

## EVALUATION OF TECHNIQUES AND ESTABLISHMENT OF LABORATORY ROUTINE

### I. METHODS OF EXAMINING FECES

#### A. Direct Wet Mounts (all diagnostic stages)

1. Use 3x2-inch slides and 22 mm square, #1 thickness coverslips.
2. Density important. Fine print should be distinct through layer of suspension. Do not use too much diluent. Saline and iodine mounts are routinely prepared.
3. Seal with 1:1 paraffin - vaseline mixture (vaspar) by applying hot mixture to edges of coverslip. Retards evaporation and prevents roll and flow of material.
4. Examine saline mount systematically and completely with low power (10X objective). Use high, dry and oil immersion magnifications for more detailed observations.
5. Solutions.
  - a. Saline - all diagnostic stages.
  - b. Iodine - cysts. Use approximately 1% iodine solutions such as Dobell's, D'Antoni's, or a 1:5 dilution of Lugol's.
  - c. Quensel's stain or Buffered Methylene Blue (Nair's Stain) - ameba trophozoites only. Requires 5 to 10 minutes to stain organisms; will overstain in about 30 minutes.
  - d. MIF (merthiolate-iodine-formaldehyde) - cysts and trophs. May be used in place of iodine.

B. Concentration Procedures (eggs, larvae, and cysts)

1. Types: flotation and sedimentation.

2. Procedures recommended for routine use.

a. Zinc sulfate centrifugal flotation

- does not recover operculated eggs or schistosome eggs
- good for *Giardia* cysts and *H. nana* eggs
- may distort cysts
- organisms begin to settle after 30 minutes

b. Formalin-ether sedimentation

- recovers all types of eggs
- is not especially good for *Giardia* cysts and *H. nana* eggs
- can be left at several stages and may fit a busy lab schedule better than the zinc sulfate method
- ether may present a fire or explosion hazard

3. Preparation of wet mounts.

a. Use pipette to remove sediment of formalin-ether concentration; use loop to remove surface film of zinc sulfate method - or - raise fluid level and cover with a coverslip.

b. Prepare unstained and iodine mounts.

c. Add saline to concentrate only if suspension is too thick to make a good mount.

C. Permanent Stains (protozoa only)

1. Hematoxylin stains
2. Trichrome (Wheatley's modification)
3. Chlorazol Black E (Kohn's Stain)  
- is not satisfactory for PVA-fixed specimens
4. Examine with oil immersion only. Should examine for 15 to 20 min.

D. Cultivation (protozoa only)

Not recommended for routine use in clinical laboratories

E. **DIAGNOSTIC STAGES RECOVERED BY DIFFERENT METHODS**

METHOD	STAGE			
	PROTOZOA		HELMINTHS	
	TROPHS	CYSTS	EGGS	LARVAE
Direct wet mount				
Concentration				
Permanent stain				

II. LABORATORY ROUTINE FOR PARASITOLOGIC EXAMINATIONS

TYPE OF SPECIMEN	METHODS		
	Direct Wet Mount	Concentration	Permanent Stain
I. Unpreserved feces			
a. Formed			
b. Soft			
c. Loose			
d. Watery			
II. Unpreserved and PVA-fixed			
III. Formalin and PVA-fixed			

CONSISTENCY OF FECES AND DISTRIBUTION OF PROTOZOA

Consistency

Cysts

Trophozoites

Formed

Soft

Loose

Watery



### III. REFERENCES

#### A. Manuals available from:

U.S. GOVERNMENT PRINTING OFFICE  
Superintendent of Documents  
Washington, D.C. 20402

#### Laboratory Procedures for the Diagnosis of Intestinal Parasites

GPO Stock No. 017-023-00091-8  
Price - \$6.45

#### Amebiasis: Laboratory Diagnosis - A Self-Instructional Lesson

Part I: Life Cycle of *Entamoeba histolytica*

Part II: Identification of Intestinal Amebae

Part III: Laboratory Procedures

Sold only in sets; not available individually

GPO Stock No. 017-023-00111-6, price: \$4.00/set.

