Examination of Feces

Waste products excreted from the digestive tract are composed of water (up to 75%), indigestible residue, undigested food, food which is digested but not absorbed, bile, epithelial cells, secretions from digestive tract, inorganic material, and bacteria. Normal amount of feces in an adult is 100-200 grams per day. **Examination of feces is helpful in the investigation of diseases of the gastrointestinal tract as follows:**

- **Detection of parasites:** Stool examination is done for detection of worms (adult worms, segments of tapeworms, larvae, ova), and protozoa (trophozoites or cysts).
- **Evaluation of chronic diarrhea:** Chronic diarrhea refers to passage of 3 or more loose or liquid stools per day lasting for more than 4 weeks. Acute diarrhea is defined as passage of 3 or more loose or liquid stools per day for less than 4 weeks. In diarrhea, stool examination is an important part of laboratory workup. Causes of chronic and acute diarrhea are listed in **Table 9.1 and Figure 9.1 respectively**. A 48- or 72-hour sample is examined for weight, **fat content, carbohydrate, osmolality, or chymotrypsin activity.**
- **Evaluation of dysentery:** Identification of causative organism is definitive in differentiating amebic from bacillary dysentery.

  Bacteriologic examination: Infection by bacteria such as *Salmonella*, *Shigella*, *Vibrio*, *Yersinia*, or *Clostridium difficile* can be identified by stool culture. Bacterial toxins (such as those released by *Clostridium botulinum* or *Clostridium difficile*) can also be identified.

  1-Diagnose the types of bacteria *Salmomella* or *Shigella* use media following:
  - A- Deoxycholate citrate agar.
  - B- Hekton enteric (HE).
  - C- Xylose- lysine – deoxcholate agar (XLD).

  2-Diagnose the species belonging to the *Campylobacter* spp using media
  - Mueller-Hinton agar with 5% sheep blood in (42C°).
  - 3-Diagnose the *Vibrio* spp.
  - Thiosulfate citrate bile salts (T.C.B.S).
- **Chemical examination:** Chemical tests can be applied on feces to detect occult blood (in ulcerated lesions of gastrointestinal tract, especially occult carcinoma of colon), excess fat excretion (malabsorption syndrome), and presence or absence of urobilinogen (obstructive jaundice).

Table 9.1: Classification and causes of chronic diarrhea

1. **Watery diarrhea**
   - *i.* ***Osmotic***
     - Carbohydrate malabsorption
     - Osmotic laxatives
   - *ii.* ***Secretory***
     - Bacterial toxins
     - Bile acid malabsorption
     - Laxative abuse
     - Hormonal disorders: VIPoma, carcinoid syndrome, gastrinoma, hyperthyroidism
2. **Inflammatory diarrhea**
   - i. Invasive bacterial and parasitic infections
   - ii. Inflammatory bowel disease
   - *iii. Pseudomembranous colitis*
   - iv. Infectious diseases
   - v. Neoplasia
3. **Fatty diarrhea** Malabsorption syndromes

