15 Laboratory Diagnosis of Parasitic Diseases

Chapter Outline

- Introduction
- Morphological identification techniques
- Culture techniques in parasitology
- Immunodiagnostic methods
- Molecular methods
- Intradermal skin tests
- Xenodiagnostic techniques
- Animal inoculation methods
- Imaging techniques
- Expected questions

INTRODUCTION

Laboratory diagnosis plays a vital role in the diagnosis of parasitic infections. Following diagnostic techniques are used for diagnosis of parasitic infections:

- Morphological identification techniques either macroscopically or microscopically
- Culture methods
- Immunodiagnostic methods
- Molecular methods
- Intradermal skin tests
- Xenodiagnostic techniques
- Animal inoculation methods
- Imaging techniques.

MORPHOLOGICAL IDENTIFICATION TECHNIQUES

The parasites can be identified by their morphology either macroscopically or microscopically. Various morphological forms of different parasites can be seen in different specimens (Table 15.1).

Microscopically they can be visualized directly by wet mount (saline/iodine) for stool specimen or either by different staining techniques.

Examination of Feces

Specimen Collection

- Stool specimens should be collected in a wide-mouthed, clean, leak-proof, screw capped containers and should be handled carefully to avoid acquiring infection from organisms present in stool
- **Timing:** Specimen should be collected before starting antiparasitic drugs and closer to the onset of symptoms
- **Frequency:** At least three stool specimens collected on alternate days are adequate to make the diagnosis of intestinal parasitic diseases (third specimen should be obtained after purgatives).
- **When to examine:** Liquid stool specimens should be examined within 15–30 minutes,

Table 15.1: Various morphological forms of parasites seen in different specimens

Specimen	Morphological form	Parasite
Feces	Trophozoite	• *Entamoeba histolytica* • *Giardia lamblia* • *Balantidium coli* • *Trichomonas hominis*
	Cyst	• *E. histolytica* • *G. lamblia* • *B. coli*
	Adult worm	• *Ascaris lumbricoides* • *Enterobius vermicularis* • *Fasciolopsis buski*
	Adult worm segments	• *Taenia solium, T. saginata* • *Diphyllobothrium latum*
	Egg	• *Schistosoma* spp. • *Fasciola hepatica* • *Fasciolopsis buski* • *Clonorchis sinensis* • *Opisthorchis felineus* • *Heterophyes heterophyes* • *Metagonimus yokogawai* • *D. latum* • *Taenia* spp. • *Hymenolepis nana* • *H. diminuta* • *Dipylidium caninum* • *A. lumbricoides* • *Ancylostoma duodenale* • *Necator americanus* • *Enterobius vermicularis* • *Trichuris trichiura* • *Capillaria* spp. • *Trichostrongylus*
Peripheral blood smear	Ring form, schizont and gametocyte	• *Plasmodium* spp.
	Amastigote	• *Leishmania* spp.
	Trypomastigote	• *Trypanosoma* spp.
	Microfilaria	• *Wuchereria bancrofti* • *Brugia malayi* • *Loa loa* • *Mansonella* spp.
Bone marrow, liver, lymph node and spleen aspirate	Tachyzoite	• *Toxoplasma gondii*
	Amastigote	• *Leishmania donovani*
Liver aspirate	Trophozoite	• *Entamoeba histolytica*
Lymph node aspirate	Trypomastigote	• *Trypanosoma* spp.
Lymph node biopsy	Adult worm	• *W. bancrofti* • *B. malayi*

Contd...

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Specimen	Morphological form	Parasite
Cerebrospinal fluid (CSF)	Trypomastigote	• *Trypanosoma* spp.
	Larva	• *Anglostrongylus* spp.
	Trophozoite	• *Naegleria fowleri* • *Acanthamoeba*
Urine	Trophozoite	• *Trichomonas vaginalis*
	Microfilaria	• *W. bancrofti*
	Egg	• *Schistosoma haematobium* • *Dioctophyma renale*
Sputum	Adult worm	• *Paragonimus* spp.
	Egg	• *Paragonimus spp.* • *Capillaria aerophila*
	Larva (migrating)	• *A. lumbricoides* • *Strongyloides stercoralis* • *A. duodenale* • *N. americanus*
	Trophozoite	• *E. histolytica*
Duodenal aspirate	Trophozoite	• *G. lamblia*
	Larva	• *S. stercoralis*
Corneal scrapings	Trophozoite	• *Acanthamoeba* spp.
Skin	Amastigote	• *Leishmania* spp.
	Microfilaria	• *Onchocerca volvulus*
	Larva in skin ulcer fluid	• *Dracunculus medinensis*
Muscle tissue	Encysted larva	• *Trichinella spiralis*
	Cysticercus cellulosae	• *T. solium*
Perianal area	Egg	• *Enterobius* spp. • *T. saginata*

semisolid stools within 1 hour and formed stools up to 24 hours after collection. On prolonged storage, trophozoites may disintegrate, become non motile and may appear as artifacts

- Several preservatives (e.g., 10% formalin or polyvinyl alcohol) can be used to maintain the morphology of the parasitic cysts and eggs
- **Specimens other than stool:**
  - **Perianal swabs (cellophane tape or NIH swab):** Useful for detecting Eggs of *Enterobius vermicularis* deposited on the surface of perianal skin. It is also used for eggs of *Schistosoma mansoni* and *Taenia* species
  - **Duodenal contents:** It is very useful for the detection of small intestine parasites like, *Giardia intestinalis* and larva of *Strongyloides stercoralis*. Duodenal fluid can be collected by intubation or by entero test (discussed in Chapter 4).

Macroscopic Examination

- **Mucoid bloody stool:** Found in acute amoebic dysentery, intestinal schistosomiasis, and invasive balantidiasis

- Dark red stool indicates upper gastrointestinal (GIT) bleeding and a bright red stool is suggestive of bleeding from lower GIT
- Frothy pale offensive stool (containing fat) found in giardiasis
- Adult worms like round worm, thread worm or segments of tapeworm may be seen.

Microscopic Examination

Direct wet mount (saline and iodine mount)

Drops of saline and Lugol's iodine are placed on two corners of a slide. A small amount of feces is mixed by a stick to form a uniform smooth suspension. Cover slip is placed on the mount and examined under low power objective (10X); followed by high power objective (40X).

Following structures can be visualized by microscopic examination of stool specimen:

- **Normal constituents:** Such as plant fiber, starch cells (stains blue black with iodine), muscle fibers, animal hair, pollen grains, yeast cells, bacteria, epithelial cells, fat globules, and air bubbles are present (Fig. 15.1)
- **Cellular elements:** Like pus cells (in inflammatory diarrhea), red blood cells (RBC) (in dysentery) may be present
- **Charcot Leyden crystals (diamond shaped):** They are the breakdown products of eosinophils and may be seen in the stool or sputum of patients with parasitic diseases such as amoebic dysentery, ascariasis, and allergic diseases like bronchial asthma (sputum)
- Trophozoites and cysts of protozoa and eggs and larvae of helminths are seen (See appendix VI).

