Concentration procedures

A routine part of complete stool examination for parasites Allows detection of small numbers of parasites that may be missed by using only a direct wet smear

Designed to separate protozoan organisms & helminth eggs & larvae from fecal debris by centrifugation and/or differences in specific gravity

There are two types of concentration procedures:

Sedimentation & Floatation

1-SEDIMENTATION METHOD

Background:

- **Concentration procedures are used to concentrate eggs, larvae and protozoan cysts to increase the sensitivity for parasite detection in stool samples, specially in cases of mild infection.**

All type of eggs and cysts can be recovered by sedimentation. Parasites settle down more rapidly by centrifugation.

Larger food particles can be removed prior to centrifugation by filtering through a sieve with a pore size enough to retain those particles. The efficiency of detection is increased by adding formalin for fixation & preservation of parasites, and ethyl acetate to remove organic material especially fat.

Materials:

- 10% formalin
- Ethyl acetate
- Centrifuge tubes
- Centrifuge stand
- Funnel
- Gauze
- Spatula
- Pipettes
- Microscope slides & coverslips

Method:

Step 1:

Emulsify 0.5-1 g of stool in 7 ml of 10% formalin in a tube.

Step 2:

Pour the stool emulsion onto a double layer of gauze in a funnel and collect in a beaker

Step 3:

Wash the stool through the gauze using formalin. This washes the parasites through but filters out the larger pieces of debris.

Step 4:

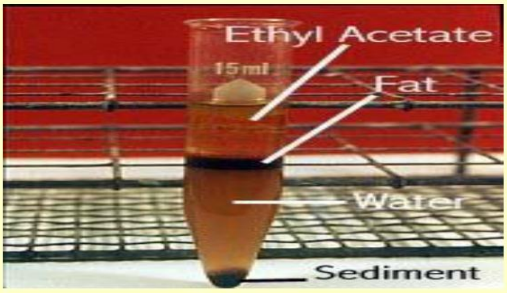
Pour the filtrate into a 15 ml centrifuge tube and add 3 ml of ethyl acetate, mix well (~1 min) by hand.

Step 5:

Centrifuge the mixture for 5 minutes at 500 g (~2000rpm).

Four layers are formed in the tube after centrifugation

(Formol-ethyl acetate concentration method)



Step 7:

Mix the sediment well

Step 6:

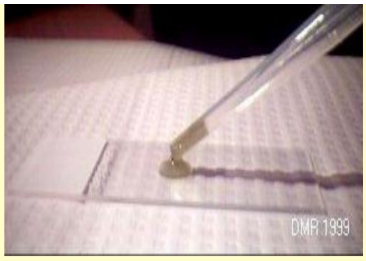
Loosen the fatty plug at the top of the tube with an applicator stick and invert the tube to discard the supernatant.

* Few drops should be kept with the sediment.

Step 9:

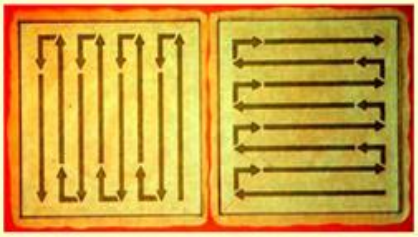
Place one edge of the coverslip on the drop and carefully pull the drop along. When the coverslip is in position gently let it drop.

Step 8:



Place a drop on the slide.

Step 11:



- Scan the entire coverslip systematically as illustrated using the 10X objective.
- If you suspect cysts or trophozoites, use higher magnification.
- 1/3 of the slide should be scanned using a higher magnification.

Step 10:

If there is a thick and thin area, the cysts and ova will generally be found in the thick portion of the mount.

Concentration Methods
2- Floatation

Floatation procedure
Permits the separation of protozoan cysts & oocysts, and certain helminthes eggs & larvae through the use of a liquid with a high specific gravity. Parasites are recovered in the surface film, and the debris remains in the bottom of the tube.

Floatation procedure (Cont.)
*** Advantage:**
- Cleaner preparation than does the sedimentation procedure.
*** Disadvantages:**
- Some helminth eggs (operculated eggs and/or very dense eggs such as unfertilized Ascaris eggs) do not concentrate well with the floatation method.
- Protozoan cysts become distorted and difficult to identify

Zinc Sulfate Floatation Method

1. Using a tongue depressor, emulsify 2 to 3 grams of feces (a size of a grape) into 5 ml of ZnSO4 solution (S.G 1.18) in a dish.
2. Push the emulsion through the strainer into a 15 ml centrifuge tube then fill the tube with zinc sulfate solution.
3. Centrifuge for 2 min at 1500 - 2000 rpm.
4. Using a headed-rod or loop, remove a sample from solution surface & place on a microscope slide.
5. Add a drop of iodine to stain cysts & ova

6-prepare the slid as the perspiration way and don the examination.

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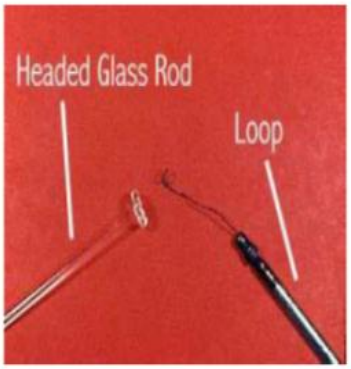

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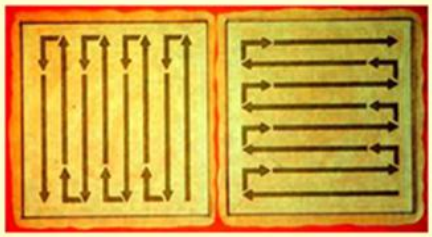

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