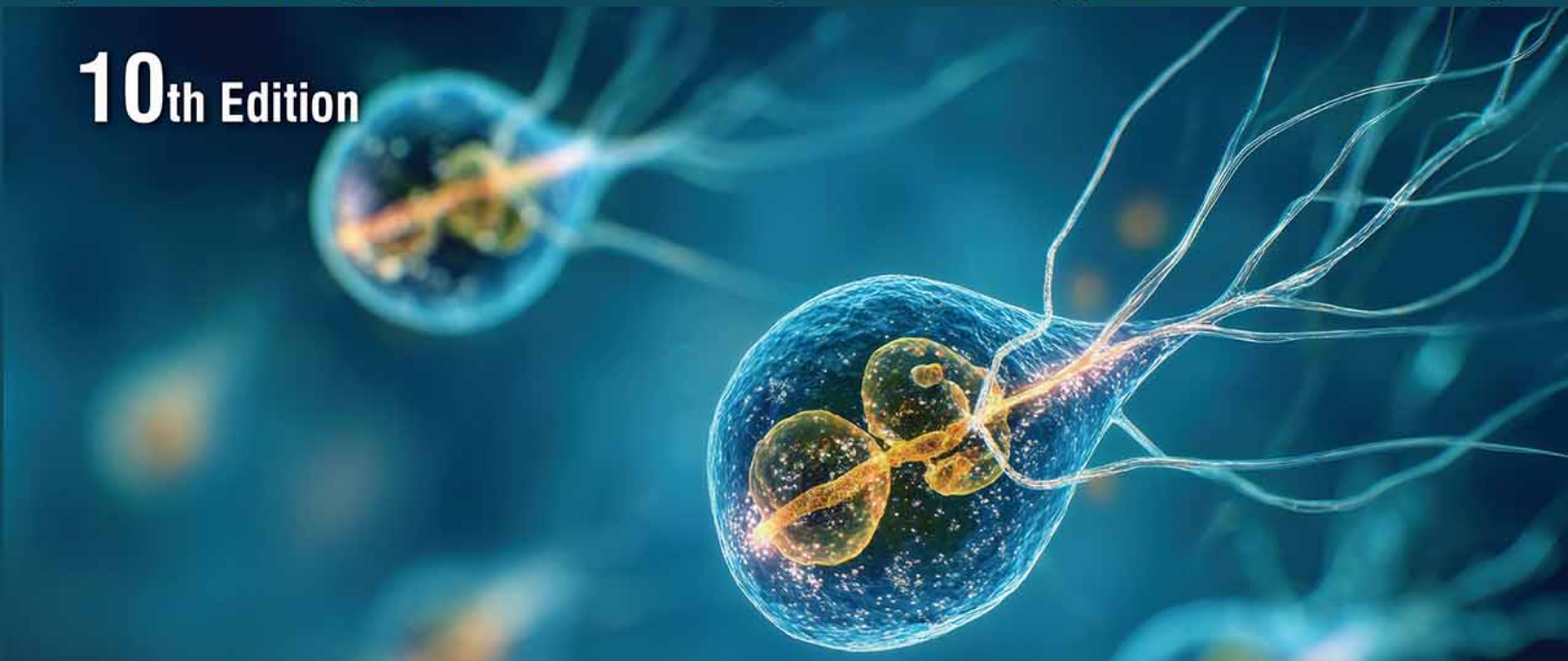
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10th Edition



Paniker's Textbook of Medical Parasitology

As per the Revised Competency-based Medical Education Curriculum (NMC)

Revised & Edited by **Sougata Ghosh**

Forewords **Pallab Ray • Jagdish Chander**

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SECTION IV

Diagnostic Methods

Chapter

29. Diagnostic Methods in Parasitology

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CHAPTER 29

Diagnostic Methods in Parasitology

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INTRODUCTION

Laboratory procedures play an important role in the diagnosis of parasitic infections, both for confirmation of clinical suspicion and for identifying unsuspected infections. The principles of laboratory diagnosis are the same as in bacterial and viral infections, but the relative importance of the different methods varies greatly.

- While isolation of the infecting agent and detection of specific antibodies are the major methods in bacteriology and virology, they are of much less importance in parasitology than **morphological identification** of the parasite by microscopy.
- Compared to bacteria and viruses, parasites are very large and possess distinctive shape and structure, which enables their specific diagnosis on morphological grounds.
- Due to their complex antigenic structure and extensive cross-reactions, serological diagnosis is of limited value in parasitic infections.
- Although many pathogenic parasites can be grown in laboratory cultures, this method is not suitable for routine diagnosis because of its relative insensitivity and the delay involved.
- Morphological diagnosis of parasites consists of *two steps:*
  (1) detection of the parasite or its parts in clinical samples and (2) its identification.
  1. ***Detection*** depends on collection of the appropriate samples and their examination by suitable techniques.
  2. ***Identification*** requires adequate skill and expertise in recognizing the parasite in its various stages and its differentiation from morphologically similar artifacts.

A description of the common diagnostic techniques in parasitology is given here.

EXAMINATION OF STOOL

Collection of Fresh Stool Specimen

- All stool specimens should be collected in a suitable, clean, wide mouthed container like a plastic container with a light-fitting lid, waxed cardboard box, or match box.
- All fresh specimens should be handled carefully because each specimen represents a potential source of infectious material.
- The specimen should not be contaminated with water, urine, or disinfectants.

Liquid stools should be examined or preserved within **30 minutes** of passage. Soft stools should be examined or preserved within **1 hour of passage** and formed stool should be examined or preserved within **24 hours** of passage.

- Normally passed stools are preferable, although samples obtained after purgative (sodium sulfate) or high saline enema may also be used.
- Examination of fresh specimens is necessary for observing motility of protozoan parasites.
- Stool should be examined for its consistency, color, odor and presence of blood or mucus.
- In some instances, parasites may be seen on gross inspection, as in the case of roundworm, pinworm, or tapeworm proglottids.

Microscopic Examination

- The microscope should be equipped with a *micrometer* eyepiece, as it is often essential to measure the size of parasites. For example, the differentiation between cysts of the pathogenic *Entamoeba histolytica* and the

nonpathogenic *E. hartmanni* is based entirely on their sizes.

- Microscopy should also include contributory findings such as the presence of *Charcot-Leyden crystals* and *cellular exudates* such as pus cells, red blood cells (RBCs) and macrophages.
- For detection of parasites, it is best to employ a combination of methods, as different methods serve different purposes.
- The methods include examination of: (i) **wet mounts**, (ii) **thick smears**, and (iii) **permanent-stained preparations**.
- Various concentration methods can be used to increase the sensitivity of microscopic examination.
- If there is a delay in examination, use of preservatives such as formalin, sodium acetate and polyvinyl alcohol is recommended.

Wet Mounts

- ***Unstained wet film:*** The unstained wet film is the standard preparation and is made by emulsifying a small quantity of stool in a drop of (0.85%) saline placed on a slide and applying a coverslip (22 mm × 22 mm) on top, avoiding air bubbles. A proper preparation should be just dense enough for newspaper print to be read through it. If the feces contains mucus, it is advisable to prepare films using the mucus part. The entire field under coverslip should be systematically examined with low-power objective (10X) under lowlight intensity. Any suspicious object may then be examined with the high-power objective.
- ***Wet saline mounts:*** Wet saline mounts are particularly useful for detecting live motile trophozoites of *E. histolytica, Balantidium coli* and *Giardia lamblia*. Eggs of helminths are also readily seen. Rhabditiform larvae of *Strongyloides stercoralis* are detected in freshly passed stool.
- ***Eosin staining:*** Eosin 1% aqueous solution, can be used for staining wet films. Eosin stains everything except living protoplasm. Trophozoites and cysts of protozoa, as well as helminth larvae and thin-walled eggs stand out as pearly-white objects against a pink background and can be easily detected. Chromatoid bodies and nuclei of amebic cysts can be seen prominently. Eosin also indicates the viability of cysts; live cysts are unstained and dead ones are stained pink.
- ***Iodine staining:*** Iodine staining of wet mounts is another standard method of examination. Either **Lugol's iodine** diluted (5 g iodine, 10 g potassium iodide and 100 mL of distilled water) or Dobell and O'Connor iodine solution (1 g iodine, 2 g potassium iodide and 50 mL of distilled water) are used. Iodine helps to confirm the identity of cysts, as it prominently stains the glycogen vacuoles and nuclei. Protozoan cyst stained with iodine show yellow-gold cytoplasm, brown glycogen material and pale refractile nuclei.

