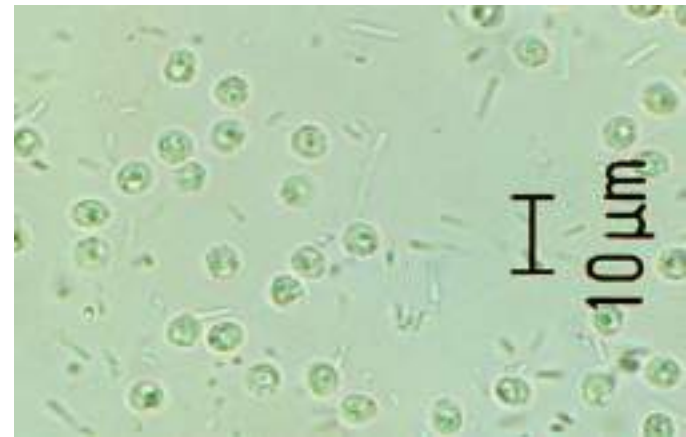
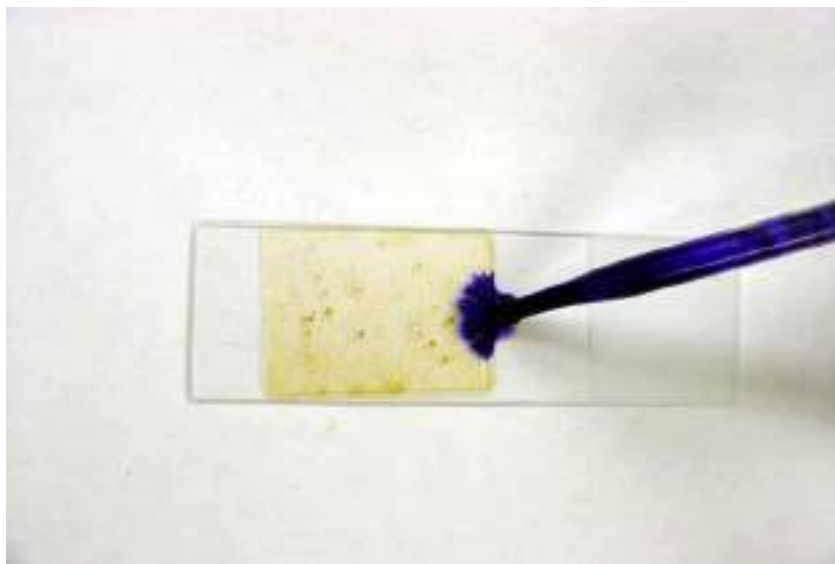


Staining

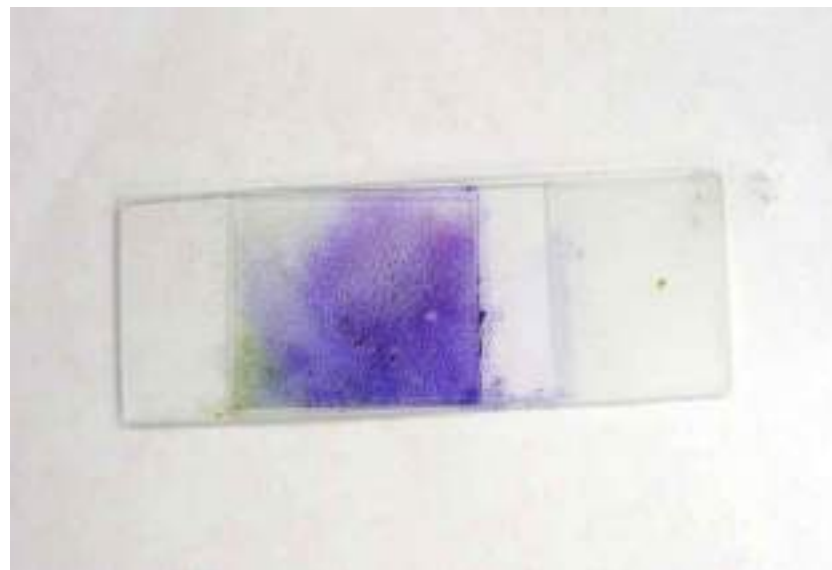
- A drop of stain can be added before the cover slip is placed or after having examined the unstained preparation.
- The stain will diffuse and then you can examine it.
- **The iodine** will stain the organisms a **dark orange brown color**.
- If you use new **methylene blue** instead, you will see organisms **contrasted against a blue background**.