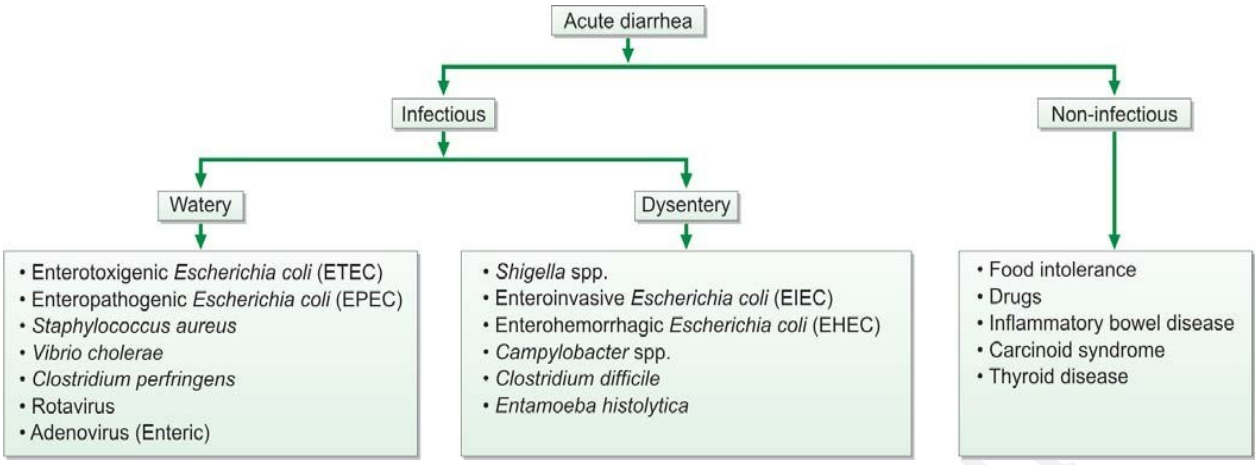


Fig. 9.1: Classification of causes of acute diarrhea

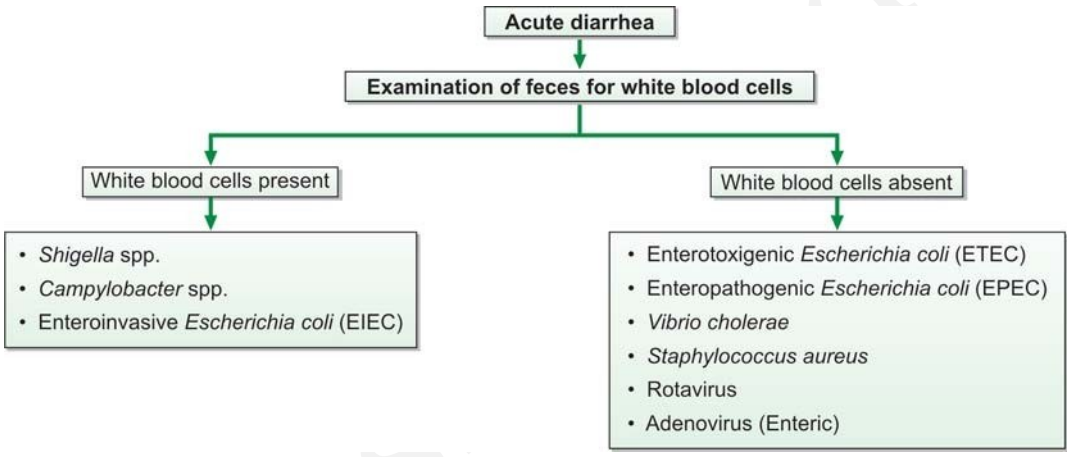


Fig. 9.2: Preliminary evaluation of acute diarrhea. Examination of feces for white blood cells is helpful in narrowing differential diagnosis in intestinal infections in acute diarrhea

• **Differentiating infection by invasive bacteria (like *Salmonella* or *Shigella*) from that due to toxin- producing bacteria (like *Escherichia coli* or *Vibrio cholerae*):** Increased numbers of polymorphonuclear neutrophils (identified by methylene blue stain) are seen in the former (Fig. 9.2).

• **Identification of Rotavirus:** Rotavirus is a common cause of diarrhea in infants and young children. It can be identified by examination of stool by electron microscopy, latex agglutination, immunofluo-rescence, or enzyme-linked immunosorbent assay (ELISA).

• **MICROSCOPIC EXAMINATION**

• **Microscopic examinations done** on fecal sample are shown in Figure9.3

• **Collection of Specimen for Parasites**

1. A random specimen of stool (at least 4 ml or 4 cm^3) is collected in a clean, dry, container with a tightly fitting lid (a tin box, plastic box, glass jar, or waxed cardboard box) and

transported immediately to the laboratory (this is because trophozoites of *Entameba histolytica* rapidly degenerate and alter in morphology).

2. About 20-40 grams of formed stool or 5-6 tablespoons of watery stool should be collected.
3. Stool should not be contaminated with urine, water, soil, or menstrual blood. Urine and water destroy trophozoites; soil will introduce extraneous organisms and also hinder proper examination.
4. Parasites are best detected in warm, freshly passed stools and therefore stools should be examined as early as possible after receipt in the laboratory (preferably within 1 hour of collection). If delay in examination is anticipated, sample may be refrigerated. A fixative containing **10% formalin** (for preservation of eggs, larvae, and cysts) or **polyvinyl alcohol** (for preservation of trophozoites and cysts, and for permanent staining) may be used if specimen is to be transported to a distant laboratory.
5. Patient should not be receiving oily laxatives, antidiarrheal medications, bismuth, antibiotics like tetracycline, or antacids for 7 days before stool examination.

Notes:

- One negative report for ova and parasites does not exclude the possibility of infection. Three separate samples, collected at 3-day intervals, have been recommended to detect all parasite infections.
- Trophozoites are most likely to be found in loose or watery stools or in stools containing blood and mucus, while cysts are likely to be found in formed stools.