Thick Smears

These are not useful for routine examination, but are valuable in surveys for intestinal helminth eggs.

The method described by Kato and Miura in 1954 is known as the ***Kato thick smear technique***.

- About 50 mg stool is taken on a slide and covered with a special wettable cellophane coverslip soaked in glycerin containing aqueous malachite green.
- The preparation is left for about an hour at room temperature, during which the glycerin clears the stool, enabling the helminth eggs to be seen distinctly under low-power magnification.
- This method is, however not useful for diagnosis of protozoa or helminth larvae.

Permanent Stained Smears

Permanent stained smears are examined normally under oil immersion (100X) objective.

- Confirmation of the intestinal protozoan, both trophozoites and cysts, is the primary purpose of this technique.
- Helminthic eggs and larvae take up too much stain and usually cannot be identified.
- Permanent smear can be prepared with both fresh and polyvinyl alcohol preserved stool specimen.
- The two methods commonly used are: (1) the *iron-hematoxylin stain* and (2) *Wheatley's trichrome stain*. The iron-hematoxylin is the older method, but is more difficult.

1. ***Iron-hematoxylin stain***

   Procedure:

   » Fecal smear on a slide is fixed in *Schaudinn's solution* for 15 minutes and is immersed successively for 2–5 minutes in 70% alcohol, 70% alcohol containing a trace of iodine, and then 50% alcohol for 2–5 minutes.

   » It is washed in water for 5–10 minutes and immersed in 2% aqueous ferric ammonium sulfate solution for 5–15 minutes.

   » It is again washed in water for 3–5 minutes and stained with 0.5% aqueous hematoxylin for 5–15 minutes.

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» It is washed for 2–5 minutes and differentiated in saturated aqueous solution of picric acid for 10–15 minutes.
» It is then washed for 10–15 minutes and dehydrated by passing through increasing strengths of alcohol, cleared in toluene or xylol and mounted.

2. ***Trichrome stain*** *(Wheatley's method)*
   » The trichrome technique of Wheatley for stool specimens is a modification of Gomori's original staining procedure for tissue.
   » It is a quicker and simpler method, which produces uniformly well-stained smears of the intestinal protozoa, human cells, yeast cells and artifact material in about 45 minutes or less.

   Procedure:
   » The smear is fixed in ***Schaudinn's solution*** and taken successively through alcohol, as earlier.
   » Trichrome stain (chromotrope 2R, light green SF, phosphotungstic acid in glacial acetic acid and distilled water) is then applied for 5–10 minutes, differentiated in acid-alcohol dehydrated, cleared and mounted **(Box 29.1)**.

- ***Modified trichrome stain for microsporidia:***
  – This staining method is based on the fact that stain penetration of the microsporidial spore is very difficult, thus more dye is used in the chromotrope 2R than that routinely used to prepare Wheatley's modification of trichrome method and the staining time is much longer (90 minutes).
  – Other staining techniques are used for special purpose. For example, modified acid-fast or Giemsa stain is employed for detection of oocysts of *Cryptosporidium* and *Isospora*.
- ***Modified Ziehl-Neelsen (acid-fast) stain (hot method):***
  – Oocysts of *Cryptosporidium* and *Isospora* in fecal specimens may be difficult to detect, without special staining. Modified acid-fast stains are recommended to demonstrate these organisms.
  – Application of heat to the carbolfuchsin assists in the staining and the use of a milder decolorizer (5% sulfuric acid) allows the organisms to retain more of their pink-red color.

Box 29.1: Reagents of trichrome stain.

- Chromotrope 2R: 0.6 g
- Light green SF: 9.3 g
- Phosphotungstic acid: 0.7 g
- Acetic acid (glacial): 1.0 mL
- Distilled water: 100 mL

- ***Kinyoun's acid-fast stain (cold method):***
  – *Cryptosporidium* and *Isospora* have been recognized as causes of severe diarrhea in immunocompromised hosts but can also cause diarrhea in immunocompetent hosts.
  – Kinyoun's acid-fast stains are recommended to demonstrate these organisms.
  – Unlike the Ziehl-Neelsen modified acid-fast stain, Kinyoun's stain does not require the heating of reagents for staining **(Box 29.2)**.

  Procedure:
  – Smear 1–2 drops of specimen on the slide and allow it to air dry.
  – Fix with absolute methanol for 1 minute.
  – Flood the slide with Kinyoun's carbolfuchsin and stain it for 5 minutes.
  – Rinse the slide briefly (3–5 seconds) with 50% ethanol.
  – Rinse the slide thoroughly with water.
  – Decolorize by using 1% sulfuric acid for 2 minutes or until no more color runs from the slide.
  – Rinse the slide with water (it may take less than 2 minutes; do not destain too much) and drain.
  – Counterstain with methylene blue for 1 minute.
  – Rinse the slide with water and air dry.
  – Examine with the low or high dry objective. To see internal morphology, use the oil objective (100X).
- ***Auramine O stain for coccidia:***
  – *Coccidia* are acid-fast organisms and also stain well with phenolized auramine O.
  – The size and typical appearance of *Cryptosporidium, Cyclospora* and *Isospora* oocysts enable auramine O-stained slides to be examined at low-power under the 10X objective.
  – The entire sample area can usually be examined in less than 30 seconds.

Box 29.2: Reagents of Kinyoun's acid-fast stain.

- 50% ethanol (add 50 mL of absolute ethanol and 50 mL of distilled water)
- Kinyoun's carbolfuchsin:
  – *Solution A:* Dissolve 4 g of basic fuchsin in 20 mL of 95% ethanol
  – *Solution B:* Dissolve 8 g of phenol crystals in 100 mL of distilled water
  – Mix solution A and B, and store at room temperature
- 1% sulfuric acid
- Alkaline methylene blue
- Dissolve 0.3 g of methylene blue in 30 mL of 95% ethanol, and add 100 mL of dilute (0.01%) potassium hydroxide

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- The low cost of the reagents, the simple staining protocol and the rapid microscopic examination also make this staining method suitable for screening unconcentrated stool specimens. Concentrated sediment from fresh or nonpolyvinyl alcohol-preserved stool may also be used.

Concentration Methods

When the parasites are scanty in stools, routine microscopic examination may fail to detect them. It is then necessary to selectively concentrate the protozoan cysts and helminth eggs and larvae. Concentration may be done using fresh or preserved feces. Several concentration techniques have been described.

They can be classified as the floatation or sedimentation methods.

- In *floatation method*, the feces are suspended in a solution of high specific gravity, so that parasitic eggs and cysts float up and get concentrated at the surface.
- In *sedimentation method*, the feces are suspended in a solution with low specific gravity, so that the eggs and cysts get sedimented at the bottom, either spontaneously or by centrifugation.

Floatation Methods

- ***Saturated salt solution technique***
  Procedure:
  - A simple and popular method is salt floatation using a saturated solution of sodium chloride, having a specific gravity of 1.2. About 2 mL of the salt solution is taken in a flat bottomed tube (or *penicillin bottle*) and 1 g of feces is emulsified in it.
  - The container is then filled completely to the brim with the salt solution.
  - A slide is placed on the container, so that it is in contact with the surface of the solution without any intervening air bubbles. After standing for 20–30 minutes, the slide is removed, without jerking, reversed to bring the wet surface on top, and examined under the microscope.
  - A coverslip need not to be applied, if examination is done immediately. Any delay in examination may cause salt crystals to develop, interfering with clarity of vision.

