

DEPMEDS LABORATORY PROCEDURES  
DEPARTMENT OF CLINICAL SUPPORT SERVICES  
U.S. ARMY MEDICAL DEPARTMENT CENTER AND SCHOOL  
FORT SAM HOUSTON, TEXAS 78234-6137

MCCS-HCM      STANDING OPERATING PROCEDURE      10 June 2002

FORMALIN ETHYL-ACETATE CONCENTRATION

1. INTRODUCTION:

The chances of locating parasites in a specimen are substantially increased if the specimen is concentrated and cleared of fecal debris.

2. PRINCIPLE:

The formalin ethyl-acetate concentration procedure is a sedimentation technique utilizing centrifugation. The specimen goes through one formalin wash and is further clarified by the addition of ethyl acetate on a second wash.

3. SPECIMEN:

The specimen required is stool; either preserved with formalin or received unpreserved, with formalin added after macroscopic examination.

4. REAGENTS AND MATERIALS:

a. Materials provided in Con-Trate kit.

- (1) Filtering devices (funnel with metal mesh).
- (2) Disposable centrifuge tubes with caps.
- (3) Reagent A (Muco Pen X).
- (4) Reagent B (ethyl acetate).

b. Materials not provided.

- (1) Cotton-tipped applicator sticks.
- (2) Microscope slides.

- (3) 22 x 40 mm coverslips.
- (4) 10% formalin.
- (5) Centrifuge.
- (6) Pipettes.
- (7) Tube rack.
- (8) Squirt bottle.
- (9) Lugols Iodine (5 g iodine, 10 g potassium iodide, 100 ml distilled water. Dilute 1:5 with distilled water for working solution.
- (10) Vortex mixer

5. PROCEDURE:

NOTE: Use the commercially available fecal concentration devices (Fekal Con-Trate System).

- a. Strain about 3 ml of the specimen suspension through a disposable-filtering device (supplied with kit) into a 15 ml conical centrifuge tube.
- b. Fill the tube to 12 ml using a squirt bottle containing 10% formalin.
- c. Centrifuge at 2000 RPM for 5 minutes.
- d. Remove tubes from the centrifuge and pour off the supernatant.
- e. Prepare slides for acid fast bacilli (AFB) staining by adding one drop of the sediment to a glass slide; allow slide to air dry.
- f. Add formalin up to the 8-ml mark.
- g. Add ethyl acetate to the 12 ml mark; stopper the tube, and vortex. Allow the tube to stand for 3 minutes.
- h. Centrifuge at 2000 RPM for 5 minutes.
- i. Remove tube from centrifuge; remove the stopper and ream the area of fecal debris from the wall of the tube with an applicator stick.

- j. Pour off the supernatant and wipe the sides of the tube with a cotton-tipped applicator stick to prevent debris and ethyl acetate from contaminating the sediment.
- k. Make wet mounts from the sediment by adding one drop of sediment to one drop of Lugol's iodine on a microscope slide. Coverslip with a 22 x 40 mm coverslip and examine the preparation under the microscope for parasites.
- l. When examining the concentration preparations, scan the whole mount on low (10x) power. Use high-dry (40x) for identification. In addition to the complete scan on low power, examine at least 75 fields under high-dry.

6. RESULTS:

- a. Any identifiable parasites should be noted on the lab slip.
- b. Any suspicious findings that are not identifiable should be noted on the upper left-hand corner of the lab slip to aid in reading of the trichrome and AFB smears.
- c. Quantification of specific ova or larvae is made from the concentrated slide preparation.
- d. Quantification of protozoans is made from the trichrome stained smear.
- e. Refer to ANNEX C for identification criteria and flow charts.

7. QUALITY CONTROL:

Known positives should be run every week to ensure adequate recovery.

8. SAFETY:

- a. Gloves should be worn when processing specimens.
- b. All specimens should be treated as potential sources of infectious disease.
- c. Iodine is corrosive. Avoid ingestion or skin contact. Avoid contact with eyes.
- d. Ethyl acetate is flammable; keep away from heat, sparks, and flame. Ethyl acetate is harmful if swallowed or inhaled; use with adequate ventilation. Avoid contact with eyes. Wash thoroughly after handling.

- e. Formalin may be fatal if swallowed and harmful if inhaled. Exposure may create a cancer risk and may cause blindness. Formalin is combustible; keep away from heat, sparks, or flame. Avoid breathing vapor. Use only with adequate ventilation. Wash thoroughly after handling.

9. REFERENCES:

- a. Brooke, M.M. and Melvin, D.M., Laboratory Procedures for the Diagnosis of Intestinal Parasites, 3rd ed., U.S. Department of Health and Human Resources, Centers for Disease Control, 1982.
- b. Con-Trate Product Instructions, Con-Trate System. Trend Scientific, Inc., P.O. Box 12266, St. Paul, MN 55112.
- c. Garcia, L. et al., Diagnostic Medical Parasitology 4<sup>th</sup> Ed.. New York: Elsevier Science Publishing Co., 2001.