Color/Appearance of Fecal Specimens

- Brown: Normal
- Black: Bleeding in upper gastrointestinal tract (proximal to cecum), Drugs (iron salts, bismuth salts, charcoal)
- Red: Bleeeding in large intestine, undigested tomatoes or beets
- Clay-colored (gray-white): Biliary obstruction
- Silvery: Carcinoma of ampulla of Vater
- Watery: Certain strains of *Escherichia coli*, Rotavirus enteritis, cryptosporidiosis
- Rice water: Cholera
- Unformed with blood and mucus: Amebiasis, inflammatory bowel disease
- Unformed with blood, mucus, and pus: Bacillary dysentery
- Unformed, frothy, foul smelling, which float on water: Steatorrhea.

Preparation of Slides

After receipt in the laboratory, saline and iodine wet mounts of the sample are prepared (Fig. 9.5). A drop of normal saline is placed near one end of a glass slide and a drop of Lugol iodine solution is placed near the other end. A small amount of feces (about the size of a match-head) is mixed with a drop each of saline and iodine using a wire loop, and a cover slip is placed over each preparation separately.

Saline wet mount is used for demonstration of eggs and larvae of helminths, and trophozoites and cysts of protozoa. It can also detect red cells and white cells. Iodine stains **glycogen and nuclei of the cysts**. **The iodine wet mount is useful for identification of protozoal cysts.**

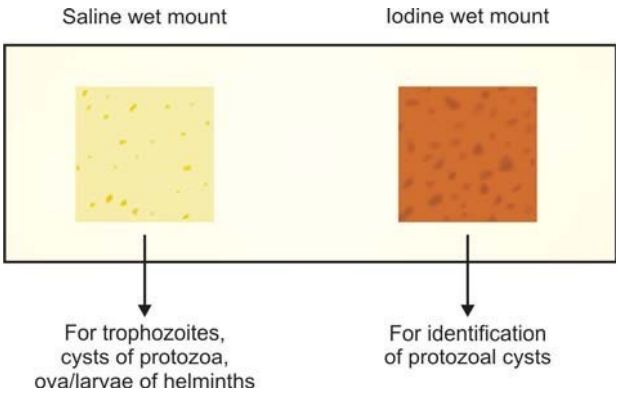


Fig. 9.5: Saline and iodine wet mounts of fecal sample

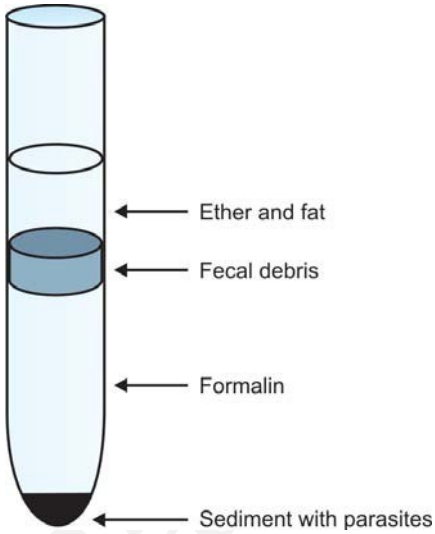


Fig. 9.6: Formol-ethyl acetate concentration technique

Concentration Procedure

Concentration of fecal specimen is useful if very small numbers of parasites are present. However, in concentrated specimens, amebic trophozoites can no longer be detected since they are destroyed.

•**Sedimentation techniques**: The most commonly used sedimentation method is formol-ethyl acetate concentration method since: (i) it can detect eggs and larvae of almost all helminths, and cysts of protozoa, (ii) it preserves their morphology well, (iii) it is rapid, and (iv) risk of infection to the laboratory worker is minimal because pathogens are killed by **formalin**.

Classification of Intestinal Parasites of Humans

Intestinal parasites of humans are classified into two main kingdoms: protozoa and metazoa (helminths) (Fig. 9.7).

PROTOZOA

Entamoeba histolytica

Infection by *E. histolytica* may be asymptomatic or may cause amebic dysentery or amebic liver abscess. Amebic trophozoites invade the large intestinal mucosa, multiply in submucosa, spread laterally and produce flask-shaped ulcers. Symptoms include low-grade fever, diarrhea with blood and mucus, weight loss, and cramping abdominal pain. In some cases, amebae penetrate the portal vessels and are transported to the liver where they form liver abscess. Amebic abscesses can also form in lungs or brain.