Note:- You can choose to look at the unstained preparation first for **motile forms**, and then **add stain** by applying it at the edge of the coverslip.



Methylene blue stain at the edge of the cover slip with a Pasteur pipette.



Stain will diffuse



Application of iodine stain

2) Dry mount technique

- The fecal smear may be examined in a dry state and stained.
- Prepare the slide as for a wet mount, but instead of placing a cover slip, **let it dry so that only a thin film is visible on the slide.**
- It should be heat fixed by passing it over a flame for a few seconds.
- Then you can stain it.

A thin fecal smear is prepared and dried



Staining

- The stains most commonly used are the **Diff Quik** or **acid fast stain**.
- If you are trying to rule out **Cryptosporidium spp.**, then **the acid fast stain** is the stain of choice.

Importance of direct fecal smear

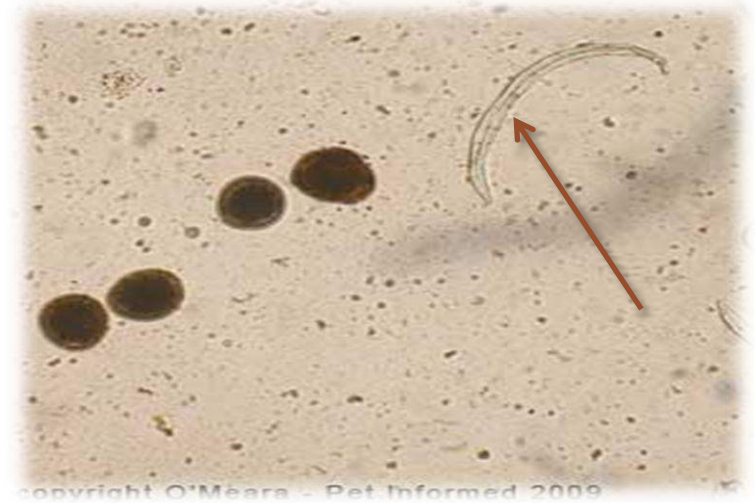
- 1) Parasitic diseases such as ascariasis, hookworms, whipworms, etc., can be diagnosed by examining stools under a microscope **for the presence of worm larvae or eggs.**
- 2) Some **bacterial diseases** can be detected with a stool examination.
- 3) Quick screening test to check for any **intestinal parasite.**



