

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

MCCS-HCM

STANDING OPERATING PROCEDURE

June 10, 2002

TRICHROME STAIN

1. INTRODUCTION:

The trichrome stain provides a method for definitive identification of protozoan trophs, as well as a permanent record of all specimens examined. All specimens submitted for routine ova and parasite testing will have two slides prepared, stained with trichrome, and examined.

2. PRINCIPLE:

The cytoplasm of thoroughly fixed and well-stained cysts should be bluish-green, tinged in purple. The nuclear chromatin will appear dark. Identification and determination of parasites is made utilizing established criteria for parasites.

3. SPECIMEN:

Feces fixed in polyvinyl alcohol.

4. MATERIALS:

- a. Histological slide carriers.
- b. Histological staining dishes with lids.
- c. Reagent grade ethanol (70%, 90%, 95%, 100%).
- d. Histoclear.
- e. Glacial acetic acid.
- f. Iodine/alcohol (70% ethanol saturated with iodine crystals to get a dark tea color).
- g. Permanent mounting media (Cytoseal 280).

- h. 22 x 40 cover slips.
- i. Microscope.
- j. Immersion oil.
- k. Trichrome stain (Meridian or Trend).

5. PROCEDURE:

- a. After drying overnight on the slide dryer, place 10 slides in a carrier and run it through the series of histological staining dishes containing the reagents, according to the following regimen:
  - (1) Iodine/70% alcohol 10 min
  - (2) 70% alcohol 10 min
  - (3) 70% alcohol 5 min
  - (4) Trichrome stain 15 min
  - (5) 90% alcohol (+ 3 drops glacial acetic acid) Quick dip
  - (6) 95% alcohol Rinse 3 times
  - (7) 95% alcohol 5 min
  - (8) 95% alcohol 5 min
  - (9) 100% alcohol 10 min
  - (10) 100% alcohol 10 min
  - (11) 100% alcohol 10 min
  - (12) Histoclear 10 min
  - (13) Histoclear 10 min
  - (14) Histoclear 10 min
  - (15) Coverslip Air dry
- b. Examine the trichrome stains under the microscope on oil immersion (1000x). Examine each slide (2 per specimen) in a methodical manner

starting at one corner of the coverslip and proceeding to the far corner. Look at approximately 100 oil immersion fields per slide. Note any parasites seen on the patient's lab slip.

6. RESULTS:

- a. Identify and report any parasite noted in the trichrome smear by genus and species.
- b. Note quantity of protozoan parasites on the slide and report using the following guidelines.
  - (1) Rare -- one or two organisms per entire smear, or organism seen in the formalin-ethyl acetate concentration but not on the trichrome.
  - (2) Few -- 2 per 10 oil immersion fields.
  - (3) Moderate -- 3-9 per 10 oil immersion fields.
  - (4) Many -- 10 per 10 oil immersion fields.
- c. Report any WBCs noted on the trichrome smear according to the above guidelines above.
- d. Branching pseudohyphae and budding yeast should be reported when they occur in large numbers in specimens that were submitted in formalin-pva preservatives.
- e. Report Charcot-Leyden crystals, if present, using the guidelines above.

7. QUALITY CONTROL:

- a. The trichrome stain provides a permanent record of patient specimens. Negative specimens are kept 90 days. Positive specimens are kept 2 years or longer. Positive controls are run each time reagents are changed. All reagents except the Iodine/Alcohol (Station 1) and Trichrome (Station 4) are changed weekly. Positive control results are logged and the slides kept at least one year.
- b. More controls may be run during the week if the results of the slides indicate the stain may be weakening. If the stain weakens, replace Stations 6, 7, and 8 and leave the lid off of Station 4 for a couple of hours, replenishing the stain to get a volume of approximately 200 ml.

8. SAFETY:

- a. Ethanol is flammable. Avoid heat, sparks, and open flame. Avoid prolonged or repeated breathing of vapor or contact with skin. Do not take internally or get into eyes.
- b. Mounting media can cause skin irritation or burn. Inhalation can cause nausea and asphyxiation. Upon skin contact, immediately flush with copious amounts of water. Flush eyes for at least 15 minutes. Use only in a well ventilated area.
- c. Iodine is very corrosive. Avoid skin contact. Flush with copious amounts of water if exposed. Do not take internally.
- d. HistoClear can cause skin dryness and irritation. Avoid contact. If skin or eye contact occurs, rinse with generous amounts of water. Do not take internally. Avoid inhaling high concentrations of vapor over long periods.
- e. Do not take trichrome stain internally. Avoid contact with skin and eyes. If contact occurs, flush area with copious amounts of water.

9. REFERENCES:

- a. Beaver, P.C. and Jung, R.C., Clinical Parasitology. 9th ed., Philadelphia: Lea and Febiger, 1984.
- b. Brooke, M.M. and Melvin, D.M., Laboratory Procedures for the Diagnosis of Intestinal Parasites. 3rd ed., U.S. Dept. of Health and Human Services, Centers for Disease Control, 1982.
- c. Garcia, L.S. and Buckner, D.A., Diagnostic Medical Parasitology. 4th ed., New York: Elsevier Science Publishing Co., Inc., 2001.