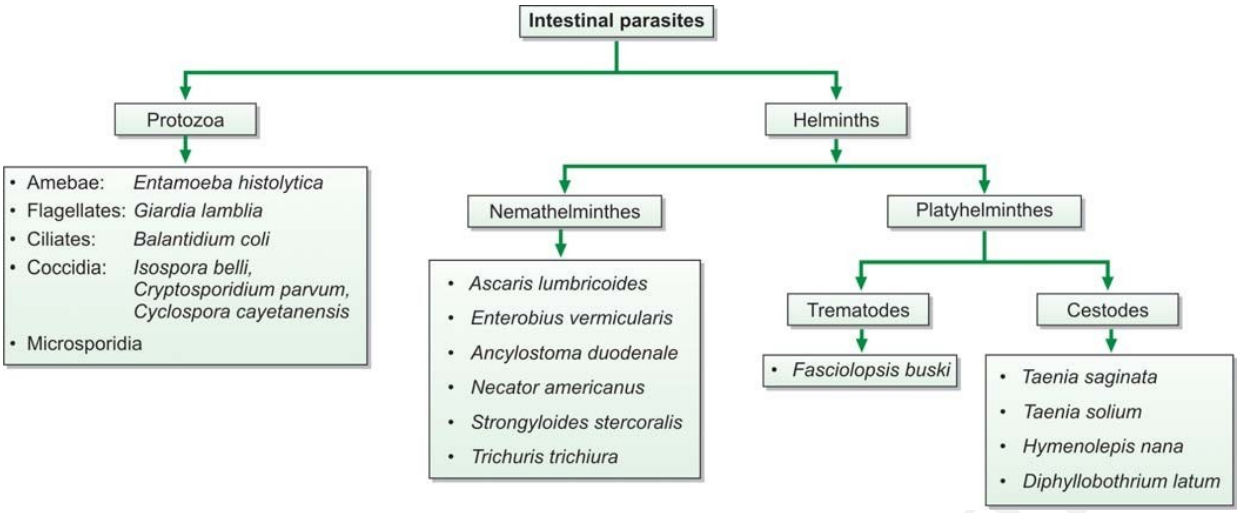


Fig. 9.7: Classification of intestinal parasites of humans

Laboratory Diagnosis

1. ***Identification of trophozoites and cysts on stool examination*:** Demonstration of trophozoites of *E. histolytica* in fecal specimens is required for diagnosis of amebic dysentery. For diagnosis, at least **three fresh stool samples** should be examined to increase sensitivity. Trophozoites vary from 15 to 40 µ in diameter. In saline wet mounts, trophozoites show motility in one direction via pseudopodia, which form rapidly. Cytoplasm shows outer transparent ectplasm and inner finely granular endoplasm. Diagnostic feature of *E. histolytica* trophozoites is the presence of ingested red cells. Nucleus is visible in the iodine preparation (Fig. 9.8). Fine granules of peripheral nuclear chromatin are evenly distributed along the nuclear membrane. Karyosome is small and centrally placed (Motility is lost in iodine mount).

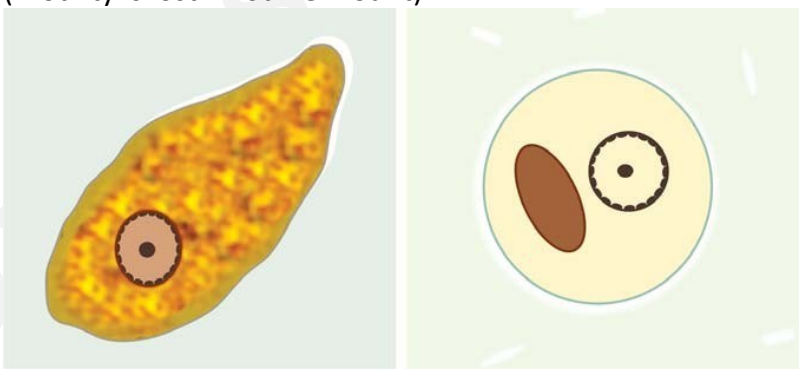


Fig. 9.8: Trophozoite (left) and uninucleate cyst (right) of E. histolytica. Nuclear chromatin is finely beaded and evenly coats regular nuclear membrane. Karyosome is central. Chromatoid body is long with rounded ends

Infection by *E. histolytica* may be **asymptomatic** or may cause amebic dysentery or amebic liver abscess. Amebic trophozoites invade the large intestinal mucosa, multiply in submucosa, spread laterally and produce flask-shaped ulcers. **Symptoms** include low-grade fever, diarrhea with blood and mucus, weight loss, and cramping abdominal pain. The cecum, ascending colon, and rectosigmoid are commonly

affected. Excessive granulation tissue may form in the intestine at the site of lesion (ameboma) to produce constriction, which may be mistaken clinically for a neoplasm