#### B. Sound-slide Program

#### Preparation and Examination of Wet Mounts for Parasitological Examinations.

Set of 2x2 projection slides with taped narration. Tape is programmed so slides change automatically if proper equipment is used. For more detailed information, contact the Laboratory Training and Consultation Division, or the Parasitology Training Branch, Center for Disease Control, Atlanta, Georgia 30333.

## IODINE SOLUTIONS FOR WET MOUNT PREPARATIONS

Iodine solutions are used to stain protozoan cysts in wet mounts. It is advantageous to have a weak rather than a strong iodine solution. The strong iodine tends to coagulate the fecal particles and to destroy the refractile nature of the organism. However, if the solution is too weak, the organisms will not stain properly. For example, Gram's iodine used for bacteriological work is not satisfactory for staining protozoan cysts.

There are several iodine solutions that can be used satisfactorily. The two described below have been widely used and are simply prepared. The one recommended by Dobell and O'Connor (1921) is a weak iodine that should be prepared fresh about every 10 days for best results. Lugol's iodine must be diluted about 5 times with distilled water, since the full strength solution is too strong. Lugol's iodine should be prepared fresh about every 3 weeks.

### PREPARATION

#### DOBELL AND O'CONNOR'S IODINE SOLUTION:

Iodine (powdered crystals)-----	1 gm
Potassium iodide-----	2 gm
Distilled water-----	100 ml

Dissolve the KI in the distilled water. Add the iodine crystals slowly and shake thoroughly. Not all of the crystals will dissolve, but the solution is ready for use when it is a strong tea color. If it is to be used with formalinized organisms, it should be slightly darker. Filter or decant before using.

#### LUGOL'S IODINE SOLUTION:

Iodine (powdered crystals)-----	5 gm
Potassium iodide-----	10 gm
Distilled water-----	100 ml

Dissolve the KI in the distilled water. Add the iodine crystals slowly and shake until dissolved. Filter and place in tightly stoppered bottle. Dilute 1:5 with distilled water for use in staining protozoan cysts.

### TECHNIQUE

Comminute a small portion of feces in a drop of the iodine solution, mount with a coverslip and seal with a melted paraffin-vaseline mixture (approximately 1:1).

In a correctly stained cyst, glycogen, if present, appears reddish brown, the cytoplasm, yellow and the nuclei as lighter refractile bodies. The chromatin particles and the karyosomes of the nuclei stain dark brown. Chromatoid bodies, however, are less visible than in saline mounts.

### REFERENCE

Dobell, C. and O'Connor, F. W. 1921, Intestinal Protozoa of Man. William Wood, New York. ix + 211 pp.

QUENSEL'S SOLUTION FOR WET MOUNT PREPARATIONS

The nuclei of amebae trophozoites are indistinct or entirely invisible in saline preparations. Iodine solution is unsatisfactory for staining this stage, since it may distort the entire organism. Quensel's solution is recommended for the study of trophic amebae in temporary preparations (Svensson, 1935) and may be used with unpreserved feces or sediment from cultures.

PREPARATION

A. Stock Solutions

1. Sudan III Saturated Alcoholic Solution

Sudan III powder -----	1.6 gm
80% ethyl alcohol -----	100 ml

Add the stain to the alcohol. Shake thoroughly. Let stand for a few hours or overnight to be sure the solution will be saturated. If all of the stain dissolves, add more powder until no more will go into solution. Filter or decant to remove the excess powder and store in a screw-cap or glass-stoppered bottle.

2. Methylene Blue, Saturated Aqueous Solution

Methylene blue powder, medicinal -----	3.5 gm
Distilled water -----	100 ml

Add the stain to the water and shake thoroughly. Let stand for a few hours, shake at intervals. If all of the stain goes into solution, add more. Filter. Store in a screw-cap or glass-stoppered bottle.

3. Cadmium Chloride Solution

Cadmium chloride (c.p.) -----	10 gm
Distilled water -----	100 ml

Dissolve the cadmium chloride crystals in the water. Store in a screw-cap or glass stoppered bottle.

