positive, and an index of negative accuracy was calculated to denote how often the test was correct when it was negative. The index of positive accuracy was 0.97, meaning only three of 100 individuals with a positive test do not have the parasites. The index of negative accuracy was 0.95, meaning a negative test result is associated with the absence of the parasite in 95.8% of the individuals tested.

Author:	**Goka AKJ, Rolston DDK, Mathan VI, Farthing MJG**
Title:	**Diagnosis of *Strongyloides* and hookworm infections: comparison of faecal and duodenal fluid microscopy**
Source:	**Transactions of the Royal Society of Tropical Medicine and Hygiene 1990;84:829-31**

Fecal microscopic diagnosis is insensitive, so the study compared duodenal fluid and fecal microscopy for detection of *Strongyloides* and hookworm infection in a group of 292 patients with GI symptoms. Fecal specimens were examined fresh, within 30 minutes of collection. Wet preparations in normal saline were examined at low and high magnification, and further examination was made following flotation on 50% zinc sulfate to concentrate ova and cysts. Aspiration of intestinal fluid was performed in the fasting state. Aspirates were examined immediately by low and high power microscopy, then centrifuged at 600 g for 10 minutes followed by further microscopical examination of the sediment.

Thirty-three (8%) had *Strongyloides* and 88 (30%) had hookworm infections. Microscopic examination for duodenal fluid was more sensitive for detection of *Strongyloides*, identifying 76%. The study indicates that microscopical examination of a single sample of duodenal fluid can identify patients with *Strongyloides* more effectively, as 67% of patients diagnosed with *Strongyloides* by duodenal fluid microscopy were not diagnosed with faecal microscopy. For hookworm, the diagnostic sensitivity was similar for both techniques, but duodenal fluid microscopy detected some patients (35%) who had not been identified by fecal microscopy.

Author:	**Jozefzoon LME, Oostburg BFJ**
Title:	**Detection of hookworm and hookworm-like larvae in human fecocultures in Suriname**
Source:	**American Journal of Tropical Medicine and Hygiene 1994;51(4):501-5**

The research project studied 804 Bushnegroes of Suriname, South America. These descendents of slaves fled to the interior of the country and remained isolated, maintaining a traditional way of life. Previous, unpublished studies by Josefzoon indicated a high prevalence of intestinal parasites. The collection of feces for examination occurred two to three months after the rainy season, when it was assumed hookworm is contracted. Examiners went to the villagers early in the morning, distributed plastic containers, and instructed villagers to collect stools, avoiding mixing the stools with urine. Filariform larvae were cultured using the Harada-Moori cultivation method and identification of the emerged larvae was based on morphologic features defined by Little. The cultures were examined between seven to ten days later, since the majority of rhabditoid larvae would have develop to filariform larvae. Test tubes were placed in hot water (50 degrees Celsius) to immobilize larvae and facilitate examination. The detection of different kinds of larvae in one culture indicates that diagnosis of hookworm infection should be made carefully and not rely solely on the morphology of worms. The Kato method did not detect the 60 persons with single infection with *S. stercoralis* but the Harada-Mori method did.

Author:	**Juckett G**
Title:	**Common intestinal helminths**
Source:	**American Family Physician 1995;52(7):2039-2048**

Typical helminths were described, with focus on Enterobiasis (pinworm), Ascariasis (roundworm), Trichuriasis (whip worm), hookworm, threadworm and tapeworm. Description includes physical characteristics, transmission routes, and diagnosis. Diagnosis involves collecting stool using plastic wrap under the toilet seat, selecting portions with a spatula, and taking these to the lab for preparation and staining.

When suspicion of helminths is quite high, fresh stool may be obtained by digital rectal examination and examined immediately.

More specific explanation is given for diagnosing *pinworm*, which is best diagnosed by examining perianal skin rather than the stool. A tongue blade covered with a segment of clear (not transparent) cellulose tape is placed sticky-side down over the unwashed perianal skin in the morning. Several specimens are collected on three separate mornings, taped to glass slides and examined in the lab. AThe glistening adult worms may also be evident if the anus is examined with a flashlight very late at night or early in the morning before the patient awakes.@

Author:	**Kato T, Kamoi R, Iida M, Kihara T**
Title:	**Endoscopic diagnosis of hookworm disease of the duodenum**
Source:	**Journal of Clinical Gastroenterology 1996;24(2):100-2**

Hookworm can be missed on stool examination, so it is important to check carefully in the distal duodenum at routine upper gastrointestinal endoscopy whenever parasitic disease is suspected but difficult to diagnose due to a limited number of eggs in feces.

