

Trichrome Stain, Wheatley Modified for Fecal Smears – Technical Memo

SOLUTION: 250 ml
Trichrome Stain, Wheatley Modified Part 10351A

Additionally Needed:

Alcohol, Ethyl Denatured, 70%	Part 10844
Acetic Acid, Glacial, ACS	Part 10010
Alcohol, Ethyl Denatured, 95%	Part 10842
Alcohol, Ethyl Denatured, 100%	Part 10841
Xylene, ACS	Part 1445

For storage requirements and expiration date refer to individual bottle labels.

APPLICATION:

Newcomer Supply Trichrome Stain, Wheatley Modified provides a ready-to-use solution for rapid staining and permanent slide preparation for the detection and identification of intestinal protozoa, flagellates and microsporidia in fecal smears.

METHOD:

Fixation: According to laboratory protocol for fecal/stool samples
a. See Procedure Note #1.

Solutions: All solutions are manufactured by Newcomer Supply, Inc.

All Newcomer Supply stain procedures are designed to be used with Coplin jars filled to 40 ml following the staining procedure provided below.

STAINING PROCEDURE:

1. Prepare within an accepted time frame, a well-made fecal smear/film per your laboratories protocol, with a focus on uniform distribution of material.
 - a. See Procedure Notes #2, #3 and #4.
2. Unless a mercuric chloride-free formulation is used, smears prepared from PVA and Schaudinn fixed material must proceed through an Iodine Alcohol Rinse to remove mercuric chloride. Prepare Iodine Alcohol Rinse; combine and mix well.
 - a. 70% Ethyl Alcohol (10844) 40 ml
 - b. Iodine Crystals; add to produce an amber color
 - c. PVA fixed smears; immerse for 10-20 minutes.
 - d. Schaudinn fixed smears; immerse for 3 minutes.
 - e. Skip this step for non-mercuric chloride fixatives.
3. Place slides in 70% ethyl alcohol; two changes 3-5 minutes each.
 - a. See Procedure Note #5.
4. Stain in Trichrome Stain, Wheatley Modified for 6-8 minutes.
5. Prepare Acid-Ethanol Solution; combine and mix well.
 - a. 95% Ethyl Alcohol (10842) 100 ml
 - b. Acetic Acid, Glacial, ACS (10010) 0.5 ml
6. Differentiate slides in Acid-Ethanol Solution; 5-10 seconds.
7. Rinse quickly in 95% ethyl alcohol; 2 dips.
8. Dehydrate in two changes of 95% ethyl alcohol; 5 minutes each.
9. Dehydrate in two changes of 100% ethyl alcohol; 3 minutes each.
10. Clear in three changes of xylene, 10 dips each; coverslip with compatible mounting medium.

PROCEDURE NOTES:

1. Stool specimens received in PVA and SAF fixatives or freshly fixed in Schaudinn Solution should be fixed and prepared according to manufacturer's recommendations.
2. Dry PVA fixed smears for 1 hour at 35-37°C or overnight at room temperature prior to staining.
3. Do not allow Schaudinn's fixed smears to dry out at any time during the procedure.
4. Drain staining rack/slides after each step to prevent solution carry over.
5. Inadequate removal of iodine in 70% ethyl alcohol may result in compromised staining. Lengthen the exposure time and/or change the 70% ethyl alcohols more frequently.

RESULTS:

Nuclear chromatin & chromatoid bodies	Red to purple
Bacteria & ingested RBC's	Red to purple
Cytoplasm of cysts	Blue/green with purple tinge
Cytoplasm of protozoan trophozoites	Blue/green with purple tinge
Microsporidia spores	Pink/red wall with clear interior
Background	Green

REFERENCES:

1. Bauer, John D. *Clinical Laboratory Methods*. 9th ed. St. Louis: Mosby, 1982. 951-952.
2. "Laboratory Identification of Parasitic Diseases of Public Health Concern." Centers for Disease Control and Prevention. November 2013. Accessed November 4, 2015. www.cdc.gov/dpdx/diagnosticProcedures/stool/specimencoll.html
3. Ryan, Norbert, G. Sutherland, K. Coughlan, M. Globan, J. Doubletree, J. Marshall, R.W. Baird, J. Pedersen, and Brian Dwyer. "A New Trichrome-Blue Stain for Detection of Microsporidial Species in Urine, Stool and Nasopharyngeal Specimens." *Journal of Clinical Microbiology* 31.2 (1993): 3264-3269.
4. Sheehan, Dezna C., and Barbara B. Hrapchak. Theory and Practice of Histotechnology. 2nd ed. St. Louis: Mosby, 1980. 250.
5. Wheatley, W.B. "A Rapid Staining Procedure for Intestinal Amoeba and Flagellates." *American Journal of Clinical Pathology* 21 (1951): 990-991.
6. Modifications developed by Newcomer Supply Laboratory.