B. Quensel's Stain Solution

Sudan III, saturated alcoholic solution -----	20 ml
Methylene blue (medicinal) saturated aqueous solution -----	30 ml
Cadmium chloride, 10% aqueous solution -----	50 ml

1. Mix the Sudan III and methylene blue.
2. Add the mixture to the cadmium chloride in an Erlenmeyer flask.

3. Gently shake the mixture now and then for 15 to 20 minutes. A voluminous flocculent precipitate develops and the fluid becomes almost colorless.
4. Filter. Note: The precipitate can be more easily removed from the paper (Step 7) if the material is filtered so that the residue (precipitate) is collected at the bottom of the filter cone rather than spread over a large area.
5. Remove all excess liquid from precipitate by placing the filter paper with the precipitate upon another filter paper or cellulose wool. Leave overnight.
6. Transfer precipitate to a fresh filter and rapidly pour through 25 to 30 ml of distilled water.
7. Dissolve the washed precipitate in 250 ml of distilled water.
8. Filter in a few days if fine crystals of cadmium chloride precipitate.

#### TECHNIQUE

A small amount of the fecal sample or culture sediment is picked up with an applicator stick, toothpick, or pipette (culture) and thoroughly comminuted in a drop of Quensel's solution. The mixture is covered with a coverslip and sealed. After about 10 to 20 minutes, the amebae trophozoites are stained a pale blue with their nuclei a deeper blue shade. The stained nuclei present the same morphologic characteristics that they do in a permanently stained hematoxylin preparation. Food inclusions within the cytoplasm are also stained. After 1/2 to 1 hour, the organisms become overstained and can no longer be identified. Occasionally, organisms fail to stain. The nuclei of Dientamoeba fragilis do not stain well, but the presence of 1 or 2 nuclei may be discernible. The use of a warm stage may aid in the staining of Dientamoeba. Blastocystis hominis stains beautifully, but ciliates, flagellates, and living cysts do not stain. Cysts preserved in formalin will stain, however, but details are not always easily seen.

#### REFERENCE

Svensson, R. 1935. Studies on human intestinal protozoa. Act. Medica. Scand. Suppl. LXX vi + 115 pp.

BUFFERED METHYLENE BLUE SOLUTIONS FOR  
WET MOUNT PREPARATIONS

The pH of the stain solution has been found to be the deciding factor in bringing out the morphologic details of the nuclei of protozoan trophozoites in wet mounts. Satisfactory results can be obtained by dissolving biological dyes, such as methylene blue, in an appropriately buffered solution (Nair, 1953). The buffered methylene blue stain can be substituted for Quensel's. For staining trophozoites of *E. histolytica* the exact pH is not critical and the optimum range may vary with the buffer system and dyes employed. Directions for preparing acetate buffers with the pH range of 3.6 to 4.8 are given below, or if desired, commercially available buffer tablets can be used. Phthalate buffers with a pH range of 2.0 to 2.5 are also satisfactory.

PREPARATION OF SOLUTIONS

A. Stock Solutions

1. Solution A, 0.2M Acetic Acid

Glacial acetic acid -----	11.55 ml
Distilled water -----	988.45 ml

Mix the acid with the water to give a 0.2M solution. Store in a glass-stoppered bottle.

2. Solution B, 0.2M Sodium Acetate

Sodium acetate ( $\text{NaC}_2\text{H}_3\text{O}_2$ ) -----	16.4 gm
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- OR -

Sodium acetate, crystalline ( $\text{NaC}_2\text{H}_3\text{O}_2 \cdot 3\text{H}_2\text{O}$ ) -----	27.2 gm
Distilled water to make -----	1,000.0 ml

Dissolve sodium acetate in 300 to 400 ml of water in a 1 liter volumetric flask. Add water to the 1 liter level. Mix thoroughly. Store in a glass-stoppered bottle.

B. Acetate Buffer Solution

For specific pH, mix the indicated portions of Solutions A and B and dilute with distilled water to a total volume of 100 ml.

pH	A	B
3.6	46.3	3.7
3.8	44.0	6.0
4.0	41.0	9.0
4.2	36.8	13.2
4.4	30.5	19.5
4.6	25.5	24.5
4.8	20.0	30.0

### C. Temporary Stain

Biological dyes like methylene blue or pyronin may be used. The quality of dye that should be dissolved in the buffer solutions can be determined by a little experience. The amount will depend on the solubility of the dye in the buffer at different pH levels. In practice a small quantity of methylene blue (0.06%) in acetate buffer at pH 3.6 has given satisfactory results.