Table 9.2: Differences between amebic and bacillary dysentery

Parameter	*Amebic dysentery*	*Bacillary dysentery*
1. Cause	*Entamoeba histolytica*	*Shigella* (most common)
2. Onset	Gradual	Acute
3. Fever/vomiting	Not significant	Significant
4. Appearance of fecal sample	Unformed with blood and mucus	Unformed with blood, mucus, and pus
5. Microscopic examination of stool		
• Red cells	Clumps	Discrete
• Pus cells	Nil or few	Numerous
• Macrophages	Not seen	Many, some with ingested red cells
• Charcot-Leyden crystals	May be present	Not seen
• Trophozoites of *E. histolytica*	Present	Not seen
• Bacteria	Many, motile	Few, nonmotile
6. Antigen test for *E. histolytica*	Positive	Negative
7. Stool culture	Negative	Positive for *Shigella*

2. ***Other findings on stool examination*:** Plenty of red cells and very few white cells are helpful in differentiating amebic from bacillary dysentery. Charcot-Leyden crystals may be seen. Differences between amebic and bacillary dysentery are listed in Table 9.2.
3. ***Detection of antigen of E. histolytica in stools*:** Direct detection of antigen specific to *E. histolytica* is possible by commercially available tests based on **enzyme immunoassay**. These tests are specific and sensitive (90%) and can differentiate *E. histolytica* from *E. dispar*.
4. ***Detection of DNA specific to E. histolytica*** is possible by polymerase chain reaction-based assays.
5. ***Serologic tests*:** Serologic tests, which detect anti- bodies to *E. histolytica*, are performed to support the diagnosis of **invasive amebiasis**. Various tests are available like latex agglutination test, indirect hemagglutination test, enzyme immunoassay, and counter immunoelectrophoresis. Enzyme immuno- assay is the method of choice since it is most sensitive and specific. Serologic tests, however, remain positive for many years after infection and thus cannot distinguish between recent and past infections.
6. ***Endoscopic biopsy of ulcer in the intestine:*** This can demonstrate trophozoites of *E. histolytica* in 50% of cases. Staining with **periodic acid Schiff stain** facilitates identification of parasites.

Giardia intestinalis (lamblia)

G. intestinalis (lamblia), a pathogenic intestinal protozoan, has a worldwide distribution. It is transmitted by fecal- oral route, and is usually water-borne. *Giardia* is resistant to chlorine levels in tap water and is commonly found in cold mountain streams. It can cause asymptomatic infection, mild diarrhea, or a severe disease with diarrhea, mal-absorption, weight loss and steatorrhea.

There are two stages in the life cycle: cyst and trophozoite. After ingestion of cysts, excystation occurs due to action of gastric acid, and trophozoites are released which migrate to the duodenum and proximal jejunum where they attach to the mucosa and replicate.

Laboratory Diagnosis

1. ***Demonstration of trophozoites or cysts:*** *G. lamblia* trophozoites are found in fresh liquid stools, particularly in flakes of mucus. Trophozoites of *G. lamblia* are pear-shaped, 12-15 µ in diameter, have 4 pairs of flagellae, 2 large and oval nuclei, 2 axonemes (axial filaments of flagella), and 1 or 2 curved median bodies. Motility is likened to that of "falling leaf". Cysts of *G. lamblia* are 8-12 µ in diameter, oval, and contain 4 nuclei, axonemes, median bodies, and remains of flagella (Fig. 9.11).

1. ***Detection of antigen of G. lamblia in stool sample*:** Antigen of *G. lamblia* can be demonstrated by enzyme immunoassay technique with high sensitivity (90- 99%) and specificity (95-100%).
2. ***Direct fluorescent antibody assay*:** This test is available commercially in a kit form and is highly sensitive and specific. Cysts are labeled with immunofluorescent antibodies and are detected under fluorescence microscope.

