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FORMALIN ETHER
CONCENTRATION TECHNIQUE
PECT



PURPOSE: PARASITIC ELEMENTS ARE CONCENTRATED THROUGH SEDIMENTATION TO ENHANCE THE RECOVERY IN FECAL SPECIMENS.

Formalin acts both a fixative and preservative of protozoan eggs, larvae and cysts. The specific gravity of protozoan cysts and helminth eggs is greater than that of water. Fecal debris is extracted into the ether phase so that the parasitic forms can be separated and then sedimented by centrifugation.

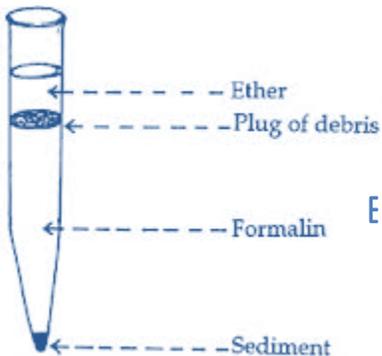


PROCEDURE:

- Mix a small portion of the stool, about the size of a marble, in 10 ml 5% - 10% formalin in a test tube. Allow to stand 30 minutes for fixation.
- Strain about 10 ml of this suspension through a small funnel containing wet gauze into a 15 ml centrifuge tube. Use two layers of gauze.
- Add 5%- 10% formalin to within ½ inch of the top of the test tube. Centrifuge for 10 minutes at 500g.
- Decant supernatant, leaving 0.5- 1.5 ml of sedimented material.
- Resuspend the sediment in saline to within ½ inch of the top of the tube. Centrifuge again for 10 minutes at 500g. This second wash is not needed if the first supernatant wash is light tan or clear in color.
- About 1 ml sediment should remain. If the amount is much larger or smaller, adjust to the correct quantity as follows:
 - **Amount too large:** resuspend the sediment in saline and pour out a portion. For example, if the amount is twice that needed, pour out slightly less than half the suspension. Then, add saline to bring the volume up to 10ml, and centrifuge again.
 - **Amount too small:** pour off the supernatant and strain a second portion of the original fecal suspension into the tube. The amount to be strained depends on the amount of sediment. For example, if it is half the amount obtained from the first centrifugation, strain another 10ml into the tube. Centrifuge again.
- After adjustment, if necessary, decant and resuspend the sediment in 5%-10% formalin, filling the tube about one half full.
- Add approximately 3 ml ethyl acetate, insert screw cap, and shake vigorously for a minimum of 30 seconds. Carefully remove the screw cap by holding tube away from yourself to avoid spraying as the stopper is removed and pressure is released.
- Centrifuge at 500g for 10 minutes. Four layers should result: Illustration on left.
 - Free the plug of debris from the sides of the tube with an applicator stick. Carefully decant the three layers. Use a cotton swab to clean debris from the walls of the tube to prevent it from settling down into the sediment.
 - Using a pipette, mix the remaining sediment with a small amount of the fluid that drains from the sides of the tube.
 - Mix the sediment well, and prepare a wet mount for examination.

MATERIALS NEEDED

Ethyl Acetate
Formalin 5-10%
NSS
Test Tube, 10 ml
Centrifuge Tube, 15ml
Stool Container
Applicator Stick
Gauze
Glass Funnel
Digital Centrifuge
Microscope



EXAMINATION RESULTS:

- Prepare a saline mount by adding 1 drop sediment to 1 drop saline. Add a coverslip. Scan the entire area under the coverslip under low power (10x) objective for eggs and larvae.
- Add 1 drop iodine to the edge of the coverslip to assist in the observation of cysts. Examine under the high- dry objective (40x- 45x).