Case report: A 31-year old man complained of epigastric discomfort and general fatigue for 3 months. Despite a low egg burden, laboratory data showed typical iron-deficiency anemia, eosinophilia, decreased cholesterol, and other conditions. Endoscopy was performed and two hookworms were found in the duodenum and removed with biopsy forceps.

Author:	**Krepel HP, Polderman AM**
Title:	**Egg production of *Oesophagostomum bifurcum*, a locally common parasite of humans in Togo**
Source:	**American Journal of Tropical Medicine and Hygiene 1992;46(4):469-72**

The purpose of the study was to examine egg production of *O. bifurcum* in humans by stool examination.
1. Stool samples were collected and cultured using the classic charcoal method. After seven days, the numbers of hookworm and *Oesophagostomum* larvae in each culture were identified by the Little method and counted.
2. Treatment and isolation of adult worms: Participants were treated with pyrantel pamoate. One day before treatment and two hours after treatment, participants were given a purgative. All stools produced during these two days were washed and sieved, and the adult worms were counted.

3. One week after treatment, two egg counts and three coprocultures were performed on another stool sample.
After treatment with pyrantel pamoate, few adult specimens of *Nector americanus* were recovered. The researchers found oesophagostomum-positive cultures in only four of 30 person but found hookworm larvae in 28 of 30 persons, so the treatment did no work effectively against hookworms.

Author: **Loukas A, Opdebeek J, Croese J, Prociv P**
Title: **Immunologic incrimination of *Ancylostoma Caninum* as a human enteric pathogen**
Source: **American Journal of Tropical Medicine and Hygiene 1994;50(1):69-77**

The incidence of human eosinophilic enteritis (EE) and abdominal pain with peripheral blood eosinophilia (PE) appear to be exceptionally high in northeastern Australia. Antibody responses were tested in three patients with confirmed dog hookworm infection (Positive control group), 25 patients with EE/PE (Group A), 42 patients with other diagnosed gastrointestinal disorders (Group B), eight with human hookworm infection (Group C), 27 with other diagnosed parasitic infections (Group D), and 100 blood donors from the State of Tasmania (Group E). They were analyzed by ELISA and Western blot for IgG and IgE antibodies to excretory-secretory (ES) antigens from adult *A. caninum*.

The study indicates that sera testing has a greater sensitivity and specificity than fecal testing. In the ELISA, sera from 88% of EE/EP patient were positive for IgG and IgE antibodies to ES antigen. In the Western blots, sera from 92% of EE/EP patients demonstrated IgG and IgE antibodies to a component of ES antigen with a molecular weight of 68kD (Ac68). ASera from most patients with EE/PE reacted positively in the ELISA, and the Western blots demonstrated IgG and IgE antibodies to a putative allergen with a molecular weight of 68 kD (that of the secreted protease from *A. caninum*). Very few control sera . . . recognize this protein. Much larger numbers of sera will be needed to be examined to quantify the specificity and sensitivity, and positive and negative predictive values of our serology . . .@ (74).

The investigators also indicate that, ABecause of the diversity of immune responses to such antigenically complex organisms as parasitic nematodes, a diagnostic assay that is both 100% specific and sensitive may be an unattainable ideal; false-positive results may be inevitable among patients with complicated and unreliable histories of exposure to a wide variety of infections and environmental agents.@

The Western blot proved more sensitive than the ELISA, identifying IgG and IgE antibodies to Ac68 which were not identified by ELISA in a positive control and in one of the three who were confirmed to be infected with dog hookworm.

The Western blot was also more specific than ELISA, because fewer sera in groups B, C, and E had antibodies to Ac68 than were falsely positive by ELISA. While 11 in group C were positive in the IgG-ELISA, in the Western blots, only three sera from two patients with strongyloides and one with schistosomiasis demonstrated IgG antibodies against Ac68. This was explained by previous undiagnosed concurrent hookworm infection than by cross-reactivity. Most Group C did not recognize Ac68 in Western blot.

