introduction

In the field of diagnostic Medical Parasitology, proper specimen collection is critical since the final laboratory results are based on parasite recovery and identification will depend on the initial quality of the samples taken. Unless the appropriate specimens is properly collected, preserved and processed, these infections may not be detected; therefore, as a part of any overall continuous programm for the laboratory, the generation of test results must begin with stringent criteria for specimens acceptance or rejection.

Laboratory procedures detects organisms within clinical specimens using morphological criteria, rather culture or biochemical tests and/or physical growth characterstics. Many clinical specimens, such as those from intestinal tract, contain multiple artifacts that complicate differentiation of parasites from surrounding derbis.
The main ways in which laboratory diagnosis of parasitic infections include:

1) ***Microscopy***:- the majority of intestinal, blood, urinary and skin parasites are usually detected by microscopically in stained or unstained; either directly or following concentations.
2) ***Culture***:- only minority of parasitic infections are diagnosed routinely by culture techniques. Relatively few of protozoa and none of the helminth parasites, can be cultured in a manner that is useful for laboratory identification. Other than strictly for research purpose , the only culture methods in general use are for the isolation of such as *E.histolytica, T.vaginalis, T.cruzi* and *Leishmania* species. are identified by this method.
3) ***Immunodiagnosis***:-it is based on the detection of :
A. Antibody in a person's serum, produced in response to a particular parasitic infection. The antibody may persist for a long period of time in the serum after an infection has ended and therefore antibody tests are unable to distinguish between past or present infection. When used to assist in diagnosing parasitic disease , antibody tests need to be interpreted with care.
B. Antigen, which is excreted by parasites and can be found in the serum, urine, CSF, feces or other specimens. Antigen tests provide evidence of present infection and are therefore greater value than antibody tests in the clinical diagnosis of parasitic infections.
Immunodiagnostic techniques are required when:

a) Parasites live in the tissue of internal organ and can not therefore easily remove for examination.

b) Parasites can be found in specimens only in certain stages of infection, e.g., in the acute stage not in the chronic stage.
c) Parasites are present intermittently or in too few numbers to be easily detected in the specimens.
d) The techniques used to detect parasite are complex or time consuming.
Those parasitic disease for which immunodiagnosis is of particular value include:

- South American trypanosomiasis , Chronic stage
- African trypanosomiasis, when parasitaemia is low
- Leishmaniasis
- Filariasis
- Amoebic liver abscess
- Toxoplasmosis
- Hydatid disease
- Trichinolosis
- Toxocarisis
- Schistosomiasis

The principal type of immunodiagnostic tests are intradermal and serological.

Stool Examination

The most frequently performed parasitological procedure is the stool examination. The detection and identification of parasites; such as adult worms, larvae, eggs, trophozoites and cysts depends on its proper collection.

Procedure

1. Provide the patients with specimen containers with tight-fitting lids.

- Three specimens are usually required at a three alternate days to detect all intestinal parasitic infections. The stool should be collected before radiological examination is carried out using barium. Stool specimens containing barium are unacceptable for examination, and intestinal protozoa may be undetectable for 5-10 days after barium is administered to the patient.

- Some substances and medications also interfer with the detection of intestinal protozoa, including mineral oil, bismuth, antibiotics, antimalarial agents and nonabsorbable antidiarrhoeal preparations.
After adminstration of any of these compounds to the patient parasite may not be recovered for a week or more.

2. Collect sufficient quantity of stool. It should contain at least 4ml Stool.
3. Examine the stool as soon as possible.

As it is not possible to predict what organisms will be present in the specimen, however, the most conservative time frames should be used for parasite recovery. The examination of liquid specimens should occur within 30 minutes of passages. Soft specimens may have a mixture of protozoan trophozoties and cysts and should be examined within 1 hour of passage. Formed specimens can be examined at any time within 24hrs.

4. If specimens cannot be examined in the above time frame, put them in available preservatives.

I. Macroscopic Examination

Stool specimen is examined with the naked eye for :

1. Presence of worms:- may have adult helminthes or segments
Example: *Ascaris, Taenia species, E.vermicularis and gravid Taenia* species.
2. Consistency (degree of moisture)- It varies in diet but certain clinical conditions associated with parasite presence may be suggested by particular consistencies.
- It will be described as hard, formed, semi-formed and diarrhoeic(watery).
3. Colour:- any abnormal colour E.g., pale yellowish passed in steatorrhoeac conditions such as giardiasis,dark or black-stools occur when iron or bismuth is taken or when there is intestinal hemorrage
4. Pathologic odour Offensive, non-offensive
5. Abnormal features seen (composition): mucus, blood or fat globules.