Saline mount

Advantages

- Useful in the detection of trophozoites and cysts of protozoa and eggs and larvae of helminths
- Motility of trophozoites and larvae can be demonstrated in acute infection
- Bile staining property can be appreciated—bile stained eggs appear golden brown and non bile-stained eggs appear colorless.

Iodine mount

Advantages

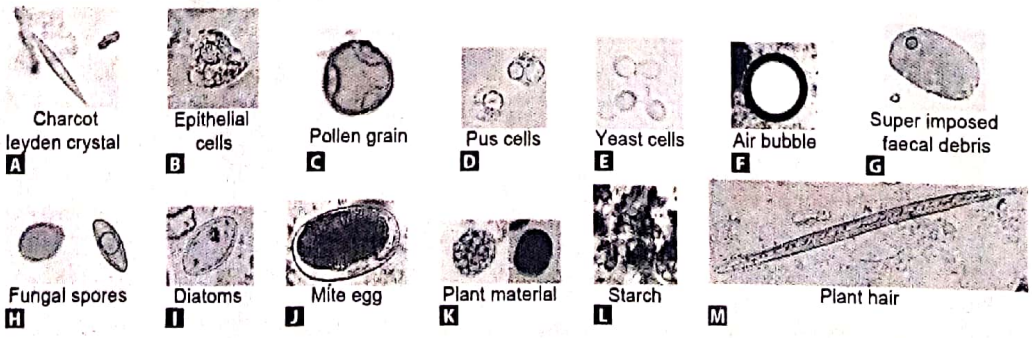
Nuclear details of cysts, helminthic eggs and larvae are better visualized; helps in species identification.

Disadvantages

- Iodine immobilizes and kills parasites, hence motility of the protozoan trophozoites and helminthic larvae cannot be appreciated
- Bile staining property cannot be appreciated.

Types of iodine stains

- **Lugol's iodine:** Potassium iodide (KI)



Figs 15.1A to M: Normal constituents and artifacts found in stool in wet mount examination

Source: A to H- Giovanni Swierczynski, Bruno Milanesi "Atlas of Human Intestinal Protozoa Microscopic Diagnosis" (*with permission*); I to M- DPDx Image Library, Centre for Disease Control and prevention (CDC), Atlanta (*with permission*)

10g + iodine crystals (5 g) + 100 mL of distilled water

- **D'Antoni's iodine:** KI 1g + Iodine crystals (1.5 g) + 100 mL of distilled water
- **Dobeil's iodine:** KI 2.0 g + iodine 1.0 g + 50 mL of distilled water.

Permanent stained smear

Permanent stained smears are required for accurate diagnosis of intestinal parasites.

Commonly used methods are:

- Iron-hematoxylin stain
- Trichrome stain
- Modified acid-fast stain

All these permanent stained smears help in the accurate diagnosis of cysts and trophozoites by staining their internal structures.

Iron-hematoxylin stain: A thin smear of feces is fixed in Schaudinn's solution (for 15 minutes) and immersed in 70% alcohol containing iodine and then in 50% alcohol for 2–5 minutes each and washed with tap water. Then the slide is immersed in 2% aqueous ferric ammonium sulphate solution for 5–15 minutes followed by washing in tap water for 5 minutes. It is then stained in 0.5% aqueous hematoxylin for 5–10 minutes and washed in tap water for 5 minutes. Finally the smear is immersed in aqueous solution of picric acid for 10–15 minutes and dehydrated by immersing in 50%, 70%, 80% and 95% alcohol for 5 minutes each. Stained smear is placed in xylene (2–5 minutes); then mounted in Canada balsam and covered with coverslip.

Trichrome stain: Fecal smear is prepared, fixed and treated with alcohol containing iodine as in case of iron-hematoxylin staining. Then it is stained with trichrome solution for 10 minutes and differentiated in acid alcohol (1 part glacial acetic acid in 99 parts of 90% alcohol) for 2–3 seconds. It is rinsed in absolute alcohol several times and dehydrated in absolute alcohol for 2–5 minutes. Stained smear is placed in xylene (2–5 minutes); mounted in Canada balsam and covered with coverslip.

Modified acid-fast stain: Modified acid-fast stain is used for detection and identification of *Cryptosporidium parvum, Cyclospora* and *Isospora belli.* The acid-fast oocyst stains red with carbol fuchsin and the non-acid-fast background stains blue.

- **Hot method:** A thin smear of feces is heat fixed and flooded with carbol fuchsin for 9 minutes. The slide is intermittently heated till carbol fuchsin starts steaming. Then slide is washed with tap water and decolorised with 5% aqueous sulphuric acid for 30 seconds, followed by washing with tap water and counter staining with methylene blue for 1 minute
- **Kinyon's cold method:** Fecal smear is methanol fixed (1 minutes), stained with Kinyon's carbol fuchsin for 5 minutes. Then it is rinsed with 50% ethanol followed by tap water. It is then decolorized with 1% sulfuric acid for 2 minutes, washed with tap water and counter stained with alkaline methylene blue for 1 minute.

Concentration Techniques

If the parasite output is low in feces (egg, cysts, trophozoites and larvae) and direct examination may not be able to detect the parasites, then the stool specimens need to be concentrated. These methods are also useful in epidemiological analysis and for assessing the response to treatment. Eggs, cysts and larvae are recovered after concentration procedures; however, the trophozoites get destroyed.

Commonly used concentration techniques are:

- **Sedimentation techniques:** Eggs and cysts settle down at the bottom following centrifugation
  - Formalin-ether concentration technique
  - Formalin-ethyl acetate concentration technique
  - Formalin-acetone sedimentation technique

- **Floatation techniques:** The eggs and cysts float at the surface due to specific gravity gradient
  - Saturated salt (sodium chloride) solution technique
  - Zinc sulphate floatation concentration technique
  - Sheather's sugar floatation technique (useful for *Cryptosporidium, Isospora* and *Cyclospora*)

Two commonly used concentration techniques are formalin-ether and saturated salt solution technique.

Sedimentation Techniques

Principle: It involves concentration of stool specimen by centrifugation. The protozoan cysts and helminthic eggs are concentrated at the bottom of the tube because they have greater density than the suspending medium.