### TECHNIQUE

A small amount of the fecal sample or culture sediment is picked up with an applicator stick, toothpicks, or pipette and thoroughly comminuted in a drop of the buffered methylene blue solution. The mixture is covered with a coverslip and sealed. After a few minutes the amebae trophozoites are stained a pale blue with their nuclei a deeper blue shade. The stained nuclei present the same morphologic characteristics that they do in a permanently stained hematoxylin preparation. Food inclusions within the cytoplasm are also stained. The nuclei of *D. fragilis* stain with this solution and the presence of 1 or 2 nuclei may be discernible. Occasionally, some organisms fail to stain.

### REFERENCE

Nair, E.P. 1953. Rapid staining of intestinal amoebae in wet mounts.  
Nature 172:1051.

## MIF SOLUTION FOR STOOL EXAMINATION

The MIF (Merthiolate-iodine-formaldehyde) solution can be used for (1) staining protozoan cysts and trophozoites in wet mount preparations, and (2) as a preservative for these stages and helminth eggs and larvae. Two slightly different formulas are recommended by the authors for these two uses (Sapero et al., 1951, 1953).

### PREPARATION OF MIF SOLUTIONS

#### A. Wet Mount Preparations

Lugol's Iodine Solution (see below)-----	0.10 ml
Formaldehyde (USP)-----	0.15 ml
Tincture merthiolate, No. 99, 1:1,000 (Lilly)*-----	0.75 ml

One ml of stain is sufficient for 25 to 30 fecal preparations. The stain is prepared fresh each day. The amounts of the three ingredients may be varied for different purposes. The greater the iodine strength, the more immediate the staining reaction, however, the cysts and trophozoites are more easily detected at a lower iodine strength. Cytoplasmic detail is more distinct at a greater formaldehyde strength.

#### B. Preservation of Specimens

##### 1. Stock "MF" solution (stable)

Distilled water-----	50 ml
Formaldehyde (USP)-----	5 ml
Tincture of merthiolate (1:1,000)* -----	40 ml
Glycerine -----	1 ml

Store in brown bottle.

##### 2. Lugol's Iodine Solution (prepared fresh every 3 weeks)

Iodine crystals (powdered) -----	5 gm
Potassium iodide-----	10 gm
Distilled water -----	100 ml

Dissolve the KI in the distilled water. Add the iodine crystals slowly and shake until dissolved. Filter.

Mix solutions 1 and 2 together as indicated below to prepare MIF preservative.

\* It is necessary to use Tincture of Merthiolate No. 99 (1:1,000) Lilly (Eli Lilly & Co., Indianapolis, Indiana) since this contains eosin which is the stain ingredient in the second stage.

## TECHNIQUE

### A. Wet Mounts

1. Place 1 drop of distilled water on slide using standard medicine dropper.
2. Add 1 drop of MIF stain.
3. Comminute a portion of the fecal specimen in this mixture.
4. Mount with coverslip, seal, and examine. The preparation may be examined later without loss of staining or details.

### B. Preservation of Specimens

1. Add 2.35 ml MF stock to 0.15 ml Lugol's solution immediately before use.

Prior addition of the iodine causes a dense precipitate to be formed and the iodine fails to act satisfactorily on the protozoa.

2. Thoroughly mix a portion of the feces about the size of a pea in the MIF solution.

Do not use too much feces. For larger samples, use increased amounts of the MIF stain in the same proportions of MF stock and iodine solutions.

3. To examine, take a drop of fluid from the top layer of the sedimented feces and place on a slide. This gives a preparation roughly comparable in density to a direct smear.

## STAIN REACTIONS

The staining reaction in both direct smears and preserved specimens is divided into two phases: (1) an iodine phase in which cysts and trophozoites stain a yellowish-green or yellow-brown color, and (2) an eosin phase which replaces the iodine and is permanent.

In the iodine phase, nuclei stain dark brown and in the eosin phase, dark red to jet black. Cytoplasm of the trophozoites and cysts change from yellowish-green or brown in iodine to an eosin color in the second stage. Occasionally, refractory mature cysts are encountered which fail to stain in the eosin phase. Nuclear elements of all species except *Dientamoeba fragilis* are fairly well defined. Glycogen appears as a dark brown area in the iodine stage and as a colorless area in the eosin stage. Chromatoid bodies are characteristic in appearance. Flagella may be detected on flagellate trophozoites. Helminth eggs also stain and retain their normal characteristics. Fecal debris stains more deeply brown than parasites and blood and tissue cells. No distortion occurs, but for good contrast, a blue filter should be used.