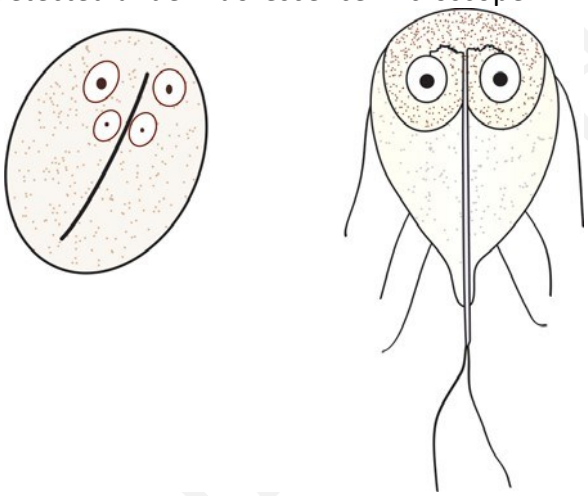


Fig. 9.11: Cyst and trophozoite of Giardia lamblia

HELMINTHS

***Ascaris lumbricoides* (Roundworm)**

This is the most common helminthic infection of humans. Children are more commonly affected than adults. Mode of transmission is fecal-oral route (ingestion of infective eggs). Adult worms live in the small intestine (duodenum and jejunum) of the host. Eggs are laid by adult female worms (about 200,000 per day), which are excreted in feces. Eggs can remain viable in soil for many years. Contamination can occur when untreated human feces are used as a fertilizer or by soiling of hands of playing children. Adult worms can live in the intestine for 1-2 years.

Life cycle: Infection is acquired by ingestion of infective eggs via contaminated food or hands. Eggs hatch to release larvae in intestine. Larvae penetrate the mucosa and enter the bloodstream. Larvae circulate, reach lungs and penetrate alveolar walls to enter the respiratory tree. Larvae migrate up the trachea to the epiglottis from where they are swallowed. Maturation of larvae to adult worms occurs in the small intestine. Female worms lay down eggs, which are excreted in feces. Eggs become embryonated (infective) in 4-6 weeks in favorable environment.

Clinical Features

- Asymptomatic if infection is light.
- Loeffler's syndrome: Migration of larvae through the lungs can induce cough, wheezing, eosinophilia, and bilateral, irregular pulmonary densities.
- Local effects: These include abdominal pain, diarrhea, intestinal obstruction due to a large mass of worms, and intestinal perforation. Sometimes worms can invade pancreatic duct or common bile duct and cause obstruction; this can lead to pancreatitis or obstructive jaundice respectively. From the bile duct, adult worms can reach liver and cause abscess. Adult worms can migrate to the appendix to cause appendicitis.
- Malabsorption

Laboratory Diagnosis

1. ***Demonstration of eggs of A. lumbricoides*:** Diagnosis of *A. lumbricoides* infection is made by demonstration of eggs on stool examination. Eggs can be demons- trated in direct wet mount of feces in moderate to heavy infections. The recommended procedure is formol-ethyl acetate sedimentation technique for concentration of eggs.

Fertilized eggs: These are oval, yellow-brown, and about 70 µ × 50 µ in size. They have outer and inner shells. Outer shell is uneven, brown (due to staining by bile), and rough (mamillated), while the inner shell is thick, smooth, and colorless. The egg contains a single central granular mass (fertilized ovum).

Unfertilized eggs: Single female worms discharge these eggs. They are slightly larger and more elongated than the fertilized eggs (90 µ in length). Outer shell is dark brown and more irregular, while the inner shell is thinner. This egg is filled with a mass of large refractile granules (Fig. 9.12).

2. ***Identification of adult worms:*** Occasionally adult worms are passed spontaneously in the feces and brought to the laboratory for identification. Adult ascaris worms are cylindrical or round, pinkish, and measure about 15 cm (male) or 30 cm (female) in length. Diameter is about 0.5 cm and tail is curved (male) or straight (female). There are three lips at the anterior end (mouth).

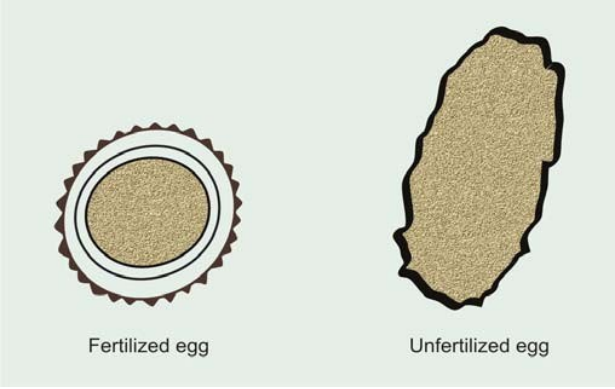


Fig. 9.12: Fertilized and unfertilized eggs of Ascaris lumbricoide

CHEMICAL EXAMINATION

Chemical examination of feces is usually carried out for the following tests (Fig. 9.17):

- Occult blood
- Excess fat excretion (malabsorption)
- Urobilinogen
- Reducing sugars
- Fecal osmotic gap
- Fecal pH

Test for Occult Blood in Stools

Presence of blood in feces which is not apparent on gross inspection and which can be detected only by chemical tests is called as **occult blood**. Causes of occult blood in stools are:

i. Intestinal diseases: hookworms, amebiasis, typhoid fever, ulcerative colitis, intussusception, adenoma, cancer of colon or rectum.
ii. Gastric and esophageal diseases: peptic ulcer, gastritis, esophageal varices, hiatus hernia.
iii. Systemic disorders: bleeding diathesis, uremia.
iv. Long distance runners.