The ELISA and the Western blot did not distinguish between dog and human hookworm infection, as all eight in group D sera (human ancylostomiasis) reacted with Ac68 in Western blots. Three of the patients were infected with *A. duodenale* (based on fecal examination), whose metalloprotease is the same molecular weight as that from *A. caninum*. It is predictable that two similar species should produce similar antigens. The other five with the human hookworm infection may have also been exposed to the dog hookworm infection, though investigators attribute Aantigenic similarity as the more likely combination.@

Author:	**Marti H, Echer E**
Title:	**Schweizerische Medizinische Wochenschrift**
Source:	**Journal Suisse de Médecine 1990;120(40):1473-6**

SAF is an alternative fixation solution to MAF for parasitological stool specimen with increased sensitivity for parasitic protozoa (*Entamoeba histolytica* 90.0% vs. 64.4%, *Giardia lamblia* 97.7% vs. 64.4%), though no increased sensitivity is detected for helminths. However, advantages include the cheap, simple formula, absence of mercury, and low toxicity. Also, contrary to the MIF fixative, the SAF fixative permits the use of a wide range of staining techniques starting from fixed specimens. The Center of the Swiss Tropical Institute have replaced the MIF fixative for the SAF due to these results.

Author:	**Parija SC**
Title:	**A review of some simple immunoassays in the serodiagnosis of cystic hydatid disease**
Source:	**Acta Tropica 1998;70:17B24**

Human cystic hydatid disease, caused by the larva (hydatid cyst), *Echinococcus granulosus*, is common in countries where sheep and cattle rearing constitute an important industry. Diagnosis is based on immunodiagnostic methods supplemented with radiological and ultrasound examination. One major exam is the ELISA, a sensitive and specific hydatid antigen-based serological exam. However, major drawbacks for testing in rural areas of developing countries include high cost, need for sophisticated equipment and trained technicians, and the use of enzyme reagents that perish in high temperatures. The article gives a review of the major diagnostic techniques, with more detailed information on the dot-ELISA, HA-DIA, and Co-A.

The Adot-ELISA,@ developed as a modification of the ELISA, tests for the demonstration of hydatid antibodies. When the test serum is layered on nitrocellulose membrane that is bound with hydatid antigen, the antibodies in infected serum will bind to the antigen dot. When an enzyme-labeled second antibody is added, the binding reaction and subsequent development of the label antibody is detected visually. The dot-ELISA has shown sensitivity and specificity by Rogan *et al.* (1991) as 94% and 90.5%, respectively and by Romia *et al.* (1992) as 88% and 96.9%, respectively. The test is a simple, rapid test that does not require any equipment and can be carried out with minimum training to technical staff. However, disadvantages include high cost and short shelf-life of heat-labile enzyme-based reagents.

Alternatives to the dot-ELISA for developing countries, where disadvantages of cost and heat are prohibitive, are the HA-DIA and the Co-A. The HA-DIA is a non-enzymatic dye-based assay in which antigen attained from bovine hydatid cysts is bound onto a nitrocellulose reactive area stuck on a plastic stick. The same antigen is adsorbed on a textile colloidal dye suspension. The

serum sample is incubated simultaneously with the stick and colloidal dye in a tube, and in the presence of hydatid antibodies, colloidal antibodies react with the nitrocellulose reactive area and a pink spot appears. The HA-DIA is highly sensitive (100%) and specific (100%), showing no false-positive reaction with cases of toxocariasis, allergic respiratory disease, high total IgE levels by no evidence of parasitic infection and healthy controls. The test is simple, with no need for heat-labile enzymes and/or instrumentation. Rapidity and cost were not mentioned.