## REFERENCES

Sapero, J.J., Lawless, D.K., and Strome, C.P.A. 1951. An improved iodine-staining technique for routine laboratory diagnosis of intestinal protozoa. *Science* 114:550-551.

Sapero, J.J., and Lawless, D.K. 1952. The "MIF" stain preservation technique for the identification of intestinal protozoa. *Am. J. Trop. Med. Hyg.* 2:613-619.

MODIFIED ZINC SULFATE CONCENTRATION  
TECHNIQUE FOR STOOL EXAMINATION

The zinc sulfate centrifugal flotation procedure was the first concentration technique developed for the recovery of both helminth eggs and larvae and protozoan cysts. Operculated eggs and those of the schistosomes are not recovered, however. The use of water for preparing the fecal suspension will destroy Blastocystis and remove some of the debris usually present. The original method developed by Faust et al. (1938) included two steps which are omitted in the modified procedure described below. These were 1) preparation of a fecal-water suspension in a beaker and 2) straining a portion of the suspension through gauze into a small tube. In the modified method, the suspension is prepared directly in the tube. In addition, unless the specimen is very oily, the number of washings by centrifugation is reduced to one.

Organisms which rise to the surface of the solution will begin to sink after about 1 hour. Therefore, for optimum recovery, slide mounts should be prepared as soon as the technique is completed. Since prolonged exposure to zinc sulfate may cause small cysts to distort and become difficult to recognize, mounts should not be allowed to stand for several hours before being examined.

PREPARATION

1. Add 331 gm of zinc sulfate (USP) to 1 liter of warm tap water. A technical grade of zinc sulfate can also be used if the insoluble salts are removed from the solution by filtering before use.
2. After the zinc sulfate is thoroughly dissolved, check the specific gravity with a hydrometer. It should read 1.18. If the reading is not correct, adjust the specific gravity by adding water or zinc sulfate as needed.
3. Some workers prefer a specific gravity of 1.20. This is prepared by using a slightly larger amount of zinc sulfate. This solution of higher specific gravity should always be used when a formalinized fecal specimen is to be examined.

TECHNIQUE

1. Using two applicators, comminute a fecal sample about the size of a small pea in a 100x13-mm tube half filled with tap water. Make certain that all obvious particles are broken up and that an even suspension is formed.
2. Add additional tap water until the tube is two-thirds full.
3. Centrifuge this preparation for 1 minute at approximately 2,500 r/min (650 g).
4. Pour off the supernatant fluid into a container holding a disinfectant, for example, cresol or amphyll.
5. Repeat this washing only if the stool is extremely oily.
6. Add enough zinc sulfate solution to fill the tube half full.

7. Using an applicator, break up the packed sediment very thoroughly and suspend it in the solution.
8. Add additional zinc sulfate solution to fill the tube to within 1/2 inch of the top.
9. Centrifuge this suspension for 1 minute at 2,500 r/min (650 g).
10. Without shaking or spilling, carefully place the tube in a rack.
11. The material in the surface layers can be removed in either of two ways:
  - a) by raising the fluid level to form a meniscus on top or b) by removing a portion of the surface film with a wire loop or pipette.

A. Raising Fluid Level

- (1) Slowly fill the tube to the brim with zinc sulfate without allowing any runover. Using a pipette or dropper, add the solution down the side of the tube to prevent disturbing the surface film.
- (2) Place a clean, grease-free #1 coverslip (22x22 mm) on top of the tube so that the under surface touches the meniscus. Leave undisturbed for about 10 minutes. No bubbles of air should be present.
- (3) Deftly remove the coverslip with a straight, upward motion. A drop containing eggs, larvae, and cysts (if present) will adhere to the underside of the coverslip.
- (4) Lower this onto a drop of iodine stain placed on a clean 3x2-inch slide. Seal the preparation.

