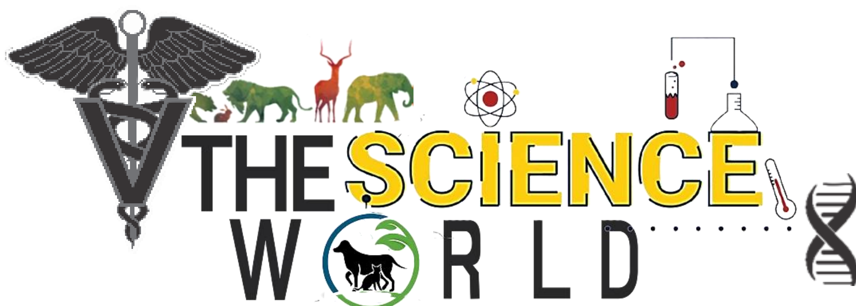

Popular Article

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Faecal Sample Examination techniques: Qualitative methods

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Clinician and technicians usually do not have the opportunity to collect faecal samples and must rely on samples brought in by clients. Regardless of how they are obtained, it is important to have fresh faeces with which to work. The need for fresh faeces seems to prevent from rapid development and changes that occur in some common parasite eggs once they are passed from the patient, as well as the death of some protozoa that can be recognized by their movement. If fresh faeces cannot be obtained, it is advisable to refrigerate the sample (not more than 24 hours). If the diagnostic center is not near the specimens should be preserved in 70% alcohol or 10% formalin and transported to the diagnostic center.

To know the different parasitic infection, we can do qualitative tests which are as follows

1. **Direct Smear method**
2. **Concentration Methods**
    a. Floatation Technique
    b. Sedimentation technique
    c. Formal ether sedimentation technique

1. Direct Smear method

The simplest method of microscopic faecal examination for detection of different stages of parasites is the direct smear, which consists of a small amount of faeces placed directly on a microscope slide. The advantages of this procedure are the short time and minimal equipment needed, as well as the small amount of faecal sample required. The main disadvantage of this procedure is its inaccuracy. Such a small amount of faeces may not contain the parasite larvae or eggs the patient is harboring and the subject may be incorrectly assumed to be free of parasites. This procedure also leaves a lot of faecal debris on the slide, which may confuse the technician.

Procedure

1. Place a drop of water or physiological saline in the middle of a slide and an equal amount of faeces next to it. A drop of iodine may also be used to help highlight protozoa.
2. Thoroughly mix the faeces and water or saline (and iodine) with the applicator stick to form a homogenous emulsion.
3. Make a smear on the surface of the slide. Be sure the smear is not too thick.
4. Remove any large faecal particles with the stick and place a cover slip over the smear. The cover slip should sit evenly over the smear.
5. Examine the smear with the microscope for parasite eggs or larvae.

Concentration Methods

The principle of these methods is to concentrate all the eggs in a given amount of faeces into as small quantity of the emulsion as possible and then examining a drop of it. There are two primary types of concentration methods used in clinical practices: **flotation and sedimentation**.

a. Floatation Technique

Floatation method is based on differences in specific gravity of parasite eggs, cysts and larvae and that of faecal debris. Specific gravity refers to the weight of an object (e.g. the parasite egg) as compared to the weight of an equal volume of pure water.

This method is useful in the examination of nematode infection only, since eggs of trematodes, most of the cestodes and even a few of the nematodes cannot be floated up. Light infections are detected by this technique invariably. The principle is to use an emulsifying fluid of a greater specific gravity than that of the contained eggs. The common fluids in use are (1) Saturated solution of common salt of Sp. Gr. 1.18-1. 19 (2) Saturated sugar solution of Sp. Gr. 1.25, (3) Saturated solution of sodium nitrate, (4) Glycerin, (5) Zinc sulphate solution of Sp. Gr. 1.18 (32.5% solution).

Most parasite eggs have a specific gravity between 1.10 and 1.20 (g/ml), while tap water is only slightly higher than 1.00. Therefore, parasite eggs are too heavy to float in tap water. To make the eggs float, a liquid with a higher specific gravity than that of the eggs must be used. Such liquids are called floatation solutions and consist of concentrated sugar or various salts added to water to increase its specific gravity. Floatation solutions usually have specific gravities between 1.20 and 1.25. In this range, parasite eggs float to the surface of the liquid and large particles of faecal material having higher specific gravity sink to the bottom.

Preparation of Saturated sodium chloride solution

Add table salt to boiling water until the salt no longer dissolves, and settles to the bottom of the pot. There is no need to adjust the specific gravity, as it cannot go above 1.200 with this solution.