II. Microscopic Examination

The detection and identification of species of parasites require microscopic examination of specimens

1. Direct Microscopic

Routine microscopic examination of stool specimen with physiological saline and Dobell's iodine solution helps to detect and identify the stages of some parasitic organisms.

Procedure

1. Place a drop of physiological saline (0.85%w/v) in the center of the left half of the slide and place a drop of Dodell's Iodine solution in the center of the right half of the slide.
2. With an applicator stick, pick up a small portion of the feces (Approximately 2mg which is about the size of a match head) and put on the drop of saline. Add a similar portion of stool sample to the drop of iodine.
3. Mix the feces with the drops to form homogeneous suspensions.

4. Cover each drop with a cover slip by holding the cover slip at an angle of 30, touching the edge of the drop, and gently lowering the cover slip onto the slide so that air bubbles are not produced.
5. Examine the saline preparations using the 10X objective for motile forms, cyst and oocyst of intestinal protozoa and for any ova or larva of helminths.
6. Examine the iodine solution preparation using 40X objective to identify the cyst stages of protozoa. The iodine will stain the nuclei and the glycogen mass of the cyst.

1.2 Modified Ziehl-Neelsen technique (Acid-Fast Stain)

Modified Ziehl-Neelsen staining of fecal smear helps to detect oocysts of *Cryptosporidium, Cyclospora* species and *I.belli*.

Procedure

1. Prepare a thin fecal smear on a slide then air dry.

2. Fix the smear with methanol for 2-3 minutes.

3. Stain the smear with cold carbol fuchsin for 5-10 minutes.
4. Wash off with clean tap water.
5. Decolorize with 1% acid alcohol for 10-15' until color ceases to flow out of the smear.
6. Rinse in tap water and counter stain with 0.5% malachite green (or methylene blue) for 30'
7. Wash off the stain with tap water.
8. Stand the slide in a draining rack for the smear to dry.
9. Examine the smear microscopically using100X objective to detect and identify oocyst.

3. Concentration Methods for Fecal Specimens

The concentration and the separation of protozoa cysts and helminths egg from other elements of the fecal specimen can be of great advantage in diagnosis. This can be accomplished by sedimentation, flotation and combination of the two. Feces normally contain a great variety of materials, most of which are either lighter or denser, smaller or larger than the cysts, eggs and larva of parasites. The concentration of parasites in parasitological specimens is sometimes called the " enrichment technique " because it enables to examine greater quantity of stools in less volume.The purpose in using a concentration technique is to separate as completely as possible parasites from all other elements of the Stool.

In general the concentration technique may be necessary:

1. To detect parasites when they are not found in a direct saline wet mount examination but the symptoms of intestinal parasitic infection continue.
2. To detect the eggs of parasites which are often few in number such as those of Schistosome or Taenia species.
3. To check whether treatment has been successful.
4. To investigate the prevalence and incidence of a parasitic infection as part of epidemiological survey .

A direct microscopical examination of stool must always be done before preparing a concentration because motile forms of flagellates, ciliates , and amoebae die during the concentration procedure.

Concentration Technique of Fecal Specimen is Divided into two :-

1. Floatation Techniques

Floatation technique concentrates the cysts and eggs of parasites at the top because their density is less than that of the suspending medium. The waste products, crystals, body cells etc. have a higher specific gravity therefore these substances will sink to the bottom. The top layer can be removed and placed on a slide to be examined under the microscope.

Zinc Sulfate Floatation Technique

Zinc sulfate floatation technique is one of the most widely used method of concentration. It has a special merit of being suitable for routine examination of both cyst of protozoa and eggs of most helminths. Operculated eggs of Trematodes and Cestodes, infertile Ascaris ova and larva of nematodes are not concentrated because they have greater specific gravity than the suspending medium. The eggs of Clonorchis, Opisthorchis and some small trematodes are satisfactorily concentrated. The technique is not also suitable for concentrating eggs or cysts in fatty faeces.

A Zinc sulfate solution which is used for the concentration of parasite has a specific gravity (relative density) of 1.180-1.200 Faeces are emulsified in the solution and the suspension is left undisturbed for the eggs and cysts to float to the surface where they are collected on a cover glass or can be collected by pasteur pipettes.