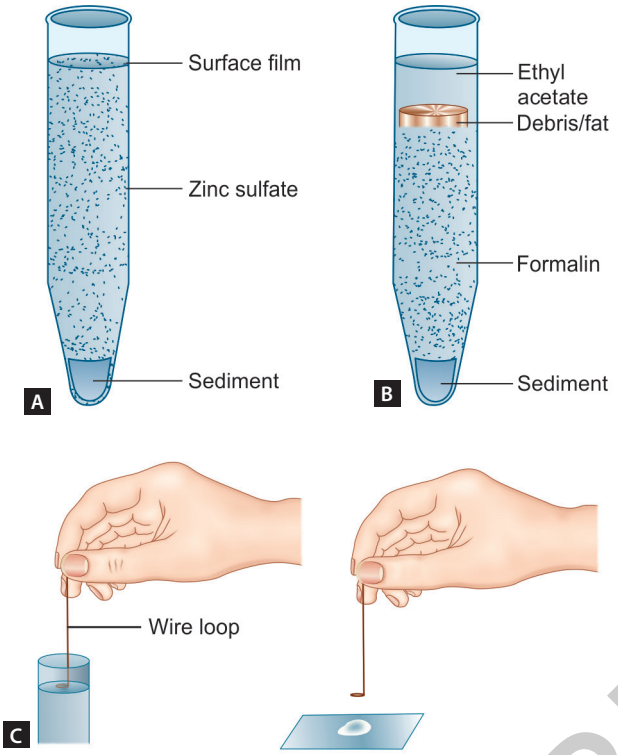
  This simple method is quite useful for detecting the eggs of the common nematodes such as roundworm, hookworms and whipworm, but is not applicable for eggs of tapeworms, unfertilized egg of *Ascaris lumbricoides,* eggs of trematodes and protozoan cysts.
- ***Zinc sulfate centrifugal floatation***
  Procedure:
  - Make a fine suspension of about 1 g of feces in 10 mL of water and strain through gauze to remove coarse particles.
  - Collect the liquid in a small test tube and centrifuge for 1 minute at 2,500 revolutions per minute. Pour off the supernatant, add water, resuspend, and centrifuge in the same manner, repeating the process, till the supernatant is clear.
  - Pour off the clear supernatant, add a small quantity of zinc sulfate solution (specific gravity 1.18–1.2) and resuspend the sediment well.
  - Add zinc sulfate solution to a little below the brim and centrifuge at 2,500 revolution per minute for 1 minute **(Fig. 29.1A)**.
  - Take samples carefully from the surface, using a wire loop, transfer to slide and examine under the microscope **(Fig. 29.1B)**. A drop of dilute iodine helps to bring out the protozoan cysts in a better way.

  This technique is useful for protozoan cysts and eggs of nematodes and small tapeworms, but it does not detect unfertilized roundworm eggs, nematode larvae, and eggs of most trematodes and large tapeworms.
- ***Sugar floatation technique:*** Sheather's sugar floatation technique is recommended for the detection of cryptosporidia infection.

Sedimentation Methods

- ***Formol-ether sedimentation technique:*** Formol-ether concentration method has been the most widely used sedimentation method **(Fig. 29.1C)**.
  Procedure:
  - Emulsify 1–2 g feces in 10 mL of water and let large particles sediment. Take the supernatant and spin at 2,500 revolutions per minute for 2–3 minutes.
  - Discard the supernatant. Add 10% formol-saline, mix well and let it stand for 10 minutes.
  - Add 3 mL ether and shake well. Spin at 2,500 revolutions per minute for 2–3 minutes. Four layers will form—(1) a top layer of ether, (2) a plug of debris at the interface, (3) the formalin-saline layer and (4) the sediment at the bottom **(Fig. 29.1C)**.
  - Carefully detach the debris from the sides of the tube and discard the top three layers.
  - Suspend the sediment in a few drops of fluid and examine wet mount and iodine preparation.
  - As ether is inflammable and explosive, its use can be hazardous. Ethyl acetate can be conveniently used in its place, with equally good results.

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Figs. 29.1A to C: (A) Zinc sulfate floatation concentration technique; (B) Method used to remove surface film in the zinc sulfate floatation concentration procedure; (C) Formol-ether sedimentation technique.

The method is useful for all helminth eggs and protozoan cysts.

- ***Baermann concentration method***
  Procedure:
  - Another method of examination of stool specimen suspected of having small numbers of *Strongyloides* larvae is the use of a modified Baermann apparatus **(Fig. 29.2)**.
  - The Baermann technique, which involves using a funnel apparatus, relies on the principle that active larvae migrate from a fresh fecal specimen that has been placed on a wire mesh with several layers of gauze, which are in contact with tap water.
  - Larvae migrate through the gauze into the water and settle to the bottom of the funnel, where they can be collected and examined.
  - Besides being used for patient's stool specimens, this technique can be used to examine soil specimens for the presence of larvae.

Egg Counting Methods

A ***semiquantitative assessment*** of the worm burden can be made by estimating the number of eggs passed in stools.

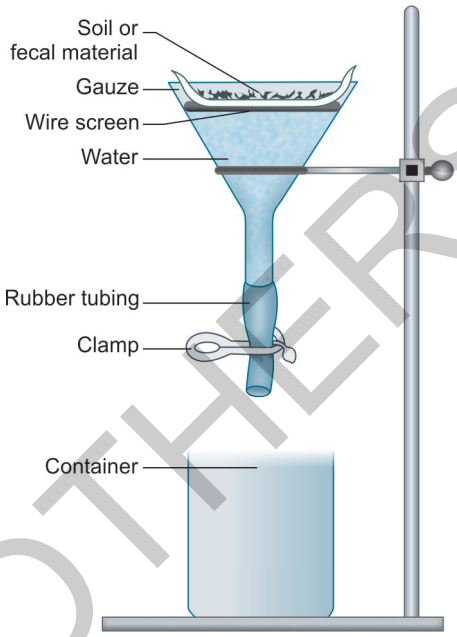


Fig. 29.2: Baermann concentration method.

This is done by *egg counts* and by relating the counts to the number of worms present by assuming the number of eggs passed per worm per day.

However, these are at best approximations and only a rough indication of worm burden can be obtained. Egg counts help to classify helminth infections as heavy, moderate, or light. Egg counts can be done by different methods.

- The ***standard wet mount*** gives rough indication of the number of eggs. Ordinarily, 1–2 mg of feces is used for preparing a wet film, and if all the eggs in the film are counted. The number of eggs per gram of feces can be assessed.
- The ***modified Kato thick smear technique*** using 50 mg of stool cleared by glycerin-soaked cellophane coverslip can be used for egg counting.
- ***McMaster's egg counting*** chamber can also be used. In this method, eggs in 20 mg of stool are concentrated by salt floatation on the squared grid on the roof of the chamber, which can be counted.
- In ***Stoll's dilution technique***, 4 g of feces is mixed thoroughly with 56 mL of N/10 sodium hydroxide using beads in a rubber stoppered glass tube. Using a pipette, exactly 0.075 mL of the sample is transferred to a slide, cover glass is applied, and all the eggs present are counted. The number multiplied by 200 gives the number of eggs per gram of feces. This figure requires to be corrected for the consistency of feces, by multiplying by 1 for hard formed feces, by 2 for mushy formed feces,

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Box 29.3: Hatching test for schistosoma eggs.

This test is used to demonstrate the viability of the miracidia within the schistosome eggs recovered from the urine or stool. Fecal or urine specimens must be processed without any preservative. The specimens are placed in 10 volumes of dechlorinated or spring water. Living miracidia may be released by hatching within few hours. The specimens are examined microscopically for presence of miracidia, which indicates active infection.

by 3 for loose stools and by 4 for liquid stools. Watery stools are unfit for counting.