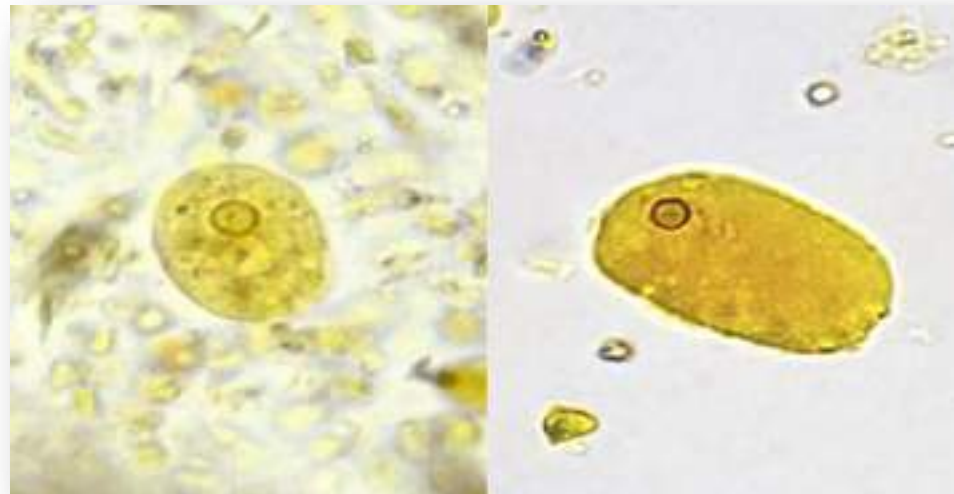
Under microscope



Schistosoma spp egg



Protozoa appear



Iodine stain. an *Entamoeba coli* trophozoite

Concentration Methods

1. Sedimentation method

- Modified Formal- Ether sedimentation technique
- Acid- Ether sedimentation technique

2. Flotation method

- Saturated Salt Solution technique
- Sheather's Sugar Centrifugal Flotation technique
- Zinc Sulphate Centrifugal Flotation technique

Sedimentation Methods

MODIFIED FORMAL- ETHER SEDIMENTATION

Modified Formal- Ether Sedimentation

- Formalin- Ether or Formalin- Ethyl acetate method is the recommended concentration procedures.
- Most types of worm eggs (round worms, tapeworms, schistosomes, and other fluke eggs), larvae, and protozoan cysts may be recovered by this method.
- **Advantages:**
 1. Speed: one sample can be processed in 5 minutes.
 2. Broad spectrum: it will recover most ova, cyst and larvae.
 3. The morphology of most parasites is retained for easy identification.
- **Disadvantages:**
 1. Requires several pieces of apparatus which does not make it an easy.
 2. The preparation contains some debris.
 3. Ether is flammable. Formalin is an irritant.
 4. *Hymenolepis nana* and *Fasciola spp.* do not concentrate well.