Formol-ether sedimentation technique

Procedure (nine steps)

Step 1: About half teaspoonful (~ 4g) of feces is transferred to a tube containing 10 mL of 5–10% formalin, mixed thoroughly and allowed to stand for 30 minutes

Step 2: Then the mixture is filtered into a 15 mL conical centrifuge tube covered with two layers of gauze. About 8 mL of the filtrate is collected (3–4 mL for formalin persevered stool)

Step 3: 0.85% saline (or 5–10% formalin) is added almost to the top of the tube containing the filtrate and centrifuged for 10 minutes at 500 × g

Step 4: The supernatant is discarded and 0.5–1 mL of the sediment is resuspended in saline or formalin (filled up to the top of the tube) and centrifuged again for 10 minutes at 500 × g

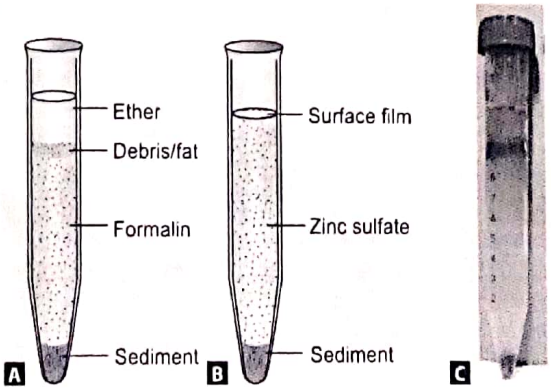
Step 5: The sediment is resuspended in 5–10% formalin (filled half of the tube) and centrifuged. This step may be eliminated if the supernatant fluid is clear after the first wash

Step 6: 4–5 mL of ether (or ethyl acetate) is added and the tube is closed with a stopper and shaken vigorously to mix well. The stopper is removed and the tube is centrifuged at 500 × g for 10 minutes

Step 7: Four layers are formed. Top layer consists of ether, second is a plug of debris, third is a clear layer of formalin and the fourth is the sediment (Fig. 15.2)

Step 8: The debris is removed from the side of the tube with the help of a glass rod and supernatant is discarded

Step 9: With a pipette, the sediment is removed and the saline or iodine mount is made and examined under the microscope.



Figs 15.2A to C: (A) Formol-ether sedimentation technique (schematic diagram); (B) zinc sulfate flotation concentration technique (schematic) diagram; (C) formol ether sedimentation technique (real image)

Courtesy: C- Dr Anand Janagond, Associate professor, Velammal Medical College, Madurai,Tamilnadu (*with permission*)

Note

- Substitute for ether: Since ether is explosive; it can be replaced by ethyl acetate or acetone or clearing agent Hemo-De, which are much safer with equal efficacy
- If the stool is formalin preserved, then the Step 1 is omitted
- If stool contains lot of mucus, then following Step 1, the mixture is centrifuged for 10 minutes at 500 × g and the sediment is directly mounted
- If the stool is polyvinyl alcohol (PVA) preserved, then following Step 1, the saline or formalin mixed stool is filtered immediately. Then the procedure is same from Step 3
- One should start monitoring the centrifugation time only after reaching the recommended speed by the centrifuge
- The woven gauze should never be more than two layers.

Advantages

- The sensitivity of detecting the ova or cysts increases by 8–10 folds
- The size and shape of the parasitic structures are maintained
- Inexpensive, easy to perform
- Fecal odor is removed
- As formaline kills the fecal parasites, no risk of acquiring laboratory acquired infection.

Disadvantages

- Trophozoite forms are killed and hence not detected in this method.

Flotation Techniques

Principle: Flotation involves suspending the specimen in a medium of greater density than that of the helminthic eggs and protozoan cysts. The eggs and cysts float to the top and are collected by placing a glass slide on the surface of the meniscus at the top of the tube.

Saturated salt flotation technique

Procedure

- About half tea spoon (~ 4g) of fresh stool is placed in a flat bottomed container of less than 1.5 inches diameter and 20 mL capacity (Fig. 15.3)

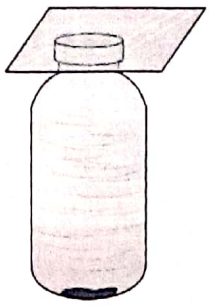


Fig. 15.3: Flotation technique (schematic diagram)

- Then, few drops of saturated salt solution (specific gravity 1.200) is added and stirred to make a fine emulsion
- More salt solution is added with stirring throughout to fill the container up to the brim, until a convex meniscus is formed
- A glass slide (3"×2") is carefully laid on the top of the container so that the center is in contact with the fluid
- Preparation is allowed to stand for 20 minutes after which the glass slide is quickly lifted, and examined under the microscope after putting a coverslip.

Disadvantages

- Flotation technique is not useful for heavier eggs that do not float in the salt solution such as:
  - Unfertilized eggs of *A. lumbricoides*
  - Larva of *Strongyloides*
  - *Taenia* eggs
  - Operculated eggs of trematodes
- If left for more than 20 minutes, protozoan cysts and thin walled nematode eggs get collapsed and become distorted due to high specific gravity of the solution

Zinc sulphate flotation concentration technique

Step 5: First five steps are same as that of formol-ether sedimentation technique

Step 6: After the second wash, the clear supernatant is poured off and the sediment

is added to a tube containing 2–3 mL of 33% zinc sulfate (specific gravity 1.18). More zinc sulfate solution is added to fill the tube up to the top and the tube is centrifuged again at 500 × g for 2 minutes

Step 7: Three layers are formed (Fig. 15.2B). Sample is taken from the surface film by a wire loop (make sure not to dip below the surface film), mounted on a glass slide. Then the supernatant is discarded and the sediment is also mounted and examined.

Note:

- The protozoan cysts and lighter helminth eggs are concentrated in the surface film where as operculated and heavy eggs, larvae are deposited in the bottom
- Zinc sulfate of specific gravity 1.20 should be used for formalin preserved stool.

Preservation of Fecal Specimen

Preservation of fecal specimens is essential for following reasons:

- To maintain morphology of the parasitic cysts, eggs and larvae
- To prevent further development of some helminthic eggs and larvae
- For teaching purpose
- For epidemiological analysis
- Transport of specimen to a referral lab for further identification.

Several preservation methods are available (Table 15.2):

- **Formalin fixative method:** 5% formalin is recommended for protozoan cysts and 10% formalin for helminthic eggs and larvae
- **Sodium acetate formalin (SAF) fixative method:** Neutral formalin buffered with sodium phosphate buffer is used to maintain the morphology of the parasites
- **Merthiolate-iodine formalin (MIF) solution fixative method:** It contains formalin and Lugol's iodine; acts both as fixative and stain
- **Schaudinn's fluid:** It is a mixture of mercuric chloride and ethyl alcohol. It fixes and

