Biological Times

Volume No. 4 | Issue No. 1 | Pages 3-5

Routine Parasitological Practice: Stool Examination

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ABSTRACT

A stool sample is used for the detection and identification of morphological stages of the protozoa and helminths (trophozoite, cyst, oocyst, egg, larvae, whole worm, or a segment of worm). There are several stool methods used for the diagnosis of parasitic diseases which are divided first into macroscopic and microscopic stool methods. The microscopic stool methods are also divided into qualitative methods (Direct stool smear, concentrated flotation method, concentrated sedimentation method, Telmman method, Barman method, and copro-culture), which are used to know the type of parasite, and the quantitative stool method (MacMaster and stoll methods), are used to determine the No. of eggs/larvae in one gram of stool to determine the severity of infection. This article aimed to overview the routine stool methods used in the Parasitology Lab.

Keywords: Parasitology, Parasitic Infection, Diagnosis, Stool Sample, Stool methods

To cite this article: Ismael SS. Routine Parasitological Practice: Stool Examination. Biological Times. 2025 January 4(1): 3-5.

Stool Samples

A stool sample is used for the detection of morphological stages of the protozoa (trophozoite, oocyst, or cyst) helminths (larvae, eggs, whole worm or segment of worm) (1).

Collection of stool samples

1. Fresh stools are collected from the ground by taking top layers only, then the gloves are turned inside out and then serve as a reservoir, ideally about 10 grams of stool should be collected (2).
2.Plastic containers may be used with lid and screw cap.
3.The stool cup should be with the following information:

- Date and time of collection.
- Patient name
- Sex
- Age
- Readable number

4. If the stool has to end or be taken to a laboratory, it should be done immediately after the sample has been taken
5. If the immediate examination is not possible, the stool samples should be stored in the refrigerator for a few hours (not more than 24 hours)
6. Any worm that appears in the feces should be preserved in 5-10% formalin or 70% alcohol solution.
7. For receiving the specimens, it is stored by adding a few drops of 3% formalin to save larvae and eggs.
8. For samples sent through the post, the addition of twice the stool volume of 10% formalin to the stool will minimize the development and hatching of eggs
9. 10% formalsaline (5%formaline + 5% saline) is prepared for protozoan preservation.
10. Searching for live protozoa trophozoites needs a direct stool examination.
11. If the patient has been treated with anti-diarrhea preparation containing bismuth or cooline, mineral or oral contrast material (barium), or antibiotics, therefore repeating stool examination 5-10 days after treatment should be done (2)

Stool Methods

There are two methods used for the examination of the stool sample: qualitative and quantitative methods (3, 4):

A. Qualitative Stool Methods

The qualitative methods are used to know the type of parasite (5, 6).

1.Macroscopic Stool Examination

- Before dilution, the entire sample should be examined, including its consistency (formed, semi-formed, or liquid) and composition (mucous, blood, pieces of tissue, undigested food, and presence of adult worm or segment of worm) (5).
- Before dilution, the entire sample should be examined: its consistency (solid, semi solid, or liquid) and composition (fecal material, mucous, blood, pieces of tissues, and undigested food); at the same time examined macroscopically for the presence of adult nematodes or segments of tapeworms (2, 5).
- The solid stool sample is used for the detection of stages of protozoa and eggs/larvae of helminths may be present.
- The semi-solid stool sample is used for the detection of all stages that may be present.
- The liquid stool samples are used for searching for the trophozoite stage of protozoa (5, 6).

II. Microscopic Stool Examination:

1.Direct Stool Smear

A pinhead amount of stool is spread on a slide mixed with a drop of iodine, tape water, or normal saline as seen in Fig. 1. (7, 8). The large particles can be removed aside, a cover slide is put on the slide and examined systematically under low power. With Iodine solution eggshell (Schistosoma) or cysts of protozoa (e.g., Giardia) are stained brown. This method is a fast and easy one. Due to the limited amount of stool examined, a negative result is not always reliable and may only detect heavy infections. Therefore, a concentration method will have to be used (9,10,).