B. Removing Surface Film (Preferred Method)

- (1) Make a small wire loop so that the loop is at right angles to the stem.
- (2) Slide the loop gently under the surface film and remove 2 or 3 loopfuls of material to a 3x2-inch slide.
- (3) A capillary pipette may also be used to transfer a portion of this film to a slide.
- (4) Add a drop of iodine, cover with a coverslip, and seal the preparation.

12. Examine for eggs, larvae, and cysts.

REFERENCE:

Faust, E.C.; D'Antoni, J.S.; Odom, V.; Miller, M.J.; Peres, C.; Sawitz, W.; Thomen, L.; Tobie, J.; and Walker, J.H. 1938. A critical study of clinical laboratory technics for the diagnosis of protozoan cysts and helminth eggs in feces. *Am. J. Trop. Med.* 18:169-183.

FORMALIN-ETHER CONCENTRATION TECHNIQUE  
FOR STOOL EXAMINATION

This concentration procedure is efficient in recovering protozoan cysts and helminth eggs and larvae, including operculated and schistosome eggs (Ritchie, 1948). Less distortion of cysts occurs with this technique than with the zinc sulfate method, and it is more effective in concentrating formalinized specimens. H. nana eggs may be missed, however, and concentration of Giardia lamblia cysts and Iodamoeba bütschlii cysts may not be very good.

TECHNIQUE FOR FRESH SPECIMENS

1. Comminute a portion of stool about the size of a large marble or a small walnut in sufficient saline so that 10 ml of suspension will yield about 1 ml of sediment upon centrifugation. The suspension can be prepared in the carton in which it is submitted (if portions for permanent staining are removed first) or in a beaker or a flat-bottom paper cup. Water may be used in place of saline.
2. Strain about 10 ml of the suspension through a small funnel, containing wet gauze or cheesecloth into a 15 ml conical centrifuge tube. Use two layers of wide-mesh gauze or one layer of narrow mesh gauze. To conserve glassware, a cone-shaped paper cup (about a 4 ounce size) with the point cut off can be substituted for the funnel.
3. Centrifuge at 2,500 r/min (650 g) for 1-2 minutes. Decant supernatant. About 1 to 1-1/2 ml of sediment should be present. If the amount is much larger or smaller, adjust to the proper quantity in the following manner:

- a. Amount too large.

Resuspend the sediment in saline (or water) and pour out a portion. For example, if the amount is twice the desired quantity, pour out about half (or slightly less) of the suspension. Add saline (or water) to bring the fluid level to about 10 ml and centrifuge again.

- b. 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 can be determined from the amount of sediment; that is, if about half of the quantity necessary is obtained with the first centrifugation, strain another 10 ml into the tube. Centrifuge again.

It is not necessary to have an exact quantity of sediment in the tube, but the quantity should approximate the amounts indicated above. Too much or too little sediment will result in an ineffective concentration.

4. Resuspend the sediment in fresh saline (or water), centrifuge, and decant as before. This step may be repeated if a cleaner sediment is desired.

5. Add about 9 ml of 10% formalin to the sediment, mix thoroughly, and allow to stand for 5 minutes or longer. At this point, the formalin-feces mixture may be stoppered and saved until a later time.

Note: Plastic squeeze bottles for saline and formalin will facilitate dispensing these solutions into the tubes.

6. Add 3 ml of ether, stopper the tube, and shake vigorously in an inverted position for at least 30 seconds. Remove the stopper with care.
7. Centrifuge at 2,000 r/min (450-500 g) for 1 minute. Four layers should result as follows: 1) layer of ether, 2) plug of debris, 3) layer of formalin, and 4) sediment.
8. Free the plug of debris from the sides of the tube by ringing with an applicator stick and carefully decant the top three layers. Use a cotton swab to clean debris from the walls of the tube and prevent it from settling down into the sediment.
9. With a pipette, mix the remaining sediment with the small amount of fluid that drains back from the sides of the tube and prepare iodine and unstained mounts in the usual manner for microscopic examination. If not enough fluid is left in the tube, a drop of saline can be added to the sediment.
10. If examination of the specimen is delayed, add 1 or 2 ml of 10% formalin to the sediment and stopper the tube. Formalinized sediments may be kept for some time if they do not dry. Remove the excess formalin before making the mounts, otherwise the specimen may be too thin for satisfactory preparations.