Table 15.2: Comparison of Stool preservation methods

Preservatives	*Advantages*	*Disadvantages*
Formalin	Easy to prepare, long shelf life Overall good for stool concentration Can be used for fecal Immunoassay kits	Not good for permanent smear Trophozoites are distorted
MIF (Merthiolate-iodine –Formalin)	Both fixes and stains the stool sample Easy to prepare, long shelf life Useful for the field study Good for stool concentration	Not good for permanent smear Trophozoites are distorted Contains mercury compounds (disposal problem) Not good for faecal immunoassay kits
SAF (Sodium acetate formalin)	Useful for – Stool concentration Permanent smear by iron haematoxylin Faecal immunoassay kits Easy to prepare, long shelf life	Adheres poorly to the slide (albumin coated slide is recommended) Not good for trichrome permanent smear Trophozoites are distorted
Schaudinn's fluid	Fixative for the fresh stool Excellent for preservation of stool	Contains mercury compounds Not good for concentration Trophozoites are distorted Not good for faecal Immunoassay kits
PVA (Polyvinyl alcohol)	Best for trichrome stain Long shelf life (In tight container at room temperature) Excellent for stool preservation, specimens can be shipped to distant places.	Difficult to prepare. Not good to preserve *Giardia* cyst, *Trichuris* egg, *Isospora* oocysts and *Strongyloides* larvae Trophozoites are distorted Not good for faecal Immunoassay kits Contains mercury compounds

preserves the specimen for 1 year or more
- **Polyvinyl alcohol (PVA) fixative method:** PVA powder is incorporated to Schaudinn's fluid. PVA is a plastic resin serves as adhesive for stool specimen where as Schaudinn's fluid helps in fixation. Liquid stool is added to PVA at 1:3 ratio
- **Modified PVA:** It uses copper sulfate or zinc base instead of mercury chloride.

Egg Counting (Egg quantification) Methods

The intensity of intestinal helminthic infection can be estimated using egg counting in the feces by following methods:

Direct smear counting method of beaver

- Smear is made by using 2 mg of feces mixed in a drop of saline on a slide and examined under microscope (low power)
- Number of eggs in 2 mg feces is counted and then multiplied by factor 500 to calculate the number of eggs per gram of feces
- It is simple and accurate when performed by an experienced technologist.

Kato's cellophane tape

Approximate number of eggs per gram of feces in a concentrated stool specimen (sedimentation technique) can be calculated by **Kato's cellophane tape** covered thick smear examination

Stoll's method or dilution egg counting method

- 4 g of feces is mixed thoroughly with 56 mL of N/10 NaOH in a calibrated Stoll's flask and a uniform suspension is made
- 0.15 mL of this mixture is transferred to the slide. The slide is kept over a mechanical stage and examined under a low power objective and the total number of eggs is counted (n)
- The number of eggs per gram of feces (N) is calculated by multiplying the count (n) with 100
- Estimated daily output of eggs is calculated by multiplying the number of eggs/gram with the weight of 24 hour fecal sample

Note: The above mentioned calculation is applied for formed feces. However, the estimate (eggs per gram) will vary according to the consistency of the stool. If feces is not formed, then 'N' is multiplied by the correction factor as given below:

- **Mushy stool (pulpy or soft):** N × 2
- **Mushy formed:** N × 1.5
- **Mushy diarrhoeic:** N × 3

Examination of Blood

Blood examination is useful in diagnosis of infection caused by blood parasites like *Plasmodium, Trypanosoma, Leishmania, Babesia, Wuchereria bancrofti, Brugia malayi, Loa loa* and *Mansonella.*

Various methods of examination of blood include:
- Direct wet mount examination
- Examination of blood smears after permanent staining
- Examination of buffy coat region (quantitative buffy coat)
- Concentration of blood.

Search for tapeworm scolex
- This is very useful for proper identification of species
- 24 hours stool sample is mixed with water, make watery suspension
- The watery suspension is filtered through a double layered sieve
- The cleansed debris is examined with hand lens to look for scolices and proglottids
- If no scolices are found, then the filtration step is repeated and cleansed debris is examined with a magnifying hand lens against the background to increase the contrast
- Tapeworm segments are picked with an applicator stick, rinsed with saline and placed between two slides and observed under low power objective

Direct Wet Mount Examination

- A drop of blood is collected by finger prick and placed on a glass slide. A coverslip is placed over the blood drop and examined under low power objective
- Useful for detection of microfilariae and trypanosomes by their motility
- Counting of microfilariae may be done by examining the blood on a neubauer counting chamber.

Examination of Blood Smears After Permanent Staining

- Thick and thin blood smears are made from peripheral blood and stained with Romanowsky stains
- Romanowsky's stains include Leishman's stain, Geimsa stain, Field's stain and Jaswant Singh and Bhattacharya (JSB) stain stain. These stains are a combination of methylene blue and eosin. They also contain oxidation products of methylene blue called **azures**; which provide further contrast in the stained peripheral smears. Stock solutions of these stains are prepared by dissolving the stains in pure methanol.
- Different types of Romanowsky stains
  - Water based stains, e.g., Geimsa, JSB, Field's stain
  - Methanol based stains, e.g., Leishman's and wright's stain.

Note:

- For staining thick blood smear, dehemoglobinization is must. While staining with water-based stains, this occurs when the stain is poured on the thick smear; but, for methanol-based stains, additional step of dehemoglobinization by adding water is necessary before pouring the stain.
- Thin blood smears must be methanol fixed before staining to prevent dehemoglobinization. When methanol-based stains are used, this occurs when the stain is poured on the smear; but for water-based stains, additional step of methanol fixation is necessary before pouring the stain
- Details of the stain composition and procedures are discussed in Appendix 4. Method to make the smears is given in Chapter 6.

Quantitative Buffy Coat (QBC)

- This involves collection of blood in a capillary tube coated internally with acridine orange stain, centrifugation at 12,000 rpm for 5 minutes and examination of the buffy coat region under ultravoilet (UV) rays
- This extremely useful for the detection of the malaria parasites and microfilariae
- Detail is given in Chapter 6.

Concentration of Blood

Concentration techniques are useful for detection of microfilariae from blood specimen. Various concentration methods are:

- Sedimentation technique
- Cytocentrifugation (cytospin)
- Knott's concentration
- Gradient centrifugation
- Membrane filtration

Sedimentation technique

5–10 mL of blood is collected and centrifuged at 500×g for 2 minutes. Supernatant is discarded and the sediment is used to prepare a smear. The smear is air dried, fixed and stained.

Knott concentration

10 mL of 2% formalin is mixed thoroughly with 1 mL of venous blood in a centrifuge tube and centrifuged at 500×g for 2 minutes. Sediment is collected; smear is prepared, stained and examined for microfilariae.

Gradient centrifugation

Heparinized venous blood (4 mL) is mixed with 4 mL of Ficoll-hypaque solution and centrifuged at 400×g for 40 minutes. Three distinct layers are formed; lower most white blood cell (WBC) layer, middle Ficoll-hypaque layer and upper most plasma layer. The middle layer is examined for presence of microfilariae.

Membrane filtration

- Blood (1 mL) is lysed by shaking gently with 10 mL of distilled water in a syringe. Then the lysed blood is passed through 25 mm membrane filter of pore size 5 µm such as millipore or Nucleopore membrane filters. The microfilariae are liberated from the blood on the filter. The filter is removed, stained and examined
- For detection of *Mansonella perstans* microfilariae, 3 µm size membrane filter is used.