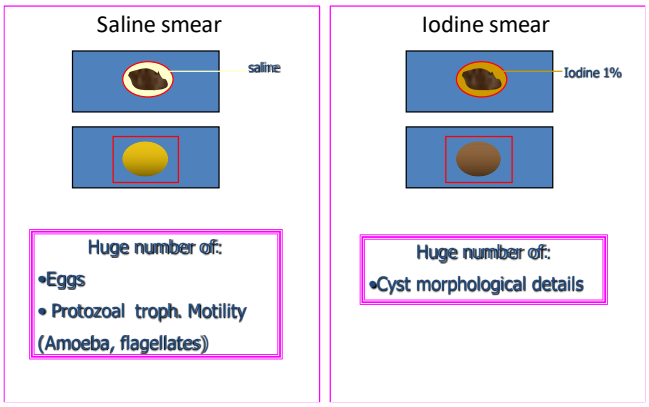

Fig. 1: Direct Stool Smear (11)

2. Concentration Stool Methods:

The concentration stool methods have advantages and disadvantages (6,7, 12)

Advantages

- It is used for detection of the light parasitic infection
- It reduces background stool debris
- It increases the number of parasites
- It preserves the morphology of parasites

Disadvantages

Which destroys the trophozoite stage of protozoa (11)

Concentration methods include

I. Flotation

In tape water, worms' eggs will sink because their specific gravity is a little higher than 1. When the stool sample is suspended in a solution with a specific gravity more than the specific gravity of eggs and cysts, the eggs and cysts will float up to the surface of the test tube (9, 11).

Common Flotation or Saturated solutions are: (11 12)

1. Saturated sodium chloride (salt): Mixed 400g of salt with 1L of hot distal water.
2. Sugar solution: 1300 g/L of D.W
3. Saturated sodium Nitrate NaNO3: (400g/ L of D.W)
4. Saturated Zinc Sulphate ZnSO4: (700g/ L of D.W)
5. Magnesium Sulphate MgSO4: (500g/L of D.W)

1.Ordinary floatation

Procedure

1.Mix about 3-5 grams of fresh stool and mix with a small amount of concentration solution.
2.Dilute then with concentration solution mix vigorously to obtain a homogenous mixture.
3.Solution will be strained through a double layer of gauze or through a fine sieve and large particle will be pressed out.
4. Pure the filtered liquid into the test tubes and cover them with a slide.
5. After 30 minutes, the cover slide is gently removed, put on a clean slide, and examined under a microscope.
6. This method is suitable for the majority of nematode eggs, cestodes, and protozoa.
7. The eggs and cysts of both class Nematoda and Cestoda will float in a saturated solution with a specific gravity between (1.10-1.2) using magnesium sulfate and sodium chloride saturated solution. The eggs and cysts of the class Trematoda are heavier and need a solution with a high specific gravity of (1.30-1.35) by using zinc sulfate or zinc chloride saturated solution.
8. A Cylinder of 25mm in diameter may be used by adding 2 grams of stool into 20 ml of flotation solution, then after 30-45 minutes examined under the microscope (13).

2. Centrifuge flotation

Procedure

1-5 grams of stool sample is mixed with 30-50 ml of water and filtered through a sieve to remove coarse stool materials, then leave the mixture for 10-15 minutes on the bench until sedimentation occurs and the supernatant is clear. Discard the supernatant and the sediment is centrifuged for one or two minutes at 1500 rpm. The surface may be touched with a cover slide. Eggs will float to the surface and stick to the cover slide (10,13).

II. Sedimentation Method

The sedimentation method is commonly used for searching for eggs and cysts with high specific gravity.

