

PROCEDURE NOTES:

1. It is very important that slides are properly fixed. Specimens that are not properly fixed will exhibit distorted morphology or poor staining.
2. Slides should be drained on a paper towel to remove excess fluid between solutions.
3. Incomplete removal of mercuric chloride will result in crystals which may make slide difficult to read and may cause a failure to identify organisms. 70% ethanol plus iodine should be changed at least weekly to maintain the strong lea color. Too long in this solution may also adversely affect staining.
4. If slides appear predominantly green, this may be due to inadequate removal of the iodine. If this occurs, increase the time in steps 2 and 3 or change the 70% ethanol more frequently.
5. It is essential the slides be quickly rinsed in 100% ethanol following the 90% ethanol plus acetic acid. This 90% ethanol plus acetic acid decolorizes the slide and it is important to rinse the slide free of acid to prevent continued destaining. Ethanol in step 6 should be changed frequently.

For assistance please call our Technical Service Department toll-free at 1-800-528-0494 between the hours of 8 A.M. and 5 P. M. Eastern Standard Time.

CAS Numbers

Trichrome Stain	
Chromotrope	4197-07-3
Light Green	5141-20-5
Fast Green	2353-45-9
Phosphotungstic Acid	12501-23-4
Acetic Acid	64-19-7
DI Water	7732-15-5

BIBLIOGRAPHY

1. ASMT, Recommended Procedures for the Examination of Clinical Specimens Submitted for the Diagnosis of Parasitic Infections. Am J Med Technol, 1978; 44:1101-1106.
2. Belding DC. Textbook of Clinical Parasitology, New York; D. Appleton-Century Company, 1947.
3. Biological Stain Commission! Staining Procedures. Baltimore: Williams and Wilkins, 1960
4. Burrows RB. Microscopic Diagnosis of the Parasites of Man, New Haven. Yale University Press. 1965
5. Garcia MS. Bruckner D. Diagnostic Medical Parasitology, 3rd ed Washington D.C.. ASM Press, 1997:594-822.
6. Gomon G. A rapid one step trichrome method American Journal of Clinical Pathology 1950;Vol 20:661-664
7. Horen WP Modification of Schaudin's Fixative J Clin Microbiol 1981;13:204-205.

8. Laboratory Manual of Special Staining Technics. Washington, D.C.:U.S. Armed Forces Institute of Pathology, 58-59.
9. Melvin DM, Brooke MM. Laboratory Procedures for the Diagnosis of Intestinal Parasites. Atlanta: U.S.H.H.S.(CDC), 1982;82:8282.
10. Paik G, Suggs MT. "Reagents, Stains, and Miscellaneous Test Procedures." 1974. Chapter 96 in Manual of Clinical Microbiology, Second Edition, A.S.M., Washington, D.C.
12. Wheatley, W.B., 1951, A rapid staining procedure for intestinal amoeba and flagellates. American Journal of Clinical Pathology, Vol. 21:990-991.

Protocol Trichrome Stain

Protocol Trichrome, 500 mL

Item

23-005-38

Other Protocol Parasitology Products

Protocol LV-PVA Vials, 20/pk
Protocol Clean Vials, 20/pk
Protocol Modified (Cu) PVA Vials, 20/pk
Protocol MF Vials, 20/pk
Protocol Zn PVA Vials, 20/pk
Protocol SAP Vials, 20/pk
Protocol C&S Vials 20/pk
Protocol 10% Buff. Formalin Vials, 20/pk
Protocol Cary Blair Vials, 20/pk
Protocol LV-PVA/ Formalin Kit, 2x10 vL/pk
Protocol LV-PVA / Clean Kit, 2x10 vL/pk
Protocol Modified (Cu) PVA/Formalin, 2x10 vL/pk
Protocol SAF / C&S Kit, 2x10 vL/pk
Protocol SAF / Clean Kit, 2x10 vL/pk
Protocol Zn-PVA 110% Formalin Kits, 2x10 vL/pk
Protocol PVA/ Formalin/Clean, 3x10 vL/pk
Protocol LV-PVA/Formalin/C&S, 3x10 vL/pk
Protocol SAFIClean/C&S, 3x10 vL/pk
Protocol Zn-PVA/ Formalin/ Clean, 3x10 vL/pk
Protocol Mod. (Cu) PVA/ Formalin/Clean, 3x10 vL/pk
Protocol Zn-PVA/ Formalin/ C&S, 3x10 vL/pk
Protocol MIF Kit
Protocol MIF Bulk Kit
Protocol LV-PVA Bulk, 500 mL
Protocol Zn-PVA Bulk, 500 mL
Protocol Bulk Tubes and caps, 15 mL
Protocol Iodine, 50 mL
Protocol 15 mL Concentration System, 50 ea
Protocol 15 mL Concentration System, 4x50 ea
Protocol 50 mL Concentration System, 120 ea
Protocol Ethyl Acetate

Item

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Protocol™

Parasitology System

Application for Trichrome Stain

PURPOSE:

Protocol Trichrome stain is a rapid staining procedure which produces uniformly stained smears of intestinal protozoa, human cells, yeast cells and artifact material. Trichrome stain provides excellent contrast facilitating the detection of intestinal parasites while differentiating organisms from fecal debris and artifacts.