- Special techniques have been described for particular purposes as for example, ***Bell's dilution-filtration count*** for schistosome eggs **(Box 29.3)**.
- ***Scotch tape method:*** This is a simple and effective method for detection of eggs and female worms of *Enterobius vermicularis* and occasionally eggs of *Taenia solium, T. saginata and Schistosoma mansoni*. In this method, a piece of transparent adhesive tape is pressed firmly against perianal skin, and the adhesive surface of the tape is spread on a glass slide **(Figs. 29.3A to D)**. The slide is then placed under microscope and observed for parasitic eggs. A drop of toluene or xylol may be placed between the tape and the slide to clear the preparation. The specimen should be collected for 3 consecutive days at night or early in the morning.

Fecal Culture

Fecal culture is not used for routine diagnosis, but for species identification, for example in differentiation between *Ancylostoma* and *Necator*.

Harada-Mori Filter Paper Strip Culture

The test detects light infection with hookworm, ***S. stercoralis, Trichostrongylus*** spp., as well as to facilitate species identification of helminths.

The Harada-Mori culture method uses strips of filter paper on which feces is smeared in the middle third. The paper strips are kept in conical centrifuge tubes with water at the bottom, in which the strips dip **(Fig. 29.4)**. The tubes are kept at room temperature in the dark for 7–10 days, during which time the larvae develop and fall into the water at the bottom, from which they can be collected. Also, caution must be exercised in handling the paper strip itself, since infective *Strongyloides* larvae may migrate upwards, as well as downwards on the paper strip.

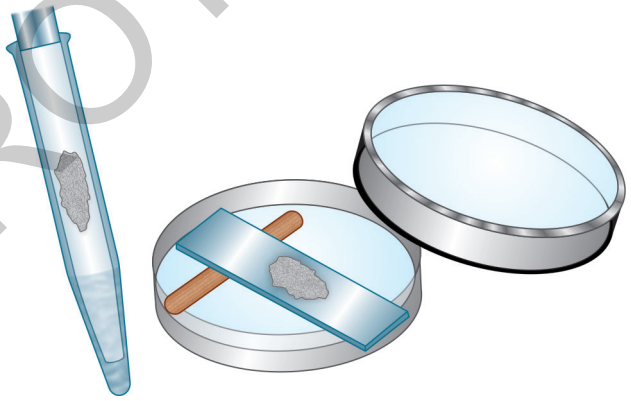
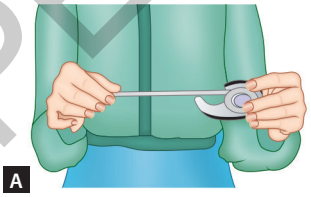
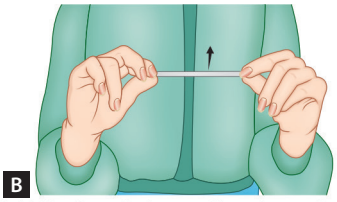


Fig. 29.4: Harada-Mori tube method and petri dish culture method.



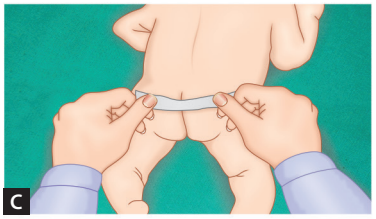
A

Use a piece of clear cellophane tape approximately 4 inches long.



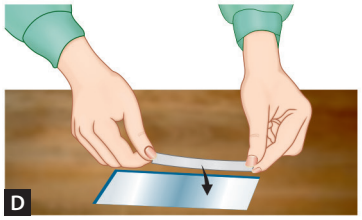
B

Hold the tape between thumbs and forefingers with sticky side facing upward.



C

Press the sticky side of the tape against the skin across the anal opening.



D

Place the sticky side of the tape down against the surface of a clean glass slide.

Figs. 29.3A to D: Method for collection of a cellophane (scotch) tape preparation for pinworm diagnosis. This method dispenses with the tongue depressor, requiring only tape and a glass microscope slide. The tape must be pressed deep into the anal crack.

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Agar Plate Culture for Strongyloides

Agar plate cultures are used to recover larvae of *S. stercoralis* and appear to be more sensitive. Approximately, 2 g fecal specimens are inoculated onto agar plates. Then the plates are sealed with tape to prevent accidental infection and placed in room temperature for 2 days. In positive cases, larvae will crawl over the agar, making visible tracks over it. For further confirmation of larvae, the plates are examined microscopically.

Charcoal Culture

Charcoal cultures are simple and efficient. Softened feces is mixed with 5–10 parts of moistened charcoal granules and packed into a suitable container and kept covered. In 7–10 days, the larvae hatch out and come to the surface. They can be collected by applying a pad of soft cotton cloth on the surface for half an hour. The cloth is removed and kept upside down on a sedimentation flask, filled to the brim with warm water. The larvae fall to the bottom of the flask, while the charcoal particles remain on the cloth.

EXAMINATION OF BLOOD

Next to feces, the largest number of parasites are found in blood. Blood examination is the routine diagnostic method in malaria, filariasis, African trypanosomiasis and babesiosis. It is sometimes positive in Chagas disease and rarely, in kala-azar and toxoplasmosis. Blood examination is done in the following ways.

Examination for Malarial Parasites

The standard diagnostic method in malaria is the examination of stained blood films—both thin and thick smears.

Collection of Blood

For demonstration of malarial parasites, blood should be collected not during the peak of fever, but optimally several hours after it. Bouts of fever follow the synchronous rupture of large number of parasitized erythrocytes, releasing their membrane shreds and contents. The emerging merozoites parasitize other erythrocytes and initiate a fresh cycle of *erythrocytic schizogony*. The timing is particularly important in *P. falciparum* infections, as here the late stages of schizogony are not seen in peripheral circulation.

- In practice, the rule is to take a blood smear when a suspected malaria patient is first seen and then again subsequently after a bout of fever.
- Smears should invariably be collected before starting antimalarial treatment.

Thin smear:

- A thin smear is prepared from finger prick or in infants from heel prick blood or ethylenediaminetetraacetic acid (EDTA) anticoagulated venous blood can also be used, provided blood films are made within 30 minutes.
- A small drop (10–15 µL) is spread on a clean *grease-free* slide with a spreader to give a uniform smear, ideally a single cell thick smear. The margins of the smear should be well short of the sides of the slide, and the tail should end a little beyond the center of its length.
- The thin smear displays blood cells and parasites clearly. Its only disadvantage is that only a small volume of blood can be surveyed, so that a light infection could be missed.
- If the smears are prepared from anticoagulated blood, which is more than an hour old, the morphology of both parasites and infected RBCs may not be typical.
- After drying, the smear is stained with Giemsa or Leishman stain.
- For ***Giemsa stain***, the smear is fixed in methanol for 3–5 minutes. After drying, Giemsa stain, diluted 1 drop in 1 mL of buffered water, pH 7–7.2, is applied for 30–45 minutes. The slide is then flushed gently with tap water, dried and examined under the oil immersion objective. The *cytoplasm* of malarial parasites is stained *blue* and the *chromatin dot* is stained *red*.
- For ***Leishman's stain***, prior fixation is not necessary as the stain is an alcoholic solution, which fixes as it stains. Leishman stain is applied for 1–2 minutes and diluted with twice its volume of buffered water, pH 7–7.2 and is kept for 10–15 minutes. The smear is then dried and examined.