Simple Floatation (Willis technique or Levitation technique)

Procedure

1. Take about 2 g (1/2 tsp.) of the faecal sample and place it in the cup. Add about 30 ml of floatation solution, using a glass rod make an emulsion by thoroughly mixing the solution with the faeces until no large pieces of faeces remain.
2. Pour the emulsion through the tea strainer into the vial.
3. Fill the vial to the top and slightly overfill it, so that a dome of liquid (a meniscus) rises above the lip of the vial without overflowing down the slide. If there is not enough fluid in the cup to fill the vial, a small amount of fresh floatation solution may be added.
4. Place a cover slip gently on top of the fluid and allow it to settle on the liquid dome.
5. Allow the cover slip to remain undisturbed on top of the vial for 10-20 minutes (sugar solution requires longer than sodium nitrate). If removed before this time, all of the eggs may not attach to the coverslip.
6. If left for more than an hour, some eggs may become waterlogged and begin to sink or become distorted.
7. Remove the cover slip carefully, picking it straight up, and immediately place it on the microscope slide. When placing it on the slide, be sure to hold the cover slip with one edge tilted slightly up and allow it to settle level on the slide gradually: this avoids air bubbles under the cover slip.
8. Examine the area of the slide under the cover slip with a microscope, as previously described, and record any eggs or larvae.
9. The procedure can be fastened up by centrifugal floatation technique where centrifuge the tubes for 5 minutes at about 1500 revolutions per minute (rpm).
10. Remove the tube from the centrifuge and gently place it in a test tube rack. A glass rod is then touched to the surface of the liquid. The drop of fluid contained in the loop or on the end of the rod is then transferred to the microscope slide. Apply a cover slip and examine microscopically.

b. Sedimentation technique

Sedimentation is mainly used to detect eggs or cysts that have too high a specific gravity to float or that would be severely distorted by floatation solution. 0.5% glycerin in water can be used for emulsifying the faeces instead of plain water. It can be used for roundworm and tapeworm eggs, but there is usually too much faecal debris hiding the eggs to make it worthwhile. For that reason, this procedure is not used routinely and has its greatest use in suspected fluke infections.

Sedimentation procedure

1. Using a glass rod, mix about 2 g of faeces with tap water in a cup or beaker. Strain the mixture through the tea strainer into a centrifuge tube as described for centrifugal flotation.

2. Balance the centrifuge tubes and centrifuge the sample at about 1500 rpm. If a centrifuge is unavailable, allow the mixture to stand undisturbed for 20-30 minutes.
3. Pour off the liquid in the top of the tube without disturbing the sediment at the bottom.
4. Using the pipette, transfer a small amount of the top layer of sediment to a microscope slide. If the drop is too thick, dilute it with a drop of water. Lugol's iodine solution may be used for dilution instead of water, to aid in identification of protozoan cysts. Apply a coverslip to the drop. Repeat the procedure using a drop from the bottom layer of the sediment.
5. Examine both slides microscopically.

c. Formal ether sedimentation technique

This technique is used in formaline fixed samples or in the samples where eggs can hatch if they are mixed with water. eg. egg of *Schistosoma sp.*

1. Wear gloves when handling stool specimens.
2. In a suitable container, thoroughly mix a portion of stool specimen about the size of a walnut into 10ml of saline solution. Mix thoroughly.
3. Filter the emulsion through fine mesh gauze into a conical centrifuge tube.
4. Centrifuge the suspension at a relative centrifugal force (RCF) of 600 g (about 2000 rpm) for no less than 10 minutes. The suspension should yield about 0.75mL of sediment for fresh specimens and 0.5 ml for formalinized feces.
5. Decant the supernatant and wash the sediment with 10 ml of saline solution. Centrifuge again and repeat washing until supernatant is clear.
6. After the last wash, decant the supernatant and add 10 ml of 10% formalin to the sediment. Mix and let stand for 5 minutes to effect fixation.
7. Add 1 to 2 ml of ethyl acetate, Stopper the tube and shake vigorously.
8. Centrifuge at 450 g RCF (about 1500 rpm) for 10 minutes. Four layers should result as follows
    a. a top layer of ethyl acetate;
    b. plug of debris;
    c. layer of formalin; and
    d. sediment
9. Free the plug of debris from the side of the tube by ringing with an applicator stick. Carefully decant the top three layers.
10. With a pipette, mix the remaining sediment with the small amount or remaining fluid and transfer one drop each to a drop of saline and iodine on a glass slide. Cover with a coverslip and examine microscopically for the presence of parasitic forms.

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Examination of Faeces for Protozoa

All of the previous procedures for microscopic faecal examination are useful for detection of cysts of intestinal protozoa. However, some protozoa do not form cysts and pass in the faeces as trophozoites. Cyst forming protozoa may also pass trophozoites in the faeces in large numbers when the host has diarrhoea. Trophozoites lack the rigid wall of cysts, and collapse and become difficult to recognize in flotation solutions.

To observe live trophozoites, a faecal smear should be prepared as previously described, but physiological saline must be used to dilute the faeces. Trophozoites are recognized by their movement, which varies among different groups of protozoa. *Giardia,* which is found in several different hosts, appears to wobble and have a sail-like structure that ripples as they move. Amoeba found in man and dogs, move by extending part of their cell body (a pseudopod) and moving the rest of the body after it.

Many methods have been used to stain and / or preserve intestinal protozoa. The simplest method to stain cysts is a direct smear stained with an iodine solution (as described under the direct smear procedure). This method does not preserve the sample but highlights any protozoa in the smear, making their identification easier. Several different iodine solutions are available (Lugol's iodine) for staining.

Faecal smears containing protozoal trophozoites may be allowed to dry and then stained with Giemsa / Wright's stain. There are many other procedures for the concentration, staining and preservation of intestinal protozoa, including merthiolate-iodine-formaldehyde (MIF) solution, polyvinyl alcohol, iron haematoxylin and others.

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