The Co-A test is a simple slide agglutination test that is being developed in the lab of Parija and Sharriff. The test demonstrates the presence of Cag in the serum, which indicates the presence of an active or recent infection. *Staphylococcus aureus* bearing protein A (SAPA) is used as a carrier particle to bind the hydatid antibodies. The test is carried out on the slide by adding a drop of test serum and an equal volume of 2% suspension of hydatid antibody-sensitized SAPA cells, rotating manually for two minutes and noting the reading. A positive reaction is indicated by the formation of large visible clumps of bacterial cells within two minutes. The Co-A was shown to be sensitive (95%) and specific (95%). Five undiluted sera from control patients with other parasitic diseases showed false-positive reactions but all gave negative results when tested after further dilution. The test is simple, rapid (30 **B** 45 minutes), and economical. It requires no special technical expertise and/or lab equipment and the reagents are thermostable, making it a test well-suited for the hot environment of tropical countries.

Author:	**Parija SC, H Srinvasa**
Title:	**Viewpoint: The neglect of stool microscopy for intestinal parasites and possible solutions**
Source:	**Tropical Medicine and International Health 1999;4(7):522-4**

Stool microscopy offers many advantages over other diagnostic methods for detecting helminths. If performed correctly, it is sensitive, simple, and economical. However, lack of motivation on the part of lab technicians, non-recognition of their work by others, lack of skills, and other problems hamper its use. There are increasing availabilities of non-microscopic, hi-tech methods, such as DNA probes, PCR and direct fluorescent antibody methods. These offer high sensitivity and specificity, but they are too expensive for use in the developing world. Increased training, increased sense of importance in lab technicians for carrying out procedures, resources for proper collection, and transport and use of concentration techniques, such as formalin-ether or salt floatation, will increase sensitivity and specificity of stool microscopy.

Author:	**Russell LJ**
Title:	**The pinworm, *Enterobius vermicularis***
Source:	**Primary Care 1991;18(1):13-24**

The pinworm is the most common intestinal parasite seen in the primary care setting. Though highly prevalent, it=s an innocuous parasite which requires little attention from a therapeutic standpoint. Pinworms can affect any system of the body.

Diagnosis requires understanding of the worm's life cycle. The most effective means of diagnosing infection is sampling from the anal verge in the morning, before stool is passed and before a bath. Parents can also observe worms in children by putting them to bed without underpants and examining the anus with a flashlight at 12am or 6am, depending on the child=s

sleep pattern. Worms obtained by parents should be placed in alcohol or vinegar and brought to the clinic for confirmation.

The current method for pinworm diagnosis is cellulose tape test, invented in 1941 by CF Graham. A clear adhesive tape is placed firmly on the perianal surface and then mounted, adhesive side down, on a glass slide and examined under a microscope using a 10x to 45x objective. Toluene solution is used to clean the preparation after it was placed on the slide.

The digital rectal exam has also been suggested as an alternative diagnostic tool, in which a gloved finger is inserted in the anus and fecal material is removed and placed on a slide using a wooden applicator stick. A microscopic slide is prepared using normal saline as in a Awet preparation@ as in a vaginal smear. This often demonstrates the parasite when the cellulose tape test is negative because mothers frequently bathe their children before doctors appointments.

Author: **Sirisinha S, Chawengkirttikul R, Haswell-Elkins MR, Elkins DB, Kaewkes S, Sithithaworn P**

Title: **Evaluation of a monoclonal antibody-based enzyme-linked immunosorbent assay for the diagnosis of *Opisthorchis viverrini* infection in an endemic area**

Source: **American Journal of Tropical Medicine and Hygiene 1995;52(6):521-4**

Current diagnosis of opisthorchiasis is done by examining the feces for the presence of eggs, a time-consuming process which is only reliable in the hands of experienced personnel. Even under optimal conditions, false-positive readings can be expected in those with light infection or with infection by a different species whose eggs are morphologically similar to those of *Opisthorchis viverrini*.

The same investigators previously reported on the use of an indirect ELISA for the detection of parasite-specific serum antibody in infected individual. Though an association between antibody levels and worm burden was observed, increased antibody levels were also found in uninfected individuals, which probably occurred because the crude *O. viverrini* antigen was used for diagnosis. A subsequent study which used a more refined antigen from culture fluid from an *in vitro* maintenance of adult worms produced more encouraging results, though the method used could not produce a mass quantity of antigen. The current study compared microscopic examination with a monoclonal antibody-based ELISA (Mmb-ELISA). Results showed that the ELISA is sufficiently sensitive and specific for diagnosis of *O. viverrini*.