Procedure: -

1. About one quarter fill the tube with the zinc sulfate solution.
2. Add an estimated 0.5 gram of faeces and using a rod or stick, emulsify the specimen in the solution.
3. Fill the tube with the zinc sulfate solution and mix well.
4. Stand the tube in a completely vertical position in a rack.
5. Using a plastic bulb pipette or pasteur pipette, add further solution to ensure that the tube is filled to the brim.

6. Carefully place a completely clean (grease free) cover glass on top of the tube, avoiding trapping any air bubbles.Leave undisturbed for 30-45 minutes to give time for the cysts and eggs to float.
7. Carefully lift the cover glass from the tube by a straight pull upward. place the cover glass face downwards on a slide.The eggs and cysts will be found adhering to the cover glass.
8. Examine microscopically the entire preparation using the 10x objective use the 40x objective and run a drop of iodine solution under the cover glass to identify the cysts.

Other flotation methods includes:
a) Brine (Saturated NaCl) flotation
b) Saturated sugar flotation
c) ZnSo centrifugal flotation

2. Sedimentation Techniques

In this technique cysts and eggs of parasites settle and are concentrated at the bottom because they have greater density than the suspending medium. The cysts and eggs can be sedimented by natural gravity or by accelerating the process by centrifugation.

Formalin-Ether Centrifugal Sedimentation Technique

This method is recommended as the best technique for concentrating the eggs and larvae of helminths and moderately satisfactory for cysts of protozoa. It is most useful for detecting the eggs of Schistosome in feces. The formalin is used for fixation and preservation of the morphology of parasites. The fecal debris absorbs ether and becomes lighter than water.

Procedure

1. Take about 2g or 2ml of stool and mix it in about 10ml of normal saline solution.
2. Filter through two layers of gauze into a centrifuge test tube
3. Centrifuge for one minute at medium speed (2000-5000 rpm) . If the supernatant fluid is very cloudy, wash the deposit again i.e. mix it with 10ml of normal saline solution ,
4. Centrifuge for one minute at medium speed and pour of the supernatant fluid.
5. Add 10ml of formaldehyde solution to the sediment.
6. Stir or mix a suspension well and let it stand for five minute.
7. Add 3ml of ether.
8. Stopper the tube, turn it on its side and shake vigorously for 30 seconds or one minute.
9. Remove the stopper carefully and centrifuge for one minute at low speed (1500 rpm).
10. Free the layer of debris by rotating the tip of a wooden applicator stick between it and the sides of the tube. Tilt the tube and pour of all the supernatant fluid. use a cotton swab to remove any debris adhering to the side of the tube.
11. Mix the remaining fluid well with the deposit by tapping the tube gently.

12. Place two drops of the deposit on a slide. Add a some drop of iodine solution to the second drop of deposit only.
13. Identify the stages and species of parasites and count the number of each type of parasites in the entire preparation and report the result.

4. Kato-thick Smear Egg Count Procedure

1. Place a small amount of faecal material on newspaper or scrap paper and press the small screen on top so that some of the faeces are sieved through the screen and accumulate on top
2. Scalp the flat- sided spatula across the upper surface of the screen to collect the sieved faeces
3. Place template with hole on the center of a microscope slide and faeces from the spatula so that the hole is completely filled. Using the side of the spatula pass remove excess faeces from the edge of the hole
4. Remove the tempate carefully so that the cylinder of faeces is left on the slide Cover the fecal material with the pre- soaked cellophane strip.
5. Invert microscope slide and filmy press the faecal sample against the hydro cellophane strip in another microscope slide or on a smooth hard surface such as a piece of tile or on a flat table. The faecal material will be spread evenly between the microscope slide and the cellophane strip.
6. Carefully remove slide by gently sliding it sideways to avoid separating the cellophane strip or lifting it off. Place the slide on the bench with the cellophane upwards. Water evaporate while glycerol clears the faeces.
7. For all ova except hookworm eggs, keep slide for one or more hours at ambient temperature to clear the faecal material prior to examination under the microscope.
9. The smear should be examined in a systematic manner and the number of eggs of each species reported. Later multiply by the appropriate number to give the number of eggs per gram of faeces (by 20 if using a 50 mg template) with high egg counts.

Identification of Eggs

1. Size- their size are generally within specific range.
2. Shape -each species has its own particular shape
3. Stage of development when passed- in some species the eggs consists of a single cell; in some there may be several cells; some species are usually embryonated.
4. Thickness of egg shell- some species like Ascaris have thick egg shell,others like hookworm , have thin shell
5. Color- Some eggs are colorless(e.g., Hookworm, Entrobius) others are bile stained (yellow-brown) e.g., Ascaris, Trichuris.
6. Presence of characterstics like opercula(lids), spines .plugs, hooklets, or mammilated outer coats

The Grham Scotch Cellophane Tape Method Technique for PinWorm (E. Vermicularis) eggs collection.

