Permanent Stained Smear Methods

PRINCIPLE

Permanent stain smears are used primarily for the identification of trophozoites, occasionally cysts, and for the confirmation of species. Small organisms missed by other examinations may be found on stain smears. Although experienced microscopists can identify certain organisms on wet prep most identification should be considered tentative until confirmed by a permanent stained slide. Permanent stains include iron hematoxylin for the detection and identification of intestinal protozoa and modified acid fast staining for the identification and detection of coccidia oocysts including *Cryptospordium, Isospora* and *Cyclospora* species.

SPECIMEN

- Stool in SAF
- duodenal aspirates in SAF
- fresh stool (within 30 minutes of passage-- by arrangement only) or aspirate specimens

MATERIAL

Reagents

Normal saline (0.85%) (commercial product, PML)
Mayer's albumin (Commercial product, PML)
If preparing in house:

1. add an equal quantity of glycerine to fresh egg white
2. mix thoroughly and gently
3. add a few crystals of thymol to prevent fungal overgrowth

Equipment:

Fume hood
Safety centrifuge
Microscope with ocular micrometer and set for Kohler illumination
Funnel filter
Applicator sticks
Centrifuge tubes and caps
Clean microscope slides (frosted) are recommended so that identifying information can be written on frosted end with an ordinary lead pencil.
Pasteur pipettes

Sharps discard
Cover slips (22x40mm)
Cotton tip applicator

QUALITY CONTROL

1. Check all reagents each time they are used and ensure that the formalin and saline solutions appear clear without any visible contamination.
2. Ensure that all reagents and chemicals used have not expired.
3. The microscope should be calibrated within the last 12 months or after any changes in the optics.
4. All QC results should be appropriately recorded (see QC binder) and “out-of-control" results (i.e. failure to recover and identify positive control organisms) should be immediately referred to the laboratory director for action. Even in the absence of reported problems, all QC results will be reviewed by the lab director or associate at least monthly.
5. Whenever possible, one technologist will read the concentrate and a different one will read the smear. Discordant results will be recorded and resolved with the lab director before reporting.
6. Whenever possible, multiple samples from the same individual should be read by a different technologist.

PROCEDURE

1. Proceed with SAF preserved stool formalin-ether concentration method until step 9.

2. Decant supernatant and drain the tube well.

3. Label one microscope slide with the sample. Prepare additional smears for each QMP-LS and any referred specimens for future internal QC.

4. Using a Pasteur or plastic transfer pipette, place 1 drop of Mayer’s albumin on a glass slide (place the Mayer’s Albumin first to avoid contamination).

5. Mix sediment with an applicator stick and transfer an equal amount of sediment to the slide and mix well with the applicator stick.

6. Spread the mixture over the slide making thick and thin areas for examination (approximately 4 to 6 bands per slide).

7. Allow the slide to air dry at room temperature THOROUGHLY before staining. You can gently warm slides (37^{o}C for 30 minutes) to aid drying.

8. Proceed with the formalin-ether concentration technique on the remaining sediment.

PROCEDURE NOTES

1. Do not heat slides to speed up drying.
2. If too much Mayer's albumin is used the smears will not dry. If too little is used the smears will not adhere well to the slides.
3. Prepare additional smears for each QMP-LS and any referred specimens to be used for future internal QC.

LIMITATIONS

1. Confirmation of intestinal protozoa (both trophozoites and cysts) is the primary purpose of this technique.

2. Most problems encountered in subsequent staining of permanent smears will result because the specimen is too old, the smears are made too densely or fixation is inadequate.

AUTHOR

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REFERENCES

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