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 02

MODIFIED ACID-FAST STAIN FOR CRYPTOSPORIDIUM

1. INTRODUCTION:

Cryptosporidium is a coccidian parasite recognized as a significant cause of diarrhea in man. Due to difficulty in differentiating this parasite from yeast, an acid-fast stain is used to make definitive identification of this organism.

2. PRINCIPLE:

Acid-fast organisms usually contain very high quantities of lipids in their cell wall making them relatively impermeable to most basic stains. However, once they are stained they retain the dyes very well and resist decolorization with acidified organic solvents. Cryptosporidium is an acid-fast organism, whereas yeasts are not.

3. SPECIMEN:

The required specimen is stool.

4. MATERIALS:

a. Centrifuge.

b. Centrifuge tubes.

c. Pasteur pipettes.

d. Microscope slide.

e. Pencil.

f. Staining carriers for staining.

g. Staining dishes.

h. Tap water.

i. Carbol Fuchsin

j. TB decolorizer

k. Methylene Blue

l. Positive and negative controls.

5. PROCEDURE:

a. Preparation of slides.

(1) Slides are prepared from the sediment obtained after the first centrifugation when performing the formalin-ethyl acetate concentration. Prepare the slides before adding ethyl acetate.

(2) Number the slide with a pencil; using a pasteur pipet, transfer one drop of the sediment from the concentration tube to the slide.

(3) Allow the slides to air dry.

(4) Prepare positive and negative control slides for each run.

b. Staining procedure.

(1) Place eight slides and a positive and negative control in a 10-slide carrier.

(2) Place the slide carrier into a staining dish containing Carbol Fuchsin for 10 minutes.

(3) Remove the carrier and place into an empty staining dish and rinse with water.

(4) Place the slide carrier into the staining dish containing TB decolorizer for one minute.

(5) Remove the slide carrier and again rinse with tap water.

(6) Place the slide carrier into the staining dish containing Methylene Blue for 5 minutes.

(7) Remove the slide carrier and rinse with tap water.

(8) Remove the slides from the slide carrier and allow to air dry.

(9) Read the slides.

6. RESULTS:

a. Cryptosporidium oocysts will appear as bright red oval or spherical structures measuring 4-6 microns in diameter. Internal structures will probably not be clearly distinguishable.

b. Yeast and fecal debris should appear as pale greenish blue.

7. QUALITY CONTROL:

a. Quality control slides consisting of positive and negative material are run with each set of slides.

b. The positive control should exhibit acid-fast Cryptosporidium oocysts and the negative control should exhibit no acid-fast organisms.

c. If either the positive or negative control fail to perform as expected, make new slides and restain.

8. SAFETY:

a. Cryptosporidium is considered to be a highly contagious organism. Care should be taken to avoid contamination.

b. The oocysts are resistant to most common disinfectants. Use 50% commercial bleach.

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. et al., "Techniques for the Recovery and Identification of Cryptosporidium Oocysts from Specimens." Journal of Clinical Microbiology. 18:285-190., 1983.

d. Garcia, L.S. and Buckner, D.A., Diagnostic Medical Parasitology. 4th ed., New York: Elseyier Science Publishing Co., Inc., 2001.