Procedures

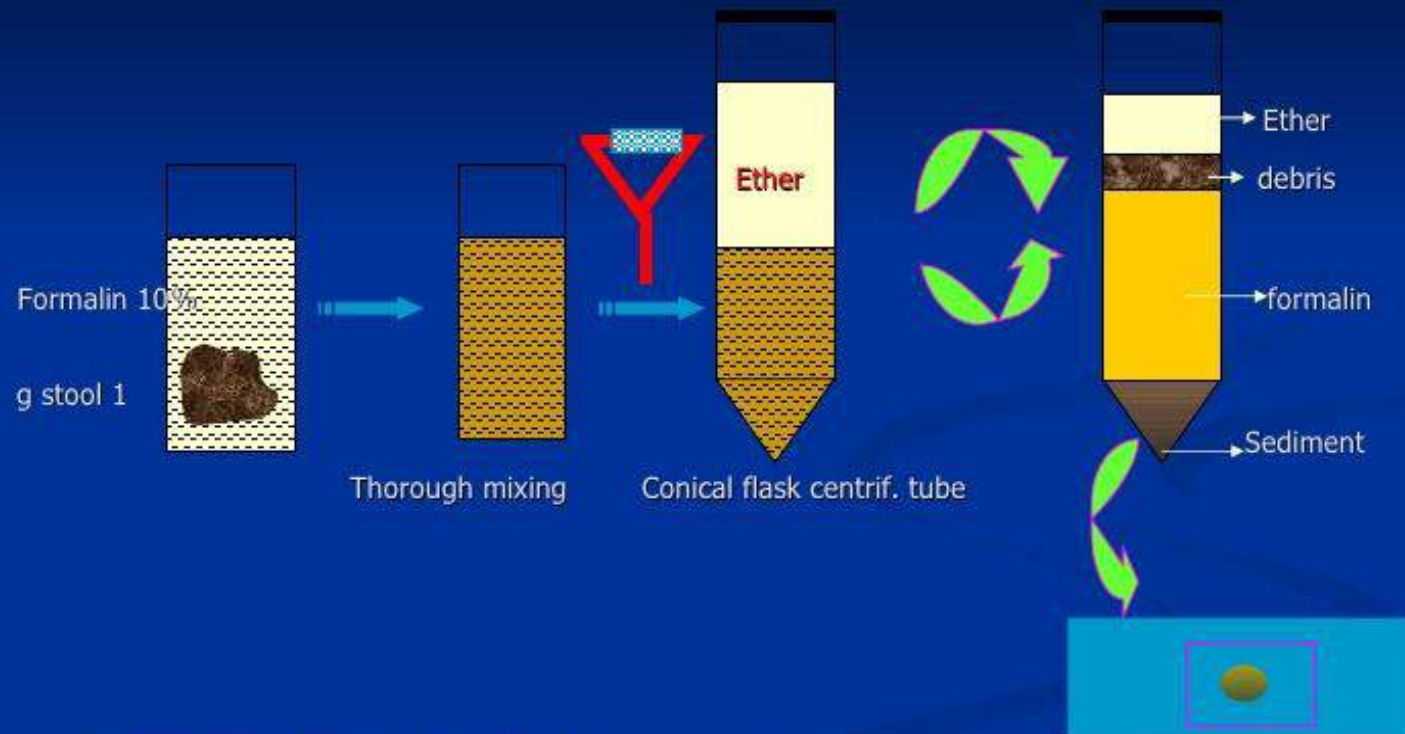
1. Emulsify 1 gm. of feces in 7 ml of 10% formalin in a centrifuge tube.
2. Strain the suspension through a brass wire sieve, and collect in beaker.
3. Pour the filtrate into a 15 ml boiling tube and add 3 ml of ether, then mix well 15 sec on vortex or whirlimixer or 1 min by hand.
4. Transfer the ether- formalin suspension back into the washed centrifuge tube, and centrifuge at 3,000 rpm for 1 min.

Procedures (cont.)

5. Loosen the fatty layer and debris at the top of the tube with an applicator stick and invert the tube quickly to discard the supernatant.
6. On righting the tube, a few drops only should remain with the sediment, mix the sediment well and transfer one drops onto a glass slide and cover it with coverslip.
7. Scan the whole coverslip using 10x objective, turning into 40x for confirmation of identification of parasites.

STOOL EXAMINATION

. Formol Ether Sed. Conc



- Ether adsorbs fecal debris & floats.
- Formalin fixes & preserves the specimen.

Flotation technique

- These method use the high specific gravity of a solution to float the lighter ova and cyst. They can be improved by centrifugation.
 - **Advantage:**
 - Easy to perform .
 - **Disadvantage:**
 - Delay in examination can result in distortion.
 - Larvae and some fluke eggs do not concentrate.
 - Frequent checking of specific gravity.

Flotation Methods

SATURATED SALT SOLUTION

Saturated Salt solution technique

■ **Advantages:**

- It is cheap preparation using simple apparatus.
- It concentrates nematode ova well.

■ **Disadvantages:**

- It doesn't concentrate cysts.

Materials and Method

1. Boil granular sodium chloride in excess in water to produce a saturated solution which when cooled has a specific gravity of 1.18 - 1.2.
2. Half fill a wide- mounted flat bottomed container with the saturated salt solution.
3. Emulsify 1gm of feces in the solution and strain it to remove the debris from the surface.
4. Pour the filtrate into meniscus and fill it to the top with saturated salt solution.
5. Lay a glass slide or large coverslip over the top, avoiding any bubbles being trapped.
6. Leave for 20 min before quickly inverting the slide.
7. Scan for ova using the 10x objectives.