Perianal fecal debris exam:-

The highest number of eggs can usually be recovered in the morning soon after waking and before bathing. Eggs can be collected from skin around the anus or from clothing by applying clear adhesive tape or saline swab. The eggs are detected microscopically by sticking the tape directly on a slide. They rarely appear in the stool.The fecal material from around the anus is usually examined for diagnosis of *Enterobius* infections. Often *Taenia* eggs, undetected by direct saline exam. are also found in perianal specimen.

The fecal swab should be collected either late at night or early in the morning before bathing or defection 3 or 4 samples collected on alternate nights, should be examined before are diagnosis for pinworm is made.

Scotch tape Method

1. Take a strip of cellophane (‘’Scotch ‘’tape) & fold it around the bottom of a test tube or Tongue depressor or glass slide with its sticky side.
2. Press the stickly surface of the ‘’Scotch ‘’ tape firmly on the skin round the anus (peri-anal –feld).
3. Cut off the middle part of the strip of cellophane tape which has been pressed on the skin.
4. Put 3 or 4 drops of Xylene or toluene on the slide & stick the middle part of the tape stickly side down on top of it.
5. Look at the strip with the low power and dry objective & you will easily see the ova

Preservation of Specimens

To preserve protozoan morphology and to prevent the continued development of some helimenth eggs and larva e, stool specimen can be placed in preservative s either immediately after passage (by the patient using collection kit) or once the specimen is received by the laboratory.

When possible, examination of faeces should be carried out immediately.

Fixatives are required for preserving parasites in faeces if:-

1. Specimens need to be sent to a reference laboratory for identification.
2. Microscopical examinations are not available locally.
3. Specimens are sent from reference laboratory to peripheral laboratories as part of a parasitology qualify assessment program.
4. Large number of faecal specimens are collected in the field for epidemiological survey.
5. Individual laboratory wants to preserve the sample for teaching purpose

Methods of Preservation:-

1. Refrigeration at 3-5C can preserve trophozoite for several days in dysenteric stools and cysts in normal feces may remain viable for more than a month. Freezing should be avoided.

2. Formalin

Formalin is an all purpose fixative appropriate for helminth eggs and larva, protozoan cyst, coccidian oocysts and microsporidian spores. Two concentrations are commonly used: 5% which is recommended for preservation of protozoan cysts; and 10%, which is recommended for helminth egg and larvae

3. 10%v/v Formol saline:-

This solution preserves cysts and eggs for some months. It alsopreserves the larvae and adult worms.

4. Merthiolate-Iodine-Formaldehyde (MIF):-

This fixative contains iodine and eosin, therefore it is used both as a fixative and a stain.

5. Sodium Acetic Acid Formaldehyde (SAF)

SAF preserved faecal specimens can be examined using both the concentration and the permanent stained smear and the fixative has the advantage of not containing mercuric chloride , as is found in Schudinn's fluid and PVA.

6. Schaudinns Fluid

This fixative is used to preserve trophozoite stages of protozoa up to one year. It is designed to be used with fresh stool specimens or samples from the intestinal mucosal surface. Permanent stained smears are then prepared from fixed material.

7. Polyvinyl Alcohol (Pva)

PVA is as plastic resin that can be incorporated into schaudinn's fixative. The PVA powder serve as an adhesive for the stool material: when the stool PVA mixture is spread onto the glass slide and allowed to dry, the stool material adheres because of the PVA component.

8. Beyer's Solution:-

It is recommended for preserving cysts and eggs in faeces. It maintains their morphology for long periods and allows the specimen to be examined as a direct preparation or after concentration by the formol ether technique.

9. Domestic Bleach Solution

S.haematobium eggs can be preserved in urine by adding 1 ml of 1% v/v domestic bleach solution to every 10 ml of urine or 1ml of undiluted formalin (37%) to each 100ml of urine or1ml of hydrochloric acid (20 drops) and 2ml of commercial bleach (40 drops) for every 100ml of urine.

- **Charcot-Leyden crystals** are formed from the breakdown of eosinophils and may be seen in the stool or sputum of patients with parasitic diseases. The crystals are slender and pointed and stain purplish-red in the trichrome stain, as shown in this image. These crystals can appear in a variety of sizes and only indicate an immune response, but the cause may or may not be a parasitic infection.