Reporting of thin blood films:

- In malignant tertian malaria, only the ring stage and gametocytes are seen in peripheral smear, while in benign tertian malaria, all stages of schizogony and gametocytes can be seen.
- Thin smear examination enables the appreciation of changes in the erythrocytes, such as enlargement, alteration of shape, fimbriation, red cells stippling (*Schuffner's dots*) as seen with *P. vivax,* and irregular stippling (*Maurer's clefts*), as seen in mature *P. falciparum* trophozoites.
- Any marked increase in white cell numbers and if indicated perform a differential white cell count.
- Parasitized erythrocytes are seen most often in the upper and lower margins of the tail of the smear.
- Count the percentage of parasitized red cells, when there is high *falciparum* malaria parasitemia (+++ or more

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parasites seen in the thick film) to monitor a patient's response to treatment.
- A minimum of 100 fields should be examined before a negative report is given.

Thick smear:
- Thick smears have the advantage that a larger quantity of blood can be tested. Increased volume of blood present on thick film may allow the malaria parasite to be detected even with low parasitemia. Compared with a thin film, a thick film is about ***30 times*** more sensitive and can detect about **20 parasites/μL of blood**.
- The disadvantages are that the red cells are lysed and the morphology of the parasites is distorted, so that species identification becomes difficult.
- A big drop of blood (**20–30** μL) from finger or heel prick is collected on a clean grease-free slide and spread with the corner of another clean slide to form a uniformly thick smear of about 1 cm^2. The thickness of the smear should be such that the hands of a wristwatch can be seen through it, but not the figures on the dial.
- The smear is dried in a horizontal position, kept covered from dust.
- Thick smears have to be dehemoglobinized before staining.
- They can be stained with ***Giemsa or Leishman's stains*** as described earlier. Wright's stain and JSB stain (so called because it was devised by Jaswant Singh and Bhattacharjee, in 1944) are very useful for staining large numbers of thick films as in malaria surveys.

  Wright's stain consists of two solutions:
  1. Solution A contains methylene blue and azure B in phosphate buffer.
  2. Solution B contains eosin in phosphate buffer. The film is immersed in solution A for 5 seconds, washed in tap water, immersed in solution B for 5 seconds, washed, dried and examined. Staining times may need adjustment. If the smear is too blue, stain longer in solution B; if too pink, in solution A.

  Jaswant Singh and Bhattacharjee stain also consists of two solutions:
  1. The first contains methylene blue, potassium dichromate, sulfuric acid, potassium hydroxide and water.
  2. The second solution is aqueous eosin.

  For staining, the smear is immersed in solution 1 for 10 seconds, washed for 2 seconds in acidulated water pH 6.2–6.6, stained in solution 2 for 1 second, washed in acidulated water, immersed again in solution 1 and washed.

Box 29.4: Plus sign scheme for reporting parasite numbers.

- 1–10 per 100 high-power fields: +
- 11–10 per 100 high-power fields: ++
- 1–10 in every high-power field: +++
- More than 10 in every high-power field: ++++

Reporting of thick blood films:
- Select an area that is well-stained and not too thick.
- Examine for malaria parasites and malaria pigment under oil immersion objective (100X).
- Examine at least 100 high-power microscope fields for parasites.
- Report the approximate number of parasites (trophozoites, schizonts and gametocytes) and also whether malaria pigment is present in white cells or not.
- The plus sign scheme that can be used to report parasite numbers are described in **Box 29.4**.

Combined thick and thin blood films:
- Combined thick and thin smears can be taken on the same slide.
- Draw a thick line with a glass-marking pencil on a slide, dividing it into two unequal parts. The thick smear is made on the smaller part and the thin smear drawn on the larger.
- Thick smear is first dehemoglobinized and the two are then stained together. An easy method is to add undiluted Leishman stain over the thin smear, and then the diluted stain flooded over to the thick smear also.
- Do not allow the methanol to contact the thick film when fixing the thin film.
- The stained thin smear is examined first. If the thin smear is negative, the thick smear should be searched for parasites.
- When a slide is positive for malarial parasites, the report should indicate the species, the developmental stages found and the density of parasites in the smear.

Examination for Microfilaria

Microfilariae may be detected in peripheral blood, both in unstained mounts and in stained smear **(Table 29.1 and Box 29.5)**.

Table 29.1: Parasites found in peripheral blood film.

Protozoa	***Nematodes***
Plasmodium spp.	*Wuchereria bancrofti*
Babesia spp.	*Brugia* spp.
Leishmania spp.	*Loa loa*
Trypanosoma spp.	*Mansonella ozzardi*

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Box 29.5: Time of collection.

In case of nocturnal periodic microfilariae, blood should be collected between 10 PM and 2 AM. In subperiodic nocturnal infection, the time of collection of blood should be between 8 PM and 10 PM and for subperiodic diurnal infection the time of collection should be ideally between 2 PM and 6 PM.

Wet Mount

- Two or three drops of blood are collected on a clean glass slide and mixed with two drops of water to lyse the red cells.
- The preparation is covered with a coverslip and sealed.
- The preparation is examined under the low-power microscope for the motile microfilariae, which can be seen ***wriggling*** about, ***swirling*** the blood cells in their neighborhood.
- The examination may conveniently be deferred till next morning, as microfilariae retain their viability and motility for 1 or 2 days at room temperature.
- By using a simple counting chamber, microfilariae in the wet mount can be *counted*.

Stained Smears

- A thick smear is prepared as for malaria, dehemoglobinized, and stained with Leishman's, Giemsa, or Delafield's hematoxylin stains.
- Stained smears have the advantage that the **morphology** of microfilariae can be studied and **species identification** can be made. Thus, for differentiation between *Mf. bancrofti* and *Mf. malayi* stained smears are necessary.
- Sometimes, microfilariae may be seen in thin smears also.
- By using a measured quantity of blood for preparing smears, as for example with a 20 cubic mm pipette and counting the total number of microfilariae in the smear, microfilaria counts can be obtained. Multiplying the number of microfilariae in a 20 cubic mm smear by 50 gives the count per mL of blood.

Concentration Methods

These methods have been developed to recover low numbers of microfilariae from blood and employ venous blood.

- ***Sedimentation method:***
  - In sedimentation method, the sample of blood is first lysed with acetic acid, saponin, or other lytic substance, or by freeze-thawing, and then centrifuged.
  - The sediment is stained and the microfilariae are counted.
- ***Membrane filtration concentration:***
  - In membrane filtration method, a measured quantity (1–5 mL) of blood is collected into an anticoagulant solution and passed through membrane filters fixed on syringes with Swinney filter holder. Blood cells and proteins sticking on to the filter are washed away by repeatedly passing saline through it.
  - The filter is removed, placed on a slide, stained with dilute Giemsa stain and examined under low-power microscope for microfilariae.
  - **Millipore** and **nucleopore membrane filters** (5 μm porosity) are available for this purpose; the latter being more sensitive, as it can screen larger volumes of blood.
  - Membrane filtration recovers most species of microfilariae; however, because of their small size, *Mansonella perstans* and *M. ozzardi* may not be recovered. Membranes with smaller pores (3 μm) have been suggested to recover these two species.
  - The membrane filter method is much more sensitive than the finger prick method as the blood samples are taken during day, it also gives reliable results even with nocturnal periodic microfilariae.
  - However, the method has the disadvantages that venipuncture is necessary, membranes are costly, and microfilariae may not be in a satisfactory condition for detailed morphological study.
  - The number of microfilariae counted divided by 10 gives the number of microfilariae per mL of blood.
  - This is the most sensitive method of detecting small numbers of microfilariae, but it is expensive for routine use.
- ***Microhematocrit tube method:***
  - Capillary blood is collected in two heparinized capillary tubes or about 100 μL is first collected into EDTA anticoagulant, and then transferred to plain capillary tubes.
  - The blood is centrifuged in a microhematocrit centrifuge.
  - The ***buffy coat*** is examined microscopically for motile microfilariae.
  - In areas where the species is known and *Mansonella* microfilariae are not found, this is a rapid technique for detecting microfilariae.
- ***Buffy coat blood film:***
  - The buffy coat containing white blood cells (WBCs) and platelets obtained after centrifugation of whole anticoagulated blood and the layer of RBCs just below the buffy coat layer, can be used to prepared thick and thin blood films in suspected infections

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with filaria, *Leishmania*, *Trypanosoma* and malaria. The sensitivity of this method is much higher than that of routine thick film.