Occult blood test is recommended as a screening procedure for detection of asymptomatic colorectal cancer. Yearly examinations should be carried out after the age of 50 years. If the test is positive, endoscopy and barium enema are indicated

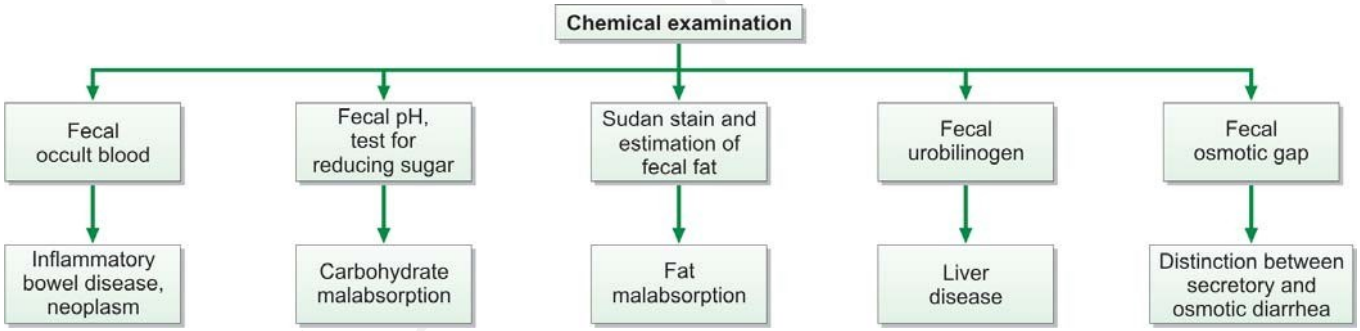


Fig. 9.17: Chemical examinations done on fecal sample

Table 9.3: Summary of laboratory tests for diagnosis of intestinal parasites

Test	*Use*
1. Direct saline mount	Trophozoites, cysts, ova, and larvae
2. Direct iodine mount	Protozoal cysts
3. Faecal concentration	Recovery of protozoal cysts, helminth ova and larvae
4. Cellophane tape technique	Ova of *Enterobius vermicularis*
5. Trichrome stain of fecal smear	Protozoal cysts and trophozoites
6. AFB stain of fecal smear	Oocysts of *Cryptosporidium, Cyclospora,* and *Isospora*
7. Detection of antigen in random stool sample	*E. histolytica, G. lamblia, Cryptosporidium*
8. Molecular methods based on polymerase chain reaction on stool sample	*E. histolytica, G. lamblia, Cryptosporidium, Microsporidia, Cyclospora*
9. Serologic methods	*E. histolytica, S. stercoralis, Cysticercus*

Tests Based on Peroxidase-like Activity of Hemoglobin to Blood detection

Principle: Hemoglobin has peroxidase-like activity and releases oxygen from hydrogen peroxide. Oxygen molecule then oxidizes the chemical reagent (benzidine, orthotolidine, aminophenazone, or guaiac) to produce a colored reaction product.

Benzidine and orthotolidine are carcinogenic and are no longer used. Benzidine test is also highly sensitive and false-positive reactions are common. Since bleeding from the lesion may be intermittent, repeated testing may be required.

Test for Malabsorption of Fat

Dietary fat is absorbed in the small intestine with the help of bile salts and pancreatic lipase. Fecal fat mainly consists of neutral fats (unsplit fats), fatty acids, and soaps (fatty acid salts). Normally very little fat is excreted in feces (<7 grams/day in adults). Excess excretion of fecal fat indicates malabsorption and is known as **steatorrhea**. It manifests as bulky, frothy, and foul-smelling stools, which float on the surface of water.

Causes of Malabsorption of Fat

i. Deficiency of pancreatic lipase (insufficient lipolysis): chronic pancreatitis, cystic fibrosis.
ii. Deficiency of bile salts (insufficient emulsification of fat): biliary obstruction, severe liver disease, bile salt deconjugation due to bacterial overgrowth in the small intestine.
iii. Diseases of small intestine: tropical sprue, celiac disease, Whipple's disease.

Tests for fecal fat are qualitative (i.e. direct microscopic examination after fat staining), and quantitative (i.e. estimation of fat by gravimetric or titrimetric analysis).