Examination of Skin Tissue

Skin Snips

Skin snips are thin horizontal slices of epidermis, which are used for demonstration of *Onchocerca volvulus*. Collected skin snips are incubated in saline, to allow the microfilariae to emerge.

Skin Biopsy

Useful for histopathological examination.

Examination of cerebrospinal fluid (CSF)

Direct wet mount examination of cerebrospinal fluid (CSF) is useful for the detection of motile free living amoebae, (*Naegleria* and *Acanthamoeba*), trypanosomes and larvae of *Angiostrongylus cantonensis*.

Examination of Aspirates from Lymph Node, Spleen, Liver and Bone Marrow

Direct Microscopy

- Wet mount preparation or stained smears are very useful in detecting intracellular parasites such as *Leishmania* and *Trypanosoma* species
- Aspirate from hydatid cyst of liver or lung is useful for diagnosis of cystic echinococcosis
- Wet mount preparation of aspirated amoebic liver pus, is useful in the diagnosis of amoebic liver abscess.

Biopsy

Histopathological examination of affected tissues is very useful in detection of localized or disseminated infections caused by the parasites.

Examination of Sputum

Examination of sputum is useful in demonstration of eggs of *Paragonimus* in cases of pulmonary paragonimiasis and trophozoites of *E. histolytica* in cases of pulmonary amoebiasis.

Examination of Urogenital Specimen

Examination of vaginal discharges is useful in detection of *Trichomonas vaginalis* trophozoites. The trophozoites can be identified by their typical jerky motility.

CULTURE TECHNIQUES IN PARASITOLOGY

Culture Methods for Protozoa

The protozoa feed on bacteria, so the culture media are supplemented with bacterial growth. Accordingly there are four types of culture media are used for protozoa:

1. **Axenic cultures:** If the parasites are grown as pure culture without any bacterial associate, the culture is referred as axenic culture
2. **Xenic cultures:** Cultures of parasite grown in association with an unknown microbe are referred as xenic cultures
3. **Monoxenic culture:** If the parasites are grown with a single known bacterium, the culture is referred as monoxenic culture, e.g., corneal biopsy specimens cultured with *E. coli* for recovering *Acanthamoeba*
4. **Polyxenic culture:** It contains multiple bacterial supplements, starch and serum providing nourishment to amoeba.

Uses of Culture Media

Culture media are not routinely used in diagnostic parasitology. They are useful in research and teaching purpose.

- Polyxenic media is used for cultivation of protozoa from the suspected patients

- Axenic culture is useful when the bacterial flora interferes with the result such as:
  - Studying pathogenicity
  - Drug susceptibility testing
  - Preparation of antigen for serological tests.

Culture Media Used for Entamoeba histolytica

Culture media used for *E. histolytica* are given in Table 15.3.

Culture Media Used for Free-Living Amoebae

- **Non nutrient agar:** Useful for the isolation of *Acanthamoeba* and *Naegleria*.
  - **Composition:** Page's saline, Difco agar, distilled water and monoxenic cultures of *E. coli* or *Enterobacter aerogenes*. Page's saline is a mixture of NaCl, $CaCl_2$, $MgSO_4$, Na_2HPO_4 and KH_2PO_4
  - **Procedure of cultivation:** The sample (CSF, contact lens solutions or tissue samples) is inoculated on the center of the nonnutrient agar plate coated with bacterial overlay. The surface of the plate is examined under low power objective for 10 days for the presence of amoebae. Thin linear tracks (areas where amoebae have ingested the bacteria) might also be seen
  - *Balamuthia* species cannot be cultured by this method. They can be grown by using tissue culture methods
- **Peptone yeast extract glucose (PYG) liquid culture media:** Useful for cultivation of *Acanthamoeba* species
- **Nelson's liquid culture medium:** Useful for cultivation of *N. fowleri*. Nelson's medium is prepared by addition of fetal calf serum or brain extract to PYG medium
- **Tissue culture techniques:** Various mammalian cell lines such as monkey kidney cell line, HEp_2 and diploid macrophage cell line are used for cultivation of *Acanthamoeba*, *Naegleria* and *Balamuthia* species.

Culture Media Used for Giardia lamblia

Giardia can be cultivated in axenic media like Diamond's media used for cultivation of *E. histolytica*.

Culture Media Used for Trichomonas vaginalis

- Lash's cysteine hydrolysate serum media
- Cysteine peptone liver maltose (CPLM) media
- Diamond's trypticase yeast maltose (TYM) media

Table 15.3: Culture media used for *Entamoeba histolytica*

Culture medium	Type	Important ingredients
Boeck and Dr bohlav's medium	Polyxenic	Solidified egg or solidified blood, Locke's solution*, Inactivated bovine calf serum
Balamuth's medium	Polyxenic	Egg-yolk-liver concentrate infusion medium
Robinson's medium	Polyxenic	Erythromycin, Bacto peptone, Phthalate solution, bovine serum, *E. coli* strain 0111 and R-medium**
Jones' medium	Polyxenic	Horse serum and yeast autolysate in phosphate buffered saline
Diamond's (TYM) medium	Axenic	Trypticase-yeast extract-maltose

***Locke's solution:** Composed of sodium chloride (8.0 g), 0.2 g calcium chloride, potassium chloride (0.2g), magnesium chloride (0.01 g), sodium phosphate, dibasic (2.0 g), sodium bicarbonate (0.4 g) and potassium phosphate, monobasic (0.3 g)
****R-medium:** Composed of sodium chloride, citric acid, potassium phosphate buffer, ammonium sulfate, magnesium sulfate and lactic acid.

- Hollander's modification of TYM medium
- Cell lines like McCoy cell line highly sensitive, can detect as low as three trophozoites/mL.

Culture Media Used for Leishmania and Trypanosoma

- **NNN medium (described by Novy, McNeal 1903 and Nicolle 1908):** It supports the growth of flagellates causing leishmaniasis and Chagas' disease
  - **Composition:** It is biphasic media composed of two part salt agar and one part defibrinated fresh rabbit blood. The medium (4 mL) is dispensed and allowed to solidify in slanted position
  - **Procedure:** Materials (blood, bone marrow aspirate, splenic pulp) are inoculated in water of condensation of the NNN medium and incubated at 37°C for 1–4 week
  - **Observation:** The culture fluid is examined every day upto 10 days of inoculation for the presence of flagellates. On the solid medium, flagellates grow as thick, grayish white mucoid spreading lawn
- **Liquid medium for hemoflagellates:**
  - *Schneider's Drosophila* medium (30% fetal calf serum) and Grace's insect tissue culture medium
  - Amastigote forms transform to promastigote forms and multiply by binary fission
  - It is found to be more sensitive and rapid than NNN media
- **Other media:**
  - Yaeger's LIT medium for Chagas' disease
  - USAMRU blood agar medium for leishmaniasis.

Culture Techniques Used for Malaria Parasites

- Culture techniques for malaria parasites are mainly used for preparation of malaria antigens
- Trager and Jensen technique using **RPMI 1640 medium** is most widely used method. In detail discussed in Chapter 6.