Diethylcarbamazine Provocation Test

Oral administration of diethylcarbamazine (**DEC**; 100 mg or 2 mg/kg of body weight) brings about mobilization of microfilariae into peripheral blood. Blood collected 20–50 minutes after the drug is given, will show microfilariae so that blood collection can be done during day time. This is a great advantage for surveys. But the drug may cause febrile reactions, particularly in brugiasis. It cannot be used in areas endemic for onchocerciasis because of the danger of provoking severe reactions.

SPUTUM EXAMINATION

Sputum is examined commonly for the demonstration of eggs of ***Paragonimus westermani***, and sometimes for detection of trophozoites of *E. histolytica* in amebic pulmonary abscess. Rarely, the larval stages of hookworm, *A. lumbricoides*, or *S. stercoralis* or the cestode hooklets may be seen in sputum samples **(Box 29.6)**.

- Concentrated stained preparations of induced sputum are commonly used to detect *P. jirovecii* and differentiate trophozoite and cyst forms from other possible causes of pneumonia, particularly in an acquired immunodeficiency syndrome (AIDS) patient.
- Normally, direct saline mount preparation is done for microscopy.
- If the sputum is thick, equal volume of 3% N-acetyl cysteine or 3% sodium hydroxide is added to the sputum to liquefy the specimen and after centrifugation, the sediment is examined for microscopic examination under low (10X) and high (40X) power magnifications.
- In a *Paragonimus* spp. infection, the sputum may be viscous and tinged with brownish flecks, which are clusters of eggs (***iron filings***) and may be streaked with blood.

Box 29.6: Parasites found in sputum.

- *Paragonimus westermani*
- *Entamoeba histolytica* (trophozoites in case of pulmonary abscess)
- *Pneumocystis jirovecii*
- Rarely migrating larvae of *Ascaris lumbricoides*
- Rarely migrating larvae of *Strongyloides stercoralis*
- Rarely migrating larvae of *Ancylostoma duodenale*
- Rarely migrating larvae of *Necator americanus*

URINE OR BODY FLUIDS EXAMINATION

- Large volume of urine samples should be allowed to settle for 1–2 hours.
- About 50 mL of the bottom sediment of the sample is taken for centrifugation.
- The highly concentrated sediment after centrifugation is examined for direct wet mount microscopy.
- May show eggs of *Schistosoma* and *Trichomonas vaginalis*. Microfilariae may be detected from chylous urine in lymphatic filariasis.

TISSUE BIOPSY

Tissue biopsies and fine-needle aspirations are taken from cutaneous ulcers of trypanosomiasis or leishmaniasis and from skin nodules of onchocerciasis and post-kala-azar dermal leishmaniasis (PKDL).

- A ***skin snip*** can be obtained to diagnose subcutaneous filariasis or leishmaniasis by grasping with a forceps or elevating a portion of skin with the tip of needle. Tip of the small cone of the skin is, then sliced with a sharp blade or razor.
- Wet mount preparation of lymph node aspirate and chancre fluid are used as rapid methods for demonstration of trypanosomes.
- Biopsies from liver, spleen, bone marrow and lymph nodes are taken in visceral leishmaniasis for demonstration of Leishman-Donovan (LD) bodies.
- All biopsy tissues must be submitted to the laboratory without the addition of formalin fixative. If there is delay in transport or processing, the specimen should be placed in polyvinyl alcohol fixative. In soft specimens, a small part should be scraped and examined as direct saline wet mount.
- *Impression smears* can be made from freshly cut tissue specimens on a glass slide and examined after fixation with Schaudinn's solution. Trichrome or other stains can be used.
- The residual part of the biopsy specimen may be processed for histopathological examination.
- Adult filarial worms can sometimes be found in section of biopsied lymph node.
- Corneal scrapings are useful in diagnosis of acanthamoeba keratitis.

MUSCLE BIOPSY

Spiral larval form of ***Trichinella spiralis***, larval form of ***T. solium*** (*cysticercus cellulosae*) and amastigote of *Trypanosoma cruzi* can be demonstrated in skeletal muscle biopsy.

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In trichinosis, muscle biopsy (gastrocnemius, deltoid and biceps) specimen must be examined by compressing the tissue between two slides and checking the preparation under low-power (10X) objective. This method does not become positive until 2–3 weeks after the illness.

DUODENAL CAPSULE TECHNIQUE (ENTEROTEST)

Enterotest is a simple method of sampling duodenal contents.

- The device is composed of a length of nylon yarn-coiled inside a **gelatin capsule**.
- The end of the yarn is affixed to the patient's face.
- The capsule is then swallowed and the gelatin dissolves in the stomach.
- The weighted string is carried into the duodenum by peristalsis.
- Bile-stained mucus is then retrieved after 3–4 hours and duodenal contents adherent to the yarn is scrapped off and examined under microscope as wet mount or as stained smear after preservation in formalin or polyvinyl alcohol.
- Usually 4–5 drops of material is obtained.
- Enterotest is used for detecting trophozoites of *Giardia*, larvae of *Strongyloides*, eggs of liver flukes and oocysts of *Isospora*.

SIGMOIDOSCOPY MATERIAL

Material obtained from sigmoidoscopy is useful in the diagnosis of ***E. histolytica*** that cannot be diagnosed by routine examination for at least 3 days.

- Material from the intestinal mucosa should be aspirated or scraped and not to be collected by cotton swabs.
- The material should be processed immediately.
- In heavy infection of *Trichuris,* sigmoidoscopy may show white bodies of the worms hanging from the inflamed mucosa of large intestine.

UROGENITAL SPECIMEN

The detection of ***T. vaginalis*** is usually based on wet preparation of vaginal and urethral discharges and prostatic specimens. Specimens should be collected in small volume of 0.85% saline and should be sent immediately for detection of actively motile organisms, as the jerky movements of *Trichomonas* begin to diminish with time.

CULTURE METHODS

Many parasites can now be grown in culture, but this has not become a routine diagnostic method in parasitic infections **(Box 29.7)**. It is sometimes employed for accurate identification of the parasite species. It is more often employed for obtaining large yields of the parasite as a source of antigen, animal inoculation, drug-sensitivity testing, experimental or physiological studies and teaching purposes. Some of the culture methods used for different parasites are indicated here.

Box 29.7: Parasites which can be cultured in the laboratory.

- *Entamoeba histolytica*
- *Giardia lamblia*
- *Trichomonas vaginalis*
- *Leishmania* spp.
- *Trypanosoma* spp.
- *Acanthamoeba* spp.
- *Naegleria fowleri*
- *Balantidium coli*
- *Plasmodium* spp.

Ameba

E. histolytica and other intestinal amebae can be grown in diphasic or monophasic media, media containing other microorganisms, or axenic cultures.

- ***Boeck and Drbohlav diphasic medium***, the classical culture medium for ameba has been modified by various workers **(Box 29.8)**.
  - The medium as used now, is basically an egg slant, with an overlay of sterile serum or liver extract in buffered saline.
  - A loopful of sterile rice powder is added to the medium just before inoculation with fresh feces or its saline centrifugal sediment.
  - Cultures can be obtained from feces-containing cysts or trophozoites.
  - The cultures are incubated at 37°C and subcultured at 48-hour intervals.
  - Amebae can be demonstrated in the liquid phase in unstained mounts or stained smears.
- ***Balamuth's monophasic liquid medium*** is also used commonly for cultivation of amebae and other

Box 29.8: Composition of Boeck and Drbohlav medium (Locke's solution).

- Sodium chloride: 9 g
- Potassium chloride: 0.4 g
- Calcium chloride: 0.2 g
- Sodium bicarbonate: 0.2 g
- Glucose: 2.5 g
- Distilled water: 1000 mL
- Egg: Four (clean and washed)

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Box 29.9: Composition of Balamuth's medium.