Microscopic stool examination after staining for fat: A random specimen of stool is collected after putting the patient on a diet of >80 gm fat per day. Stool sample is stained with a fat stain (oil red O, Sudan III, or Sudan IV) and observed under the microscope for fat globules (Fig. 9.18). Presence of 60 fat droplets/HPF indicates steatorrhea.

Quantitative estimation of fecal fat: The definitive test for diagnosis of fat malabsorption is quantitation of fecal fat. Patient should be on a diet of 70-100 gm of fat per day for 6 days before the test. Feces are collected over 72 hours and stored in a refrigerator during the collection period. Specimen should not be contaminated with urine. Fat quantitation can be done by gravimetric or titrimetric method.

In gravimetric method, an accurately weighed sample of feces is emulsified, acidified, and fat is extracted in a solvent; after evaporation of solvent, fat is weighed as a pure compound.

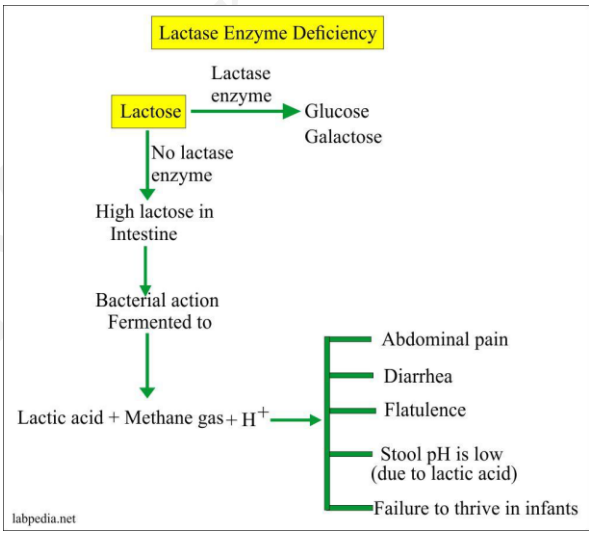
Titrimetric analysis is the most widely used method. An accurately weighed stool sample is treated with **alcoholic potassium hydroxide to convert fat into soaps**. **Soaps** are then converted to **fatty acids** by the addition of **hydrochloric acid**. Fatty acids are extracted in a solvent and the solvent is evaporated. The solution of fat made in neutral alcohol is then titrated against sodium hydroxide. Fatty acids comprise about 80% of fecal fat. Values >7 grams/day are usually abnormal. Values >14 grams/day are specific for diseases causing fat malabsorption.

Test for Urobilinogen in Feces

Fecal urobilinogen is determined by Ehrlich's aldehyde test (see lecture 1 "Examination of Urine"). Specimen should be fresh and kept protected from light. Normal amount of urobilinogen excreted in feces is 50-300 mg per day. Increased fecal excretion of urobilinogen is seen in **hemolytic anemia**. Urobilinogen is deceased in biliary tract obstruction, severe liver disease, oral antibiotic therapy (disturbance of intestinal bacterial flora), and aplastic anemia (low hemoglobin turnover). Stools become pale or clay-colored if urobilinogen is reduced or absent.

Test for Reducing Sugars

Deficiency of intestinal enzyme lactase is a common cause of malabsorption. Lactase converts lactose (in milk) to glucose and galactose. If **lactase is deficient**, lactose is converted to **lactic acid with production of gas**. In infants this leads to diarrhea, vomiting, and failure to thrive. Benedict's test or ClinitestTM tablet test for reducing sugars is used to test freshly collected stool sample for lactose. In addition, oral lactose tolerance test is abnormal (after oral lactose, blood glucose fails to rise above 20 mg/dl of basal value) in lactase deficiency. **Rise in blood glucose indicates that lactose has been hydrolysed and absorbed by the mucosa**. Lactose tolerance test is now replaced by lactose breath hydrogen testing. In lactase deficiency, accumulated lactose in the colon is rapidly fermented to organic acids and gases **like hydrogen. Hydrogen is absorbed and then excreted through the lungs into the breath. Amount of hydrogen is then measured in breath; breath hydrogen more than 20 ppm above baseline within 4 hours indicates positive test.**



Fecal Osmotic Gap

Fecal osmotic gap is calculated from concentration of electrolytes in stool water by formula 290-2([Na+] + [K+]). (290 is the assumed plasma osmolality). In osmotic diarrheas, osmotic gap is >150 mOsm/kg, while in **secretory diarrhea**, it is typically below 50 mOsm/kg. Evaluation of **chronic diarrhea.**

Fecal pH

Stool pH below 5.6 is characteristic of carbohydrate malabsorption.