Culture Techniques used for Larval-Stage Nematode

- Fecal culture methods (copro-culture) are especially useful for specific identification of hookworm, *Strongyloides stercoralis* and *Trichostrongylus* species
- Eggs hatch out into rhabditiform larvae in the culture medium which can be used to differentiate between hookworm, *Strongyloides stercoralis* and *Trichostrongylus* species
- Further rearing of nematode larvae leads to transformation into filariform larvae which can be used to differentiate *A. duodenale* and *N. americanus*.

Harada-Mori Filter Paper strip Culture (Fig. 15.4A)

- Smear is made with 0.5 g to 1 g of fresh feces in the center of a narrow strip of filter paper (15 cm × 1.5 cm)
- The filter paper is placed in a conical centrifuge tube with sterile water in such a way that the lower end dips in water
- This preparation is incubated for 7–10 days at room temperature after sealing the mouth of the tube
- Larvae develop on the filter paper migrate and are liberated in water, which can be examined under a microscope.

Petri Dish/ Slant Culture Method (Little et al.) (Fig. 15.4B)

- Fresh stool material is placed on the microscope slide shaped filter paper
- The filter paper is then placed on the slanted glass slide, kept in a glass petri dish plate containing water
- This technique allows direct examination of the culture system with a dissecting microscope to look for nematode larvae in the fecal mass.

Charcoal Culture

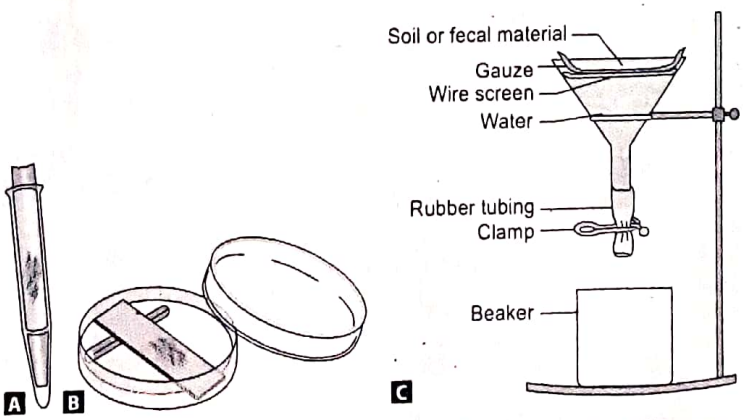
- 20 g of fresh stool mixed in water to form thick suspension, which is then added to storage dish containing granulated charcoal. Water is added to provide moisture. The dish is covered, and placed in dark for 5-6 days
- The hookworm and *Strongyloides* infective stage larvae can be harvested
- The condition of this culture technique provides an environment that mimics natural condition, efficient to harvest large numbers of infective-stage larvae.

Baermann Technique (Fig. 15.4C)

- This technique is useful for examining a stool specimen suspected of containing small numbers of *Strongyloides* larva
- This technique exploits the property of the *Strongyloides* larva to migrate from cooler to warmer area
- **Procedure:**
  - This method uses glass funnel fitted with a rubber tubing and clamp
  - A round wire screen is kept on the surface of the funnel, above which a piece of gauze is placed
  - 5 g of feces is placed on the gauze
  - Funnel is filled with warm water, left for 1-2 hours to give time for *Strongyloides* larvae to emerge from the feces
  - Clamp of the tubing is opened and 7-10 mL of fluid is collected in a beaker. Larvae in the stool migrate downward to the bottom in the fluid
  - After centrifugation, sediment is examined microscopically
- **Modification of Baermann Technique:** Funnel used in the original version is replaced by a test tube with a rubber stopper, which is perforated to allow insertion of a plastic pipette tip. The tube containing the fecal suspension is inverted over another tube containing 6 mL of saline solution and incubated at 37°C for 2 hour. Centrifuged saline solution is screened for larvae.

Agar Plate Culture for Strongyloides Stercoralis

- Agar plates are composed of 1.5% agar, meat extract (0.5%), peptone (1%) and NaCl (0.5%)
- Approximately, 2 g of fresh stool specimen is placed onto agar plates; the plates are sealed and held for 2 days at 26-33°C
- The plates are examined under the microscope for the presence of tracks (bacteria carried over agar by migrating larvae)



Figs 15.4A to D: Schematic diagram of techniques (A) Harada-Mori filter paper strip culture; (B) petri dish/slant culture method; (C) Baermann technique

- Then the surface of the agar is washed with 10% formalin, centrifuged and the sediment is screened for the presence of nematode larvae
- Daily search for furrows on agar plates for up to 6 consecutive days results in increased sensitivity for diagnosis of both *S. stercoralis* and hookworm larvae.

IMMUNODIAGNOSTIC METHODS

This method involves detection of parasite specific antibodies in serum, and detection of circulating parasitic antigen in the serum.

Immunodiagnostic methods are useful when:

- Parasites are detected only during the early stages of the disease
- Parasites occur in very small numbers
- Parasites reside in internal organs and morphological identification is not possible
- When other techniques like culture are time consuming.

Antibody Detection Tests

Antibodies are detected in various parasitic infections mainly from serum, sometime from other sites like CSF (neurocysticercosis) or pleural fluid (paragonimiasis).

Various antibody detection methods are:

- **Older methods:** Agglutination tests, complement fixation tests (CFT), gel electrophoresis, indirect hemagglutination assay (IHA) and counter-current Immuno-electrophoresis (CIE), less sensitive and specific
- **Recent techniques:** Indirect fluorescent antibody test (IFA), enzyme-linked immunosorbent assay (ELISA) (Table 15.4 and 15.5), rapid Immunochromatographic

Table 15.4: ELISA for detection of antibodies

Disease	*Antibody against*	*Comments*
Amoebiasis	Lectin antigen of *Entamoeba histolytica*	Sensitivity 75–85% Specificity > 85%
Visceral Leishmaniasis	Antigen of *Leishmania donovani*	Sensitivity (90%) Specificity (low)
Chagas' disease	*Trypanosoma cruzi* specific recombinant and synthetic peptide antigens	Less specific as it cross reacts with *T. rangeli* infection, leishmaniasis
Toxoplasmosis (double-sandwich IgM-ELISA)	Recombinant *Toxoplasma gondii* specific antigen and anti IgM capture antibody	Highly specific and sensitive in Diagnosing acute toxoplasmosis
Cryptosporidiosis	*Cryptosporidium parvum* oocyst antigen	Useful for seroepidemiology
Neurocysticercosis	Crude extract of cysticerci or vesicular fluid of *Taenia solium* detecting antibodies in serum and CSF	Sensitivity 75–90% Specificity (low)
Hydatid disease	Crude *Echinococcus granulosus* cyst fluid antigen	Sensitivity—variable (60–90%)
Schistosomiasis HAMA- FAST- ELISA (falcon assay screening test)	Using *Schistosoma hematobium* adult worm microsomal antigen (HAMA)	Useful for seroepidemiology.
Paragonimiasis	Purified adult excretory-secretory antigen of *Paragonimus westermani*	High sensitivity especially with pleural fluid than serum
Strongyloidiasis	Crude larval antigens	Sensitivity (95%) Useful when stool microscopy is negative
Lymphatic filariasis	• Crude parasitic extract • Recombinant *Wuchererla bancrofti* antigens	Useful for seroepidemiology Also detect *Brugia* infection