Procedure

1- Mix 3-5 grams of stool with 30-60 ml of tap water or normal saline in a cup or beaker and filter and pour into a centrifuge tube. The filtered liquid is centrifuged at 1500 rpm for five minutes.
2. Discard the liquid without disturbing the sediment.
3. Transfer a small amount of sediment by pipette and put it in the center of the slide and if the drop is too thick, dilute it with a few drops of water.
4. Placed a cover slide to the drop and examined under the microscope (13, 14).

III- Baerman Method

It is used for searching for the larval stages of nematodes from the stool sample, especially for lungworms. Because their eggs are similar in shape, therefore diagnosis depends on larval shapes and measurement (13, 14)

Materials used in the Baerman method are:

- The Baermann apparatus consists of a eglass or plastic funnel clamped to a stand; to the button of funnel rubber tube is fitted (10cm long) ending with a dropper.
- The rubber tube can be pinched, for instance by a clip.
- The funnel is covered with a fine sieve (or wire gauze). The sieve is covered with a double layer of gauze (,15, 16, 17).

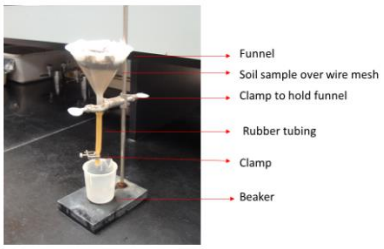

Fig. 2: Baerman Apparatus (17)

Procedure (14)

1- Apply to the gauze 5-15 g of fresh stool.
2- Gently fill the funnel with tap water or normal saline (38C-40C).
3- leave it overnight. The larvae will come out of the soaked feces and fall through the meshes into the funnel neck.
4- Clip is released (first 3-4 drops) are collected on a microscopic slide and examined under low power without using a cover slide.
5- A few drops of 3.3% HCL (1ml HCL/30 ml of water) are added to the solution. Parasitic larvae will stay alive, and non-parasitic larvae will die immediately (16, 17)

IV. Fecal Culture or Copro-Culture Method

The identification of nematodes Genera and Species in living host requires the development of infective larvae (L3) from nematode eggs, therefore two techniques are used for the culture of the infective larvae (18):

A. Technique One

1- Put about 30 grams of fresh stool sample in a jar and moist with tap water.
2- Cove the jar with cellphone paper and incubate at room temperature away from direct sunlight for seven days, then take a few drops from the jar and put on the slide and cover with cover slide pass it over the flame of a Bunsen burner once or twice to kill the larvae then examined under the microscope for searching of nematode larvae (17, 18).

B. Technique Two

Spread feces on the middle third of a moist filter paper placed in a petri dish with a top, covered then stored at 25 - 27°C. Humidity is checked each day. After storage for 7 to 10 days, the dish is flooded with water and the larvae are harvested as before and examined (by Bearman apparatus) (16, 18).

V. Telmman Method

Materials are used are (18):

1- Acetic acid and Ether
2- Centrifuge and a centrifuge tube
3- Fine sieve mesh
4- Stool

Procedure

1- One gram of stool is added to 5ml of 5% acetic acid solution, mix the suspension.
2- Let the suspension settle for one minute and filter through the fine sieve.
3- Pour the fluid into centrifuge tube.
4- Unequal amount of ether is added
5-The mixture is shaken vigorously and centrifuged for one minute at 1500 rpm.
6- The sediment containing the eggs and oocysts above the acid layer and ether layer.
7- Between the acid layer and ether layer there will be a layer of dirt.
8- The supernatant (ether, acid, and dirt) is decanted all at once as shown in Fig. 3.
9- The sediment is diluted with a small amount of water and mixed.
10- A few drops of this suspension were applied to a slide and examined under low power.
11- The sediment contains different types of eggs, oocysts of coccidia…. etc. (18).