100. (Figure 2)

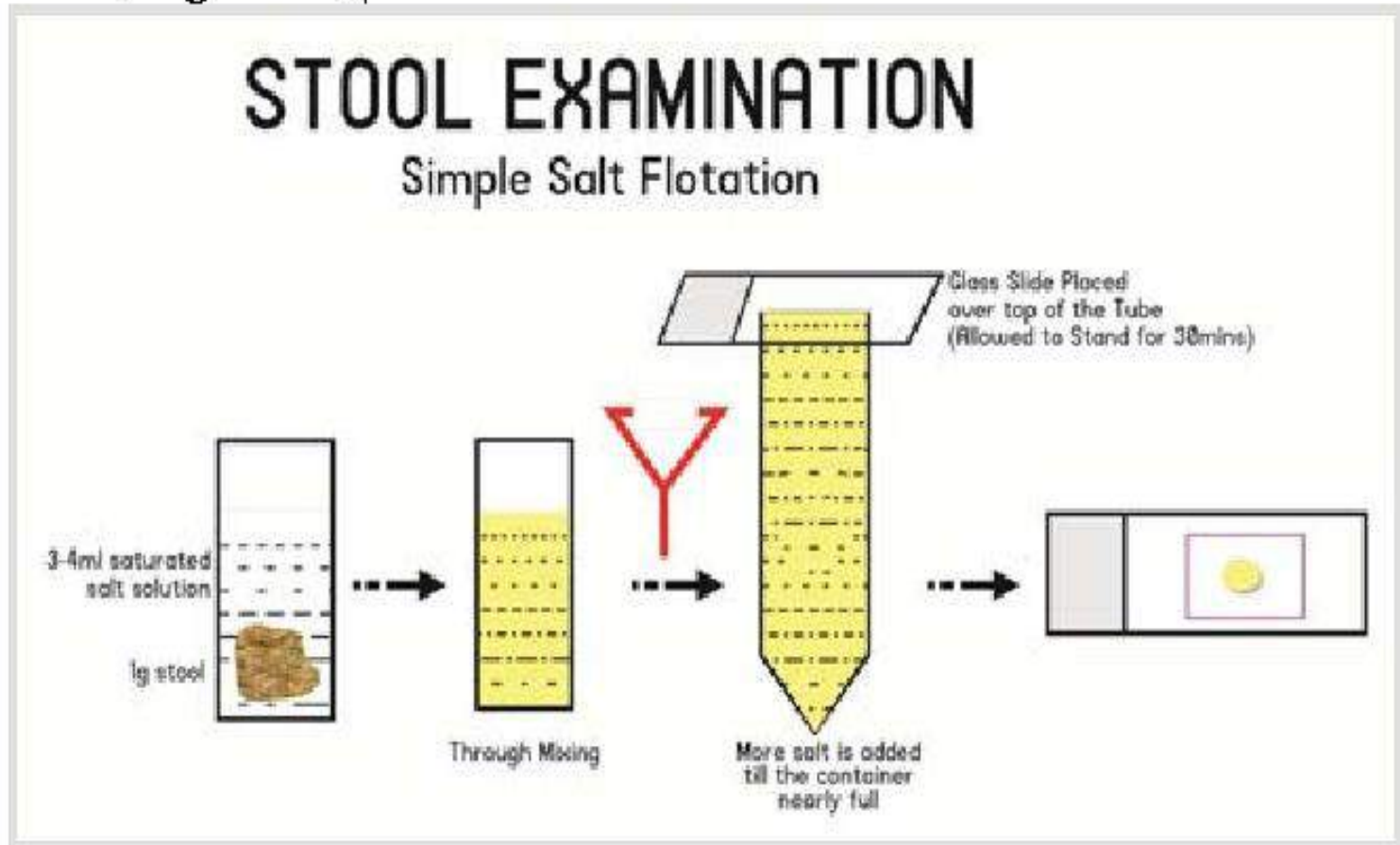


Figure 2: Salt flotation technique