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Section: Parasitology Manual	Subject Title: Laboratory Procedures for Stool Examination	
Issued by: LABORATORY MANAGER	Original Date: March 13, 2000	
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Smears For Microsporidia (Modified Trichrome or Chromotrope Stain)

PRINCIPLE

Microsporidia (such as *Enterocytozoon bieneusi*, and *Encephalitozoon intestinalis*) are parasites that are important opportunistic pathogens in immunocompromised patients particularly those with AIDS. It is difficult to recognize Microsporidia in fecal samples because of background debris and their relatively small size (in the order of microns). Scanning electron microscopy is considered to be the gold standard in making a diagnosis, however it lacks sensitivity and is expensive and time consuming. Several staining methods of stool or biopsy samples can be used, however at the current time a modified chromotrope stain with 10X the usual amount of Chromotrope 2R is gaining favor as the most sensitive method to facilitate identification.

SPECIMENS

- Stool in SAF
- Duodenal aspirate in SAF

MATERIALS

Reagents:

Chromotrope 2R (Sigma)
Fast green (VWR)
Phosphotungstic acid (Sigma)
Glacial acetic acid (VWR)
90%, 95% ethyl alcohol (VWR)
methanol (VWR)
xylol (VWR)

Chromotrope stain

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| 1. Chromotrope 2R | 6gms |
| 2. Fast green | 0.15gms |
| 3. Phosphotungstic acid | 0.7gms |
| 4. Glacial acetic acid | 3mls |
| 5. Mix ingredients gently and allow to stand for 30 minutes. Then add 100 mls distilled water. | |

Acid alcohol for Chromotrope 2R stain

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| 1. ethyl alcohol | 995.5mls |
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2. Glacial acetic acid 4.5 mls
3. Mix gently.

Equipment:

Light microscope with ocular micrometer and set for Kohler illumination
Glass microscope slides
Pasteur pipets
Glass cylinders
Staining containers
Covers slips 22 X 40 mm
Slide container
Sharps container

QUALITY CONTROL

1. Positive control slides should be used every time the staining procedure is performed. If the control slides are not satisfactory, the unknowns cannot be interpreted.
2. All staining dishes should be covered to prevent evaporation of reagents. The reagents should be made up on Monday morning to ensure accurate staining.
3. All “out-of-control” results should be reported to the Laboratory Director for action.
4. When the smear is thoroughly fixed and the stain is properly prepared the spores will be ovoid and refractile with the spore wall being bright pinkish red. Occasionally the polar tube will be seen as a stripe or a diagonal line across the spore.
5. The microscope should be calibrated (within 12 months or anytime there is an alteration of the optics).
6. Known positive control slides, Kodachrome slides, photographs and reference books are available to help with morphologic identification.

PROCEDURE

This is considered to be a non-routine procedure therefore it should only be performed by experienced personnel

Special Safety Notes

Methanol, ethanol and xylol are flammable. The acid alcohol solution is corrosive.

1. Shake the SAF stool to resuspend thoroughly.
2. Prepare thin smear with 10-20 µl of the stool (unconcentrated) on a slide.
3. Air dry the smear thoroughly at room temperature or 60 C.

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STAINING

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| 4. Fix the dried smears in absolute methanol | 5 minutes |
| 5. Rinse in tap water | 1 minute |
| 6. Hematoxylin stain | 10 minutes |
| 7. Rinse in tap water | 1 minute |
| 8. Chromotrope stain | 90 minutes |
| 9. Rinse in acid-alcohol | 10 seconds |
| 10. Rinse in 95% methanol | 10 seconds |
| 11. Dehydrate in 95% methanol | 5 minutes |
| 12. Dehydrate in 100% methanol | 5 minutes |
| 13. Dehydrate in 100% methanol | 5 minutes |
| 14. Dehydrate in xylol | 5 minutes |
| 15. Dehydrate in xylol | 5 minutes |
| 16. Drain and coverslip with Entellan mount. | |
| 17. Examine under oil immersion lens. | |

EXAMINATION

Examine smears under oil immersion (x1000) with overlapping fields and examine at least 200 fields.

REPORTS

1. The microsporidial spore wall should stain pink to red and may display a horizontal or diagonal stripe which represents the polar tube. These can be distinguished from spores by the overall morphology and shape.
2. Report the organism. Example: Microsporidia present (*Enterocytozoon bieneusi*, or *Encephalitozoon intestinalis* are probable agents if specimen is feces.)

PROCEDURE NOTES

1. Positive control slides of microsporidia in SAF preserved stool are included every time the staining procedure is performed. If the controls are not satisfactory the patient samples can not be interpreted.
2. Because of difficulty in getting the stain to penetrate the spore wall, prepare thin smears and do not reduce the staining time. Make sure slides are not left too long in decolorizing agents (acid-alcohol).

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3. In the final stages of dehydration, the 100% ethanol and xylol should be kept as free from water as possible. Tight fitting caps to prevent evaporation and absorption of moisture are essential. Change these solutions weekly.

LIMITATION OF PROCEDURE

1. It is preferred to use unconcentrated stool since there are anecdotal concerns that concentration procedures may actually result in a loss of microsporidial spores.
2. Definitive species identification is only possible with electron microscopy or molecular methods.

AUTHOR

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