

WHEATLEY'S MODIFIED TRICHOME STAIN

DESCRIPTION

Wheatley's Trichrome Stain technique for fecal specimens is a modification of the Gomori staining procedure for tissue. Wheatley's modification provides a rapid procedure that produces uniform, well-stained smears of intestinal protozoa, human cells, yeast cells, and artifact material. It is known to be the best stain to support stool examinations for intestinal protozoa.

SPECIMEN NOTES

1. Dry PVA fixed smears for 1 hour at 35-37°C or overnight at room temperature prior to staining.
2. Do not allow Schaudinn's fixed smears to dry out at any time during the procedure.
3. Fecal smears prepared from SAF-preserved stool material do not require the iodine step.
4. If the smears fall off slide, the specimen might have been inadequately dried on the slide (particularly in the case of PVA-fixed specimens) or the slides may have an oily film residue

STAINING PROCEDURE

Smears prepared from PVA and Schaudinn's fixed material must proceed through an Iodine Alcohol Rinse to remove mercuric chloride. If slides are produced from SAF preserved or mercuric chloride free fixative, skip to Step 2.

1. Prepare Acid Alcohol, 0.5% Solution and save until needed. Combine and mix well.

95% Ethanol	100 mL
Acetic Acid, Glacial	0.5 mL
2. Immerse slide in Iodine Alcohol

PVA fixed smears	5-20 minutes
Schaudinn's fixed smears	3 minutes
3. Immerse slide in 70% Ethanol (2 changes) 5 minutes each
4. Stain in Wheatley's Modified Trichrome Stain 6-8 minutes
5. Differentiate slides in Acid-Ethanol Solution 5 seconds
6. Dehydrate in 95% ethyl alcohol (2 changes) 3 minutes
7. Dehydrate in 100% ethyl alcohol (2 changes) 3 minutes
8. Clear in xylene (2 changes) 1 minute
9. Coverslip with acrylic mounting medium

RESULTS

Bacteria, Red Blood Cells	Red to purple
Nuclear chromatin, Chromatoid bodies	Red to purple
Cytoplasm of Cysts	Blue to green with a hint of purple
Cytoplasm of Protozoan trophozoites	Blue to green with a hint of purple
Microsporidia spores	Pink-red wall with colorless interior
Background	Green

REFERENCES:

1. "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.
2. 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.
3. NCCLS. 1997. Procedures for the recovery and identification of parasites from the intestinal tract, Approved Guideline, M28-A, National Committee for Clinical Laboratory Standards, Villanova, PA

Cat. No.	Description	Size
ES37263	Wheatley's Modified Trichrome Stain	8oz