Table 15.5: ELISA for Detection of antigens

Disease	Antigen detected	Comments
Amoebiasis	170 kDa of lectin antigen of *Entamoeba histolytica* in stool or serum	Stool • Sensitivity > 95% • Specificity > 95% Serum • Sensitivity 65% (early stage) • Specificity > 90%
Giardiasis	Cyst wall protein antigens in stool	Sensitivity (90–100%) Specificity (99–100%)
Chagas' disease	*Trpanosoma cruzi* specific antigens from serum and urine	Useful for acute and congenital infection and drug response monitoring
Toxoplasmosis	*Toxoplasma gondii* specific antigens in blood/body fluid or amniotic fluid	Useful for diagnosis of acute and congenital infection
Cryptosporidiosis	*Cryptosporidium parvum* oocyst antigen in stool	Sensitivity (66–100%) Specificity (> 90%)
Intestinal taeniasis	*Taenia* specific antigen in stool by using polyclonal *Taenia* antibodies	More sensitive than stool examination But cannot differentiate between *Taenia saginata* and *Taenia solium*
Schistosomiasis	Circulating cathodic antigen (CCA) in urine Circulating anodic antigen(CAA) in serum Soluble egg antigen in serum	Sensitivity of 90% (serum) and 94 % (urine) Indicates recent infection used for monitoring the treatment
Lymphatic filariasis	Detects filarial antigens by using monoclonal $Og4C_3$ and $AD1_2$ antibodies	Highly sensitive (100%) and specific (99–100%) Can be detected in day time Differentiates between current from past infections- Used for monitoring response to treatment Can be detected in urine

Table 15.6: Rapid immunochromatographic tests in parasitic diagnosis (Principle of ICT is discussed in detail in malaria chapter)

Disease	Target	Comments
Antigen detection		
Malaria	Histidine rich protein-2 (Pf. HRP 2) *Plasmodium falciparum* specific Parasite lactate dehydrogenase (pLDH) common to all species	Sensitivity >90% (at parasite density >100/μL) pLDH—monitor response to treatment HRP-2—diagnose malaria in pregnancy
Triage parasite panel (for amoebiasis, giardiasis and cryptosporidiosis)	Simultaneous detection of *Giardia lamblia, Entamoeba histolytica* and *Cryptosporidium parvum*	Sensitivity (83–96%) Specificity (99–100%)
Lymphatic filariasis	Detects filarial antigens by using monoclonal Og4C3 and AD12 antibodies	Highly sensitive (96–100%) and specific (95–100%) Can be detected in day time Differentiates current from infection—used for monitoring response to treatment. Can be detected in urine
Antibody detection		
Visceral Leishmaniasis	*Leishmania infantum* recombinant kinesin 39 (rk39)	Sensitivity (98%) Specificity (90%)

tests (ICT) (Table 15.6) and Immunoblot (Western blot) (Table 15.7), more sensitive and specific.

Antigens used for the antibody detection are obtained:

- Crude antigen from cultured parasites, by animal inoculation or by natural infections to humans
- Recombinant or synthetic antigens
- From related parasites or related bacteria

Limitations of antibody detection techniques:

- Cannot distinguish between acute and chronic infection. [However, immunoglobulin M (IgM) based assay can diagnose recent infection accurately]
- Less specific: Cross reactive antibodies are found in unrelated infections due to heterogenicity of parasitic antigens
- Speciation is not possible.

Antigen Detection Tests

Most commonly used antigen detection tests are:

- ELISA (Table 15.5)
- Direct fluorescent antibody assays (DFA)
- ICT (Table 15.6).

Advantages of antigen detection tests:

- Detection of circulating parasitic antigen in serum, urine, genital specimen or feces
- Provides information about acute/recent infection
- Used to monitor the response to treatment
- Useful when microscopy is negative
- To diagnose congenital infection
- Assessing the severity of infection.

MOLECULAR METHODS

Molecular methods most frequently used in diagnostic parasitology include:

Table 15.7: Western blot (Enzyme linked immunotransfer blot/EITB) in parasitic diagnosis

Basic Principle of Western blot		
Step-1: PAGE (Polyacrylamide gel electrophoresis)→ parasitic antigens are mobilized electrophoretically and separated into smaller antigen fragments **Step-2:** Nitrocellulose membrane (NCM) blotting → antigenic fragments are blotted on NCM **Step-3:** Enzyme immunoassay → Detects antibodies against the antigenic fragments		
Disease	***Target***	***Comments***
Chagas' disease	Peptide antigenic fragments	Highly specific, confirms the diagnosis
Neurocysticercosis	Lentil lectin purified seven glycoprotein (LL-Gp) antigenic fractions of *Taenia solium*	Sensitivity—98% (when > 3 cysticerci detected in CNS) Specificity (nearly 100%)
Hydatid disease	Antibody against antigen B fragment of *Echinococcus granulosus* (produces 8-12kDa band)	Sensitivity (92%) Specificity (100%) Useful for seroepidemiological study
	Em-18 antigenic fragment of *E. multilocularis*	Sensitivity (97%) Specificity (100%) Doesn't cross react with *E. granulosus*
Schistosomiasis HAMA-EITB	Using *S. hematobium* adult worm microsomal antigen (HAMA).	Confirms the diagnosis Useful for seroepidemiology.
Paragonimiasis	Adult worm homogenate antigen of *Paragonimus westermani*	Highly sensitive and specific

- DNA probes
- Polymerase chain reaction (PCR).

DNA Probe

DNA probe consists of radiolabelled nucleotide sequences that is complementary to a part of the parasitic DNA present in the clinical samples. This is highly specific and reproducible. Currently, DNA probe based methods are available for the detection of *P. falciparum, W. bancrofti* etc.

Polymerase Chain Reaction (PCR)

Polymerase chain reaction (PCR) is an *in-vitro* procedure for DNA amplification.
The procedure involves

- Parasitic DNA extraction
- **Amplification:** This involves repeated cycles of i)Denaturation of ds DNA, ii)Annealing of primers (oligonucleotide sequences that hybridizes with the complementary regions present in the extracted target DNA), iii) Extension of the primers with the help of Taq DNA polymerase.
- Detection of the amplified products by gel electrophoresis.

Advantage: It is more sensitive and specific (can detect even few parasitic DNA), rapid, used for speciation, drug resistance detection and for research purpose.

PCR is now days increasingly used for the diagnosis of various parasitic infections (Table 15.8).