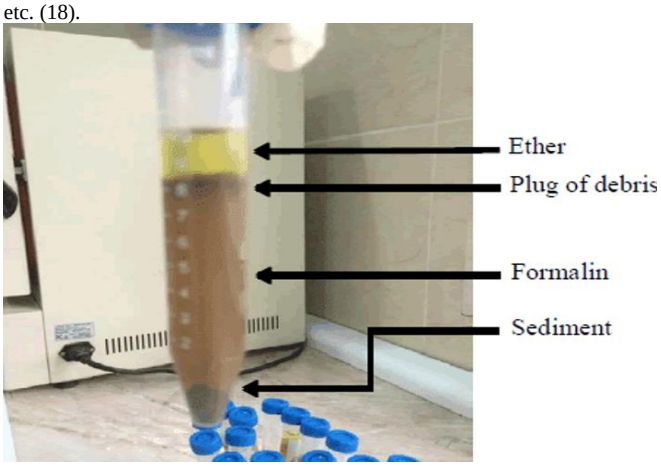

Fig. 3: Telmman Method (19)

B. Quantitative Stool Methods

It is used to count the number of eggs or larvae per gram of stool is performed (EPG, LPG).

The number of eggs or larvae per gram of stool may be counted for further examination as well as for determination of the severity and size of the infection. The method used for quantitative is (20):

1.MacMaster Method

MacMaster counting slide consisting of two glass or plastic slides joined together; between the marked areas of the upper slide and button slide, two or three chambers of 0.15 ml volume each are formed (10*10*1.5mm) (20). As shown in Fig. 4.

Fig. 4: MacMaster slide (21).

Procedure

1- Suspend 2 grams of stool in 60 ml of saturated sodium chloride solution.
2- The suspension is strained through a fine sieve.
3- Pasteur pipettes fill one compartment of the counting cell at once.
4- Let all bubbles escape.
5- Repeat the same operation to fill the second counting chamber
6- After a few minutes, the eggs float up to the surface and stick to the cover slide.
7- Count the number of eggs or larvae under low power (20, 22)

Calculation

2 grams of stool are dissolved in 60ml
Thus: 1g in 60/2 or 30 ml
1gram of stool represents in other words the content of 0.15ml
Number of eggs in one gram of the stool = X
X*60/2*1/0.15= EPG
EPG= X*200
X= Number of eggs in one counting cell
When 2 or 4 counting cells are filled and counted:
X= Total number of eggs/Total No. of counting cells (20, 22).

2. Stoll Method

Procedure

A 100ml graduated measuring cylinder is charged with 5g of accurately weighted fresh feces.
2. Add a 0.1 N (4%) solution of sodium hydroxide NaOH in water up to 75ml (the hydroxide will dissolve the slime without affecting the eggs).
3. By shaking vigorously the liquid with glass beads, a suspension as homogenous as possible is obtained.
4. Apply 0.15 ml suspension to a microscopic slide and cover with a large size cover slide.
5. Examination of the preparation completely under low magnification. It is advisable to check four preparations.
6. The average number of eggs or larvae found multiplied by 100= number of eggs or larvae per gram of feces (EPG, LPG) (20).

Interpretation of the egg count: (23)

It is impossible to calculate from the EPG the precise size of the worm population in the host because of many factors influencing egg production. These factors are:

- The male is not involved in the calculation
- Worms produce various numbers of eggs according to the species
- Immune responses
- Different amounts of egg production according to seasons, temperature, animal hormonal status
- Animal nutritional conditions and consistency of feces
- Using of anthelmintics
- EPG of cestode infections has only a diagnostic value

III. Other Stool Tests

Adhesive tape method or Cellophane Method

Adhesive tape method or Cellophane Method:
It is used for the detection of eggs of Enterobius vermicularis (pinworm) (24).
Because the females of pinworm protrude from the anus and deposit their eggs on the skin around the anus. The pinworm eggs usually are not seen in the routine fecal examination. Therefore, this tape around the anus can be placed on the slide with a small drop of water and examined under the microscope (The examination should be made in the morning, before the patient has washed or defecated) (24).

Conclusion

Stool samples represent gastrointestinal parasitic infection**.** This article highlighted the main methods used for the diagnosis of intestinal parasitic diseases.

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