- Liver concentrate powder: 1 part
- Egg yolk medium: 9 part
- *Phosphate buffer*
- Tribasic potassium phosphate: 212 g
- Monobasic potassium: 136 g
- Distilled water

intestinal protozoa. This is an egg yolk-liver extract infusion medium **(Box 29.9)**.

- Both protozoa and bacteria present in stools grow in the earlier media.
- Bacterial growth can be reduced by addition of penicillin or other antibiotics that do not inhibit protozoa.
- Axenic cultures (pure cultures without bacteria or other microorganisms) were first developed by Diamond in 1961. Axenic cultivation has enabled precise antigenic and biochemical studies on amebae.
- *B. coli* grows well in Balamuth's medium. *G. lamblia* had been established in association with *Candida* and *Saccharomyces*, but axenic cultures were developed in 1970.
- *T. vaginalis* grows very well in several commercially available media such as trypticase serum media.
- *Naegleria* and *Acanthamoeba* from cerebrospinal fluid (CSF) can be grown on agar plates heavily seeded with *Escherichia coli*.

Leishmania and Trypanosomes

- ***Novy-MacNeal-Nicolle medium:*** The classical *Novy-MacNeal-Nicolle (NNN) medium* first described in 1904 for cultivation of *Leishmania,* is equally satisfactory for trypanosomes also. This is a defibrinated rabbit blood agar medium **(Box 29.10)**. Several modifications of this medium have been introduced.
  - Two bottles of culture are aseptically inoculated with 0.1 mL of specimen in each and incubated at 24°C for 4 weeks.
  - The primary culture is examined every 4 days for promastigotes in leishmaniasis and for epimastigote stages in trypanosomiasis for up to 30 days.

Box 29.10: Composition of Novy-MacNeal-Nicolle (NNN) medium.

- Bactoagar (Difco): 1.4 g
- Sodium chloride: 0.6 g
- Double distilled water: 90 mL
- Defibrinated rabbit blood (10%):10 mL.

Box 29.11: Composition of Schneider's insect tissue culture medium.

- Schneider's *Drosophila* tissue culture medium: 80 mL
- Fetal calf serum: 20 mL
- Antibiotic-antimycotic solution: 1.2 mL

- ***Schneider's insect tissue culture medium:*** It is recommended *in vitro* culture of *Leishmania*. This medium is said to the more sensitive than NNN medium **(Box 29.11)**.

Malaria Parasites

- Cultivation of malaria parasites was first obtained by Bass and Jones in 1912. A simple method of cultivation is as follows:
  - About 10–12 mL of defibrinated or heparinized blood rich in ring forms of malaria parasite, mixed with 0.2 mL of 50% dextrose solution are incubated at 37°C in a sterile test tube in an upright position.
  - The blood separates into the erythrocytes below, plasma above and the buffy coat in between.
  - Malaria parasites grow in the erythrocyte layer immediately below the buffy coat.
  - Smears are collected from this layer at intervals, without tilting the tube.
  - Segmented schizonts are usually observed after incubation for 24–36 hours.
- The breakthrough in cultivation of malarial parasites came in 1976 when Trager and Jensen successfully maintained *P. falciparum* in continuous cultures in human erythrocytes using Roswell Park Memorial Institute **(RPMI)** 1640 medium.
  - The cultures are incubated at 38°C with 10% human serum at pH 6.8–7.2 under an atmosphere with 7% carbon dioxide and 1–5% oxygen.
  - A continuous flow system is used in which the medium flows slowly and continuously over the layer of erythrocytes. The method has been applied to various species of *Plasmodia*.
  - It has been employed for preparation of antigens, drug-sensitivity studies, vaccine tests and many other purposes.

ANIMAL INOCULATION

Animal inoculation is not a routine diagnostic procedure in parasitic infections, but can be used in some instances because of its sensitivity.

- ***Toxoplasmosis:*** Animal inoculation can be used for isolating *Toxoplasma gondii* from infected persons.

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Lymph node or other biopsy materials are inoculated intraperitoneally into immunosuppressed mice. Peritoneal fluid obtained 7–10 days later, may show the parasite in Giemsa-stained smears. However, serial passages may be necessary for its isolation. Brain smears may be examined for cysts after sacrificing the mice 3–4 weeks after inoculation. Seroconversion of the animal inoculation also indicates a positive result.

- ***Visceral leishmaniasis:*** Bone marrow, liver, spleen, or lymph node aspirates from kala-azar patients, injected intraperitoneally into hamsters is a very sensitive method for diagnosing visceral leishmaniasis. Even a single amastigote can establish the infection in the animal. Spleen smears taken 4–6 weeks later show Leishmania donovani (LD) bodies.
- ***Trypanosomiasis:*** Blood from patients with trypanosomiasis can be injected intraperitoneally or into the tail vein of mice, rats and guinea pigs, etc. These animals are susceptible to infection by *T. brucei rhodesiense.* Parasitemia can be demonstrated in 2 weeks.

XENODIAGNOSIS

This method involves the diagnostic infection of a vector, in which the parasite multiplies and can be demonstrated. In ***T. cruzi,*** diagnosis may be established by letting the vector **reduviid bug** feed on suspected patients. In 4–5 weeks, live flagellate forms can be seen in the feces of the bugs.

IMMUNOLOGICAL DIAGNOSIS

Serology

Several serological tests have been developed for detection of antibodies to parasites using antigens from cultured parasites or from natural or experimental infections in animals or humans. In some cases, antigens are obtained from related parasites or even sometimes from bacteria. Advances in cultivation of parasites have made parasitic antigens more readily available. Cloning of parasitic antigens promises to be a new source.

In some instances, diagnosis is attempted by serological demonstration of parasitic antigens in blood, tissues, or secretions of suspected patients.

Virtually, all types of serological reactions have been used. However, serodiagnosis in parasitic infections has only limited value due to various factors:

- Parasites are complex antigenically and exhibit wide range of cross-reactions, so that serological tests are not sufficiently specific.
- Another difficulty is in distinguishing between past and current infections. This has been solved partly by looking for immunoglobulin M (IgM) antibody, as in amebiasis and toxoplasmosis.
- In general, indirect hemagglutination (IHA), enzyme-linked immunosorbent assay (ELISA) and counter-immunoelectrophoresis (CIEP) are most sensitive; indirect immunofluorescence (IF), direct agglutination test (DAT) and complement fixation test (CFT) are moderately sensitive; and simple precipitation in gel and coated particle agglutination tests are least sensitive. Serology has not been very useful in the diagnosis of individual cases, but has been valuable as a screening method in epidemiological surveys. However, in some infections where parasites are seldom demonstrable in patients, for example in toxoplasmosis and hydatidosis, serology is of great help. Listed here are some of the applications of serology.

Amebiasis

Serology is of no value in the diagnosis of acute amebic dysentery or luminal amebiasis. But in invasive amebiasis, particularly in liver abscess, serology is very useful.

- *Indirect hemagglutination is most widely employed.* Titers of 1:256 or more are significant in cases of amebic liver abscess and have prognostic value.
- **Tech Lab *E. histolytica*** test was able to detect galactose lectin *(GalNAc) antigen* in almost all patients of amebic liver abscess.

Giardiasis

Enzyme-linked immunosorbent assay and *indirect immunofluorescence (IIF) test* have been developed for detection of *Giardia.*

Commercially available ELISA (**ProSpec T/*Giardia***) kit detects *Giardia-specific antigen 65 (GSA 65)*. The sensitivity of the test is 95% and specificity is 100%, when compared to conventional microscopy.

Trypanosomiasis

Serological tests used to detect trypanosomiasis are *IHA, indirect fluorescent antibody (IFA)* and *ELISA*.

- Specific antibodies are detected by these tests in the serum within 2–3 weeks infection.
- Specific antibodies can be demonstrated by *IFA* and *ELISA* in *CSF.*

Leishmaniasis

Indirect hemagglutination, CIEP, and *DOT-ELISA* are usually positive in kala-azar.

