

## 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 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 provided staining procedure.

**STAINING PROCEDURE:**

- Prepare a well-made fecal smear from a fresh or fixed specimen with focus on uniform distribution of material.
  - Fix fresh smears according to recommendations.
- Allow smears to dry for an hour at 35-37°C or overnight at room temperature.
- Place slides in 70% ethyl alcohol; two changes 3 minutes each.
  - See Procedure Note #2.
- Stain in Trichrome Stain, Wheatley Modified for 8-10 minutes.
- Prepare Acid-Ethanol Solution; combine and mix well.
  - Alcohol, Ethyl Denatured, 95% (10842) 100 ml
  - Acetic Acid, Glacial, ACS (10010) 0.5 ml
- Differentiate slides in Acid-Ethanol Solution; 3-5 seconds.
- Rinse quickly in 100% ethyl alcohol; 2 dips.
- Dehydrate in two changes of 100% ethyl alcohol; 3 minutes each.
- Clear in three changes of xylene, 10 dips each; coverslip with compatible mounting medium.

**PROCEDURE NOTES:**

- Stool specimens received in modified polyvinyl alcohol (PVA) and sodium acetate-acetic acid-formalin fixatives (SAF) or freshly fixed smears in a modified Schaudinn Solution should be fixed and prepared according to manufacturer's recommendations.
- Drain slides after each step to prevent solution carry over.

**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:**

- Bauer, John D. *Clinical Laboratory Methods*. 9th ed. St. Louis: Mosby, 1982. 951-952.
- "CDC - DPDx - Diagnostic Procedures - Stool Specimens," [www.cdc.gov/dpdx/diagnosticprocedures/stool/staining.html](http://www.cdc.gov/dpdx/diagnosticprocedures/stool/staining.html).
- 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.
- Sheehan, Dezna C., and Barbara B. Hrapchak. *Theory and Practice of Histotechnology*. 2nd ed. St. Louis: Mosby, 1980. 250.
- Wheatley, W.B. "A Rapid Staining Procedure for Intestinal Amoeba and Flagellates." *American Journal of Clinical Pathology* 21 (1951): 990-991.
- Modifications developed by Newcomer Supply Laboratory.