Fecal specimen from 207 apparently healthy villagers in northeastern Thailand were analysed in a double-blind test for the presence of *O. viverrini* eggs by microscopic examination and for antigen by Mab-ELISA. The procedure for the ELISA was essentially the same used previously by the team, however the microtiter plate were coated with MAb from a single clone (6B1, Aspecific for the carbohydrate moiety of the 89-kD glycoprotein@) instead of the mixture of MAbs used previously.

Of the 207 individuals who participated in the study, 66 (32%) were found to be *O. viverrini* egg-negative in two microscopic examinations. The MAb assay detected 88.3% of egg-positive stools from 141 individuals. ABased on the in vitro production of the 89-kD antigen, it was estimated that the method was theoretically sensitive enough to detect the infection in humans harbouring only a single mature parasite. This level of infection may be below detection limits of any microscopic egg count technique . . .It was previously determined that up to 52.6% of the

infections with less than 20 *O. viverrini* worm were not detected by Stoll-dilution technique@(523).

There are additional advantages to this monoclonal assay. The clone 6B1 did not cross-react with other human liver flukes, a very important consideration, as the eggs of these human liver flukes and other commonly encountered minute intestinal fluke are virtually indistinguishable from those of *O. viverrini*. Also, unlike earlier antibody detection methods, this assay only detect current infections.

In general, lower cost, increased speed and probably higher sensitivity of the ELISA compared with microscopy make it the preferred method.

Author: **Stoltzfus R, Chwaya H, Tielsch J, Schulze K, Albonico M, Savioli L**
Title: **Epidemiology of iron deficiency anemia in Zanzibari schoolchildren: the importance of hookworms**
Source: **American Journal of Clinical Nutrition 1997;65:153-9**

In children were tested for anemia in Pemba Island, Zanzibar, a strong association was found between low iron status and prevalence of hookworms. For helminth assessment, children were asked to bring a fecal sample to class. These were stained the same day and examined within one hour of staining by the AKato-Katz@ (the cellophane fecal thick smear) method. Hookworm, *Trichuris trichiura* (whipworm), and *Ascaris lumbricoides* (round worm) were counted. If more than 10,000 eggs per gram feces were found, the sample was diluted using a modified AStoll@ technique and reexamined. Specificity and sensitivity were not given.

Schistosomiasis was screened by collecting urine samples and testing with Hemastix test strips. This techniques gave a sensitivity of 69% and specificity of 89%.

Author: **Tietze PE, Tietze PH**
Title: **The roundworm, *Ascaris lumbricoides***
Source: **Primary Care 1991;18(1):25-41**

The roundworm is the most prevalent parasite infecting humans. Disease results from larval migration and intestinal obstruction by adult worms.

Checking a stool sample for eggs can be done in a physician's office. Either fresh stool sample or stool from digital rectal exam can be prepared for microscopic study by a method described by JE Jones. A small sample from digital rectal exam is mixed with water and viewed under a low power microscope.

More involved procedures to identify ova are used by reference laboratories, including centrifuge and sedimentation techniques, formalin-ether sedimentation, formalin-ether with preserved specimens and others. With intestinal obstructive symptoms, the cause of obstruction may be obscure. Plain radiographs may reveal obstruction in the bowel. Ultrasonography and esophogogastroduodenoscopy reveal obstruction in the pancreas, bile duct or appendix.

Author:	**Wahlquist SP, Eberhard M**
Title:	**Sodium azide: ineffective as a faecal preservative for parasitological diagnosis**
Source:	**Annals of Tropical Medicine and Parasitology 1991;85(3):365-8**

Use of formalin is a significantly better choice than sodium azide for preserving parasites. Sodium azide was compared with 10% formalin as a faecal preservative on direct wet-mount preparations after 1.5, 6.5, and 11.5 weeks. Sodium azide did not preserve the morphology of either helminths or protozoa as well as formalin, though sodium azide prevented embryogenesis of helminth eggs while helminth eggs in 10% of formalin contained larvae. Use of 10% formalin is a significantly better choice than sodium azide for preserving parasites when accurate identification of parasites and biosafety are the main concerns.

