Smears For Coccidia

PRINCIPLE

Coccidial infections, particularly those caused by *Cryptosporidium* species are considered a significant cause of acute and chronic diarrhea. In immunocompromised hosts, particularly AIDS patients, these infections can be chronic and life threatening. Oocysts of cryptosporidia, cyclospora, isospora, and sarcocystis are acid fast and will be detected with a modified acid fast stain. They are not reliably detected using a iron hematoxylin stain.

SPECIMEN

- SAF-Preserved stool
- Fresh stool (within 30 minutes of passage-- by arrangement only)
- bile
- duodenal aspirates or small bowel biopsies
- external QC samples
- rarely Cryptosporidium may be identified from respiratory tract samples from compromised hosts.

MATERIALS

Reagents:

Basic fuchsin (commercial product, VWR)
Liquid phenol (commercial product, VWR)
95% ethyl alcohol (commercial product, VWR)
10% Tween 80 (commercial product, VWR)
Malachite green (commercial product, VWR)
Decolorizer (commercial product, PML)
Distilled water
Carbolfuchsin Stain Solution (PML, or if unavailable use following)

1. Basic fuchsin 21gms
2. Liquid phenol (85%) 85.2mls
3. 95 % alcohol 150mls
4. 10% Tween 80 7.5ml

5. Gently heat mixture in 45 C incubator for 24 hours to dissolve the crystals. Add distilled water to bring volume to 1.8L.

Malachite Green

1. Malachite Green (Commercial Product) 0.5 gms
2. Distilled water 100 mls
3. Mixed gently and thoroughly until dissolved.

Equipment:

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. A quality control slide of *Cryptosporidium parvum* is performed each week or whenever stains are changed.
2. Cryptosporidia and other coccidia stain pink-red. Oocysts of Cryptosporidia measure 4 to 6 microns and often 4 sporozoites may be identified internally.
3. Record all QC results. Report any "out-of-control" results to Laboratory Director for action.
4. Known positive slides and reference books are available to aid in morphologic identification.
5. The microscope should be calibrated (within twelve months or when ever the optics are altered).

PROCEDURE

Special Safety Notes: Assume samples are biohazards and use universal precautions. Handle the decolorizer solution with care since it contains sulphuric acid. Always dilute acid into water. Never add water to concentrated acid.

1. Fix the smear (from formalin-ether concentrate step 9) in absolute methanol for five minutes.
2. Flood the slide with carbolfuchsin and stain for five to ten minutes at room temperature.

3. Gently wash with running tap water for 60 sec.
4. Decolorize with decolorizer for 30-60 seconds (until no more color runs from the slide).
5. Gently wash with running tap water for 60 sec.
6. Counterstain with 1% malachite green for one minute.
7. Gently wash with running tap water for 60 sec.
8. Air dry slides in vertical position and mount with Entellan.

EXAMINATION OF SMEARS

1. Examine as per permanent stains for iron hematoxylin.

REPORTING

1. The oocysts of *Cryptosporidium* and *Isospora* species will stain pink to red. If Cyclospora oocysts are present they resemble cryptosporidia but are larger and lack definite internal structure.
2. Report the organism and stage. Do not use abbreviations, eg. *Cryptosporidium parvum* oocysts, *Isospora belli* oocysts.
3. Yeast will stain green.

LIMITATIONS OF PROCEDURE

1. Light infections with cryptosporidia and cyclospora may be missed. Immunoassays may be more sensitive.
2. Multiple specimens must be examined since the number of oocysts may vary day to day.
3. Other organisms that stain modified acid fast positive including Nocardia, and Microsporidia may be difficult to identify.

AUTHOR

Ian Crandall

REFERENCES

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Weber, R, et al Improved light microscopical detection of microsporidia spores in stool and duodenal aspirates. NEJM 326: 161-166 1992