INTRADERMAL SKIN TESTS

Skin tests are useful when a reliable antibody detection methods are not available. They are employed for research and epidemiological purpose. Positive intradermal skin tests are

Table 15.8: Polymerase chain reaction in parasitic diagnosis

Disease	*Target*	*Comments*
Amoeblasis	Nested PCR targeting small subunit rRNA genes	Can differentiate the subspecies—*E. histolytica/dispar/moshkovskii* Sensitivity > 90% Specificity 90–100%.
	Real-time PCR	More sensitive, takes less time with less contamination rates
Visceral Leishmaniasis	*Leishmania* specific kinetoplast (mitochondrial) DNA	Sensitivity 70–93% Available only in limited laboratories
Malaria	Using PBRK1 primer	Not for routine use Used for speciation, drug resistance detection and for research purpose
Cryptosporidiosis	*C. parvum* genes from both clinical and environmental samples	More sensitive, can differentiate various genotypes of *C. parvum,* hence useful in outbreak situations
Hydatid disease	PCR-RFLP targeting mitochondrial DNA of *Echinococcus granulosus*	Can differentiate genotypes of *E. granulosus* (G1 to G10)
Clonorchiasis	Multiplex PCR	Can differentiate *Clonorchis* and *Opisthorchis*
Strongyloidiasis	Real-time PCR to detect *Strongyloides* DNA in stool	Nearly 100% specificity and high sensitivity Used for research purpose
Lymphatic filariasis	PCR-RFLP based assays by using ITS 1- rRNA genes as primers	Can differentiate all the filarial species

Abbreviations: PCR, polymerse chain reaction; DNA, Deoxyribonucleic acid; RFLP, restriction fragment length polymorphism

Table 15.9: Intradermal skin tests in parasitic diagnosis

Skin tests showing immediate hypersensitivity in	Showing delayed hypersensitivity in
Hydatid disease (Casoni's test)	Leishmaniasis (Montenegro test)
Filariasis	Trypanosomiasis
Schistosomiasis	Toxoplasmosis
Ascariasis	
Strongyloidiasis	
Trichinellosis (Bachman test)	

suggestive of past exposure. As they remain positive for longer duration, so they cannot differentiate old and recent infection. More so, non standardized crude antigens are used, hence they lack sensitivity and specificity. There is always a danger of provoking an anaphylactic reaction in the patient (Table 15.9).

XENODIAGNOSTIC TECHNIQUES

Principle

Xenodiagnosis uses laboratory reared arthropod vectors to detect low levels of parasites during chronic stages of the disease, when their numbers in the blood will be very low.

- This technique is employed to diagnose Chagas' disease
- This technique may be useful in endemic areas, but not in routine diagnostic laboratories

Xenodiagnosis in Chagas' Disease

Procedure

- Laboratory reared *Triatomine* (reduviid) bugs are starved for 2 weeks and then fed on the patient's blood, suspected to have Chagas' disease
- If Trypanosomes are present in the blood, they will multiply and develop into epimastigotes and trypomastigotes in about 30 days and are passed in the feces of the reduvid bug
- After 1–2 months, feces from the bugs are examined over a 3 month period for the developmental stages of the parasite, in the hind gut of the bug. The bugs may also be dissected and examined microscopically.

ANIMAL INOCULATION METHODS

Animal Inoculation techniques are not routinely used in diagnosis of parasitic infections; but useful in some parasitic infections (Table 15.10).

IMAGING TECHNIQUES

Being noninvasive methods, imaging techniques such as the X-ray, ultrasound (USG), computed tomography (CT) and magnetic resonance imaging (MRI) are extensively used various space occupying parasitic infections (Table 15.11).

Table 15.10: Animal inoculation methods in parasitic diagnosis

Parasite tested	Animal used	Route of inoculation	Specimen inoculated	Method of demonstration	Observation after inoculation
Toxoplasma gondii	Mice and Rats	0.5 mL of material injected intraperitoneally	Body fluid, blood, lymph node fluid or cerebrospinal fluid	Peritoneal fluid obtained after 7–10 days, stained with Romanowsky's stain	If animal survives after 6 months, the serum of the animal shows presence of antibodies

Contd...

Contd..

Leishmania donovani	Young hamsters (2–3 months old)	0.5–1 mL of material injected Intra-peritoneally	Aspirates or biopsy obtained from cutaneous ulcers, lymphnodes, spleen, liver or bone marrow	Splenic impression smears are prepared after 4–6 weeks, stained with Romanowsky's stain	Positive cases animal dies several days after the inoculation
Trypanosoma species	Mice and Rats, Guinea pigs	Intra-peritoneal or in tail vein	Blood, lymph node aspirate or spinal fluid	Blood sample is collected after 2 weeks, smears are prepared and stained with Romanowsky's stain	Stained smear shows presence of the parasite
Trichinella spiralis	Rats	Feed orally	Infected muscle tissue	Rats are examined for *Trichinella spiralis* larvae in the muscle of the infected rat	Mainly, *Trichinella spiralis* larvae can be demonstrated in the diaphragm

Table 15.11: Imaging methods in parasitic diagnosis

Disease	*Imaging method used*	*Comments*
Amoebic liver abscess	USG	Detects the location of abscess and its extra hepatic extension
Hydatid disease	X-ray, USG, CT scan and MRI	• ***X-rays:*** It is simple, inexpensive, yet useful technique to detect hepatomegaly and calcified cysts and cysts in lungs • ***USG:*** It is the imaging method of choice because of its low cost and high diagnostic accuracy. It detects both single and multiple cystic lesions, floating membrane (Water lily sign) and daughter cysts. It is also useful to monitor the response to treatment and for epidemiological studies • ***CT scan:*** It is superior to detect smaller cysts, calcified cysts, extrahepatic cysts and to differentiate from other cystic lesions. Also used as a prognostic marker • ***MRI:*** It has a higher contrast resolution, which makes cysts clearer. It can be used as an alternate to CT scan.
Trichinella spiralis	X-ray	Detects calcified muscle cysts
Neurocysticercosis	CT scan and MRI	***Detects:*** Number, location, size, of the cysts and extension and stage of the disease ***CT scan:*** For calcified cysts ***MRI:*** It is superior to CT scan, to detect extraparenchymal cysts, vesicular, necrotic lesions and non-cystic lesions
Paragonimus westermani infection	X-ray, MRI and CT scan	***X-ray:*** Pulmonary cysts ***MRI and CT Scan:*** locate extrapulmonary cysts (CNS)
Clonorchis and *Opisthorchis*	Cholangiography	Detects site of the lesion and obstruction of the biliary tract
Filariasis	USG	• Serpentine movement within the lymphatic vessels of scrotum (filarial dance sign) • Dilated and tortuous lymphatic vessels

Abbreviations: USG, ultrasonography; CT, computed tomography; MRI, magnetic resonance imaging; CNS, central nervous system