#### TECHNIQUE FOR FORMALIN PRESERVED SPECIMENS

1. Thoroughly stir the formalinized specimen.
2. Depending on the size and density of the specimen, strain a sufficient quantity through wet gauze into a conical 15 ml centrifuge tube to give the desired amount of sediment indicated below. Usually 4 to 5 ml is sufficient unless the fecal suspension is thin.
3. Add tap water to make 10 ml of suspension, mix thoroughly, and centrifuge at 2,000 to 2,500 r/min (500-650 g) for 1 to 2 minutes.
4. Decant supernatant and, if desired, wash again with tap water. The amount of sediment should be about 1/2 to 3/4 ml. If too much or too little is present, adjust the quantity by the same methods described in step 3 for fresh material. In formalin-preserved specimens, the formalin has already clarified the feces to some extent and further clarification is caused primarily by the ether. Therefore, the reduction of the sample (or sediment) is not as great as with fresh feces, and the initial quantity must be less.
5. Add 9 ml of 10% formalin (preferably buffered) to the sediment and mix thoroughly.

6. Add 4 ml of ether, stopper the tube, and shake vigorously in an inverted position for at least 30 seconds. Remove the stopper with care.
7. Centrifuge at 2,000 r/min (450-500 g) for about 1 to 2 minutes. Four layers should result as stated above (Step 7).
8. Free the plug of debris from the sides of the tube by ringing with an applicator stick, and carefully decant the top three layers. Use a cotton swab to remove debris adhering to the glass.
9. Mix the remaining sediment with the small amount of fluid that drains back from the sides of the tube (or add a drop of saline, if necessary) and prepare iodine and unstained mounts in the usual manner for microscopic examination. After decanting the upper three layers (Step 8), a small amount of formalin may be added to the sediment, the tube stoppered, and mounts prepared later.

#### REFERENCE

Ritchie, L.S. 1948. An ether sedimentation technique for routine stool examinations. Bull. U.S. Army Med. Dept. 8:326.

LAND QY160 M531 1978  
Melvin, Dorothy M.  
Evaluation of techniques and  
establishment of laboratory

CONCENTRATION OF MIF PRESERVED SPECIMENS

Merthiolate-iodine-formaldehyde (MIF) preserved specimens can be concentrated in the same ways as the usual formalinized material. One of the best methods is the MIF-ether concentration (MIFC) technique which is similar to the formalin-ether technique except that MIF solution replaces the formalin. Since MIF preserves trophozoites as well as cysts, larvae and eggs, trophozoites may be found frequently in the sediment.

A. SEDIMENTATION

A certain amount of concentration is accomplished by simple gravity sedimentation of the specimen and taking the sample to be examined from the top layer of sediment since eggs, cysts, and trophozoites tend to collect in the upper layers.

Sedimentation can be speeded up by centrifuging the specimen (or a portion of it) at about 2,000 to 2,500 r/min (500-600 g) for 1 minute.

B. MIF-ETHER CONCENTRATION

1. Thoroughly mix the MIF fecal suspension and strain 10 ml into a 15 ml centrifuge tube. If the quantity of specimen is insufficient, add fresh MIF preservative to make 10 ml.
2. Add 3 ml of ether. Stopper with a rubber stopper and shake thoroughly for at least 1 minute. Remove stopper with care.
3. Centrifuge at 2,000 r/min (450-500 g) for 1 minute. Four layers will result: (1) ether (at the top), (2) plug of debris, (3) MIF solution, and (4) sediment (bottom).
4. With the tube in a horizontal position, loosen the plug with an applicator and carefully pour off everything except the sediment. Use a cotton swab to remove debris adhering to the glass.
5. Thoroughly mix the sediment with a capillary pipette and remove a portion to a slide, cover with a coverslip, seal, and examine.

It is not necessary to make an iodine preparation since the MIF solution is both a preservative and a stain.

REFERENCE

- Blagg, W., Schloegel, E.L., Mansour, N.S., and Khalaf, G.I. 1955. A new concentration technic for the demonstration of protozoa and helminth eggs in feces. *Am. J. Trop. Med. Hyg.* 4(1):23-28.