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- *Complement test using Witebsky, Klingenstein and Kuhn (**WKK**) antigen* from the acid-fast Kedrowsky bacillus are relatively less sensitive.
- Indirect fluorescent antibody test is positive very early in the disease, even before the appearance of symptoms and becomes negative within 6 months of cure.
- ***rK39 micro ELISA test*** is a qualitative immunochromatographic assay for detection of antibodies to *Leishmania*.

Malaria

Indirect immunofluorescence, ELISA and *IHA* are sensitive and specific, but are not useful for diagnosis of acute malaria because antibodies persist for some years after cure.

- A negative test may, however help to exclude malaria.
- Serological tests are useful in epidemiological surveys for malaria.
- Molecular assays such as antigen capture for detection of *histidine-rich protein II (**HRP-2**)* and *Plasmodium lactate dehydrogenase (**pLDH**)* have been applied for developing rapid dipstick tests (e.g. ParaSight-F in malignant tertian malaria).

Toxoplasmosis

Serological tests offer the most useful diagnostic method in toxoplasmosis.

- The original ***Sabin-Feldman dye test***, though very specific and sensitive, is no longer in use. *IIF, IHA* and *CFT* were other useful tests. The dye test remains positive for life, while CFT becomes negative soon after active infection.
- At present, *ELISA* is routinely used in *Toxoplasma* serology. It is very informative, as it provides titers of IgM and IgG antibodies separately for better interpretation of the results.

Cryptosporidiosis

Indirect fluorescent antibody and *ELISA* using purified oocysts as antigens have been used to detect circulating antibodies specific to *Cryptosporidium parvum*.

Intestinal Helminths

Antibodies can be demonstrated in most intestinal helminthiases, but extensive cross-reactions limit their use in diagnosis.

Trichinosis

Serology is very useful in diagnosis of trichinosis. *Bentonite flocculation slide tests* and *CFT* become positive 3–4 weeks after infection.

- *Indirect immunofluorescence* becomes positive even earlier.
- *Enzyme-linked immunosorbent assay* is also available.

Demonstration of seroconversion is diagnostic.

Toxocariasis

High titers in serological tests are obtained in visceral larva migrans, but specificity is low due to cross-reactions with intestinal nematode antigens.

Filariasis

Indirect hemagglutination and *bentonite flocculation tests* with antigen from *Dirofilaria immitis* gives positive reaction in patients, and high titers in tropical pulmonary eosinophilia. But cross-reactions are frequent.

*Immunochromatographic card test (**ICT**)* is a new and rapid filarial antigen test that detects soluble *Wuchereria bancrofti* antigens in the serum of infected humans.

Echinococcosis

Several serological tests have been developed using hydatid fluid or scolex antigens from hydatid cysts in sheep. *IHA, IIF, CIEP* and *ELISA* are very sensitive. Cross-reactions occur with cysticercosis.

SKIN TESTS

Intradermal tests have been used in many parasitic infections. They are sensitive and persist for many years, sometimes even for life. But specificity is relatively low.

- ***Casoni's test:*** This test had been used widely in the diagnosis of hydatid disease since its original description in 1911. The antigen is sterile hydatid fluid drawn from hydatid cysts from cattle, sheep, pig, or humans, filtered and tested for sterility. Intradermal injection of 0.2 mL of the antigen induces a wheal and flare reaction within 20 minutes in positive cases. A saline control is used. False-positive tests are seen in schistosomiasis and some other conditions. Casoni's test is now largely replaced by serological tests.
- ***Leishmanin (Montenegro) test:*** This test is used to measure delayed hypersensitivity. *Leishmania* test is sensitive and relatively specific. The antigen is obtained from cultured *Leishmania* and consists of killed promastigotes in phenol saline. Intradermal injection of 0.1 mL induces a papule of 5 mm or more in diameter in 48–72 hours. This delayed hypersensitivity test is positive in cutaneous leishmaniasis and negative in diffuse cutaneous and visceral leishmaniasis.
- ***Fairley's test:*** This skin test is group-specific and gives positive results in all schistosomiasis. The intradermal

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allergic test uses antigen infected snails, cercariae, eggs and adult schistosomes from experimentally infected laboratory animals.

- ***Skin test in Bancroftian filariasis:*** Intradermal injection of filarial antigens (extracts of microfilariae, adult worms and third-stage larvae of *Brugia malayi,* or the dog filaria, *Dirofilaria immitis*) induce an immediate hypersensitivity reaction, but the diagnostic value of the skin test is very limited due to the high rate of false-positive and negative reactions.

MOLECULAR METHODS

Nucleic acid-based diagnostic tests are mainly available in specialized or reference centers. Nucleic acid probes and amplification techniques such as polymerase chain reaction (**PCR**) and multiplex PCR, western blot and deoxyribonucleic acid (**DNA**) **hybridization** techniques are increasingly used to detect parasites in specimens of blood, stool, or tissue from patients.

- Loop-mediated isothermal amplification (LAMP) techniques are increasingly used in diagnosis of parasitic disease. Particularly in field settings, as it does not require a thermal cycler.
- These test are useful for detecting subspecies or stain level identification which is important for epidemiological studies and are also used to detect parasitic drug resistance. For example, specific 17 kDa and 27 kDa sporozoite antigens are employed for seroepidemiological studies in cryptosporidiosis using western blot technique.
- Deoxyribonucleic acid probe is a highly sensitive method for the diagnosis of malaria. It can detect even less than 10 parasite/μL of blood.
- **B1 gene** of *T. gondii* can be detected by PCR of the amniotic fluid in case of congenital toxoplasmosis. PCR have been developed for detection of filarial DNA from patients blood. If parasite cannot be identified by microscopy, amplification of babesial 18S ribonucleic acid (RNA) by PCR is recommended.
- **Drug resistances** in malaria are detected now by PCR techniques. PCR is increasingly used now for species specification and for detection of drug resistance in malaria. Chloroquine resistance in *P. falciparum* has been attributed to mutation in the *Plasmodium falciparum* chloroquine resistance transporter (PfCRT), a transporter gene in the parasite. Point mutation in another gene *Plasmodium falciparum* multidrug resistance protein 1 (PfMDR1) has also been implicated in determining resistance *in vitro*. Pyrimethamine and sulfadoxine resistances are associated with point mutations in dihydrofolate reductase (DHFR) and dihydropteroate synthase (DHPS) genes respectively. Mutation in PfATPase gene is associated with reduced susceptibility to artemisinin derivatives.

REVIEW QUESTIONS

1. **Enumerate the various methods employed for examination of stools and describe in detail the concentration methods of stool examination.**
2. **Describe various skin tests used for diagnosis in many parasitic infections.**
3. **Write short notes on:**
   a. Scotch tape method
   b. Blood examination for malarial parasite
   c. Blood examination for microfilaria
   d. Enterotest
   e. Casoni's test
   f. Floatation method of stool examination

MULTIPLE CHOICE QUESTIONS

1. **Time of collection of blood is important in:**
   a. Microfilaria
   b. *Trypanosoma* spp.
   c. *Leishmania* spp.
   d. *Babesia* spp.
2. **Modified acid-fast stain is used for the diagnosis of:**
   a. *Entamoeba histolytica*
   b. *Toxoplasma gondii*
   c. *Cryptosporidium parvum*
   d. *Leishmania donovani*

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3. Sputum examination is commonly done for detecting the eggs of:

a. *Strongyloides stercoralis*
b. *Entamoeba histolytica*
c. *Paragonimus westermani*
d. *Ascaris lumbricoides*

4. Larval forms of which parasite can be found in muscle biopsy:

a. *Ascaris lumbricoides*
b. *Taenia solium*
c. *Trichuris trichiura*
d. *Ancylostoma duodenale*

Answers

1. a	**2. c**	**3. c**	**4. b**

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