Author:	**Walden J**
Title:	**Other roundworms: trichuris, hookworm and strongyloides**
Source:	**Primary Care 1991;18(1):53-74**

A. Whipworm diagnosis (trichuriasis): Find eggs in the stool by means of direct stool examination or stool preserved in formalin. AThe presence of worms in the rectum, as detected by anoscopy, provides a quick, safe, simple, and effective method for detecting heavy trichuris infection.@

B. Hookworm diagnosis (ancylostomiasis, unicinariasis, necatoriasis) : Direct fecal film on microscope slide mounted in saline or iodine solution or in stained smears of PVA-preserved stool specimen. Concentration methods such as zinc sulfate flotation or the formalin ether technique are useful, especially in light infections.

For Acutaneous larva migrans . . ., sputum examination for the presence of larvae should be undertaken in the patient with rare pulmonary involvement.@

C. Strongyloidiasis (threadworm): Any immunocompromised host who has a relevant history of gastrointestinal symptoms, pneumonia, bacteremia or meningitis should be considered for strongyloides.
1. Stool sample: As with other helminths, eggs provide the essential diagnostic clue. The actively motile rhabditoid larvae are found in stool by direct smear. Direct stool is optimal, as the larvae disappear rapidly from stool kept under refrigeration. Formalin-ether concentration techniques can be used, or parasites can be cultured in charcoal. Some think the Baermann larval extraction techniques is the most sensitive stool test.
2. Duodenal aspiration: Larvae may be recovered by duodenal aspiration. Enterotest, otherwise known as the string test, Asignificantly increases the yield in diagnosing patients with strongyloides.@ An Aencapsulated string@ is swallowed by the patient and withdrawn four hours later for examination for larvae on the terminal portion.
3. Serological (ELISA) analysis: Not widely available, but it is shown to be reliable and inexpensive in diagnosis. (No reference given).
4. Sputum examination by concentration method is recommended if pneumonia is present in the immunocompromised patient.

Author:	**Ware B, Jones J**
Title:	**The office diagnosis of common intestinal parasitic diseases**
Source:	**Primary Care 1991;18(1):25-41**

Faecal specimen are collected, applied to a slide, and studied within a few hours in a laboratory or office (confirmation of the diagnosis by laboratory results is mandatory in most cases).

A. Diagnostic methods

1. Faecal collection for slide preparation
    a. Digital Rectal Exam: a gloved finger lubricated with K-Y jelly is placed at the anal verge, inserted 1 to 2 cm into the anus, and rotated. A wooden applicator stick is used to remove faecal debris from the gloved finger. If faecal smears are negative, the procedure should be repeated.
    b. Patient can also collect sample in saran wrap and then use a spatula to pick up a sample of faeces and put it in a collection cup. Patient must follow instructions and must understand that no water or urine can touch the sample.
2. Proctoscopic examination (visual exam of anus): useful in identifying adult nematodes and particularly useful for detecting pinworms.
3. Sigmoidoscopic exam (inspection through an endoscope of the interior of a sigmoid colon): useful as an indicator of mucosal invasion by such protozoa as amoebiasis.
4. Collection of perianal specimen using adhesive tape test.
5. Serological testing, including ELISA: limited due to lack of balance in sensitivity and specificity, difficulty in Aobtaining the required antigens.@ Useful for trichinosis.

B. Reasons why sample is unacceptable: These include use of antibiotics, antiparasitic drugs, laxatives, antidiarrheal agents, enemas, frozen stool, samples contaminated with water and urine, and those collected after certain procedures, such as gastrointestinal radiographs. Specimen collected with cotton swabs are unacceptable because the parasites are likely to be absorbed in the cotton.

C. Preserving Sample

If a sample cannot be examined within a few hours, it should be preserved, preferably with a Atwo vial method@ (one of formalin and another of polyvinyl alcohol [PVA]). Formalin is found to be effective in preserving helminth eggs, larvae, and protozoan cysts. PVA is effective in preserving protozoan cysts and trophozoites.

D. Preparing the slide (great detail is provided in the article)

1. Direct wet mount (stained and unstained)
2. Concentration methods (flotation and sedimentation)
3. Permanent stained smear