PRINCIPLES:

Diagnosis of enteric parasitic disease is confirmed by isolation and identification of pathogenic organisms in fresh stool specimens. The detection and identification of intestinal parasites frequently depends on the examination of the permanent stained smear. These slides provide not only a permanent record of organisms but may also be used for consultation if necessary. Unless the appropriate stain is used, the stained smear may be difficult to examine and identify. Smaller protozoan organisms that may be easily missed with direct smear or concentration methods are frequently seen on the stained smear. For this reason, it is recommended that a permanent stain is performed on every specimen submitted for parasite examination

CONTENTS:

Each bottle contains 500 ml- of Trichrome stain. Directions for use are also included

SPECIMEN PREPARATION:

Fresh Specimen

1. Prepare a thin smear of fresh material and immediately place in Schaudin's fixative- Allow to fix for a minimum of 30 minutes Specimen's may be fixed overnight. Smear should be thin enough so that newsprint is legible through focal material.
2. Slides should be placed in 70% alcohol to remove excess Schaudin's fixative prior to placement in iodine-alcohol (used with mercury-based fixatives).
3. if specimen is liquid, place 3-4 drops of PVA on the slide and mix with several drops of specimen. Allow slide to dry for several hours in a 37°C incubator or overnight at room temperature.
4. Proceed with the Trichrome stain procedure by placing in iodine-alcohol.

PVA-Fixed Specimen

1. Allow specimen to fix for at least 30 minutes in LV-PVA. Mix thoroughly with two applicator sticks. Pour a small amount of the LV-PVA fixed material onto a paper towel and allow to stand for three minutes. (Allow for absorption of the excess PVA.) Do not eliminate this step.
2. Using an applicator stick, apply (Do Not Smear) some of the stool material from the paper towel onto one or more clean glass slides to the edge of the slides.
3. Dry the slides overnight at room temperature or for several hours in a slide warmer or 37°C incubator. Accelerated drying is not recommended and may cause distortion of the specimen morphology. Do not proceed until the slides are completely dry.
4. Once the slides have dried completely, proceed with staining by placing into iodine-alcohol. There is no need for 70% alcohol rinse, as the PVA-fixed specimens are already dry.

NOTE: Slides made from very watery specimens may require additional time to dry completely.

SAF-Fixed Specimen

1. Allow specimen to fix for at least 30 minutes in SAF. If specimen appears mucoid, add 10 drops of surfactant and mix gently by swirling tube.
2. The Protocol SAF vials may be used with 15 mL or 50 mL conical centrifuge tubes (Protocol 15 mL Concentrate System or 50 mL Concentrate System work well for this application). Filter the SAF fixed specimen. Add at least 5 mL of filtered specimen to the centrifuge tube (the entire specimen may be used if desired).
3. Centrifuge the specimen for 10 minutes at 500 x g (1800-2200 rpm). Decant the supernatant. The final sediment should be approximately 0.5-1 mL. If necessary, adjust by adding specimen or resuspending the sediment in physiological saline and remove part of the suspension.
4. Place 1 drop of Mayers Albumin on the slide and wipe so that only a thin layer remains on the slide. Add 1 drop of

the SAP fixed sediment to the slide and allow the smear to air dry at room temperature for 30 Minutes prior to staining. (The SAF stool smear can also be post-fixed in Schaudin's fixative prior to staining).

5. After drying, the smear can be placed directly into 70Q/ alcohol to coagulate the albumin, prior to staining. When proceeding with staining, the iodine-alcohol step may be eliminated.

MIF-Fixed Specimen

1. Place 1 drop of Mayers Albumin on the slide and wipe so that only a thin layer remains on the slide. Add 1 drop of the MIF fixed sediment to the slide and allow the smear to air dry at room temperature for 30 minutes prior to staining,
2. After drying, the smear can be placed directly into 70% alcohol for up to 30 minutes to fix the slide. Proceed with staining by placing in trichrome stain and dip once in deionized water before acid alcohol step.

STAIN PROCEDURE:

- | | |
|---|---------------|
| 1. 70% ethanol plus iodine | 5-10 minutes |
| (Used with mercury-based fixatives to remove mercuric chloride) | |
| 2. 70% ethanol* | 5 Minutes |
| 3. 10% ethanol* | 3 Minutes |
| 4. Trichrome Stain | 10 Minutes |
| 5. 90% ethanol plus acetic acid | 1-3 Seconds |
| (Drain slides immediately and proceed with step 5.) | |
| 6. 100% ethanol | Dip 2-3 times |
| 7. 100% ethanol | 3 Minutes |
| 8. 100% ethanol* | 3 Minutes |
| 9. Xylene* | 5-10 Minutes |
| 10. Xylene* | 5-10 Minutes |
| 11. Mount with coverslip and mounting medium. | |
| (Securemount, Mounting Media X or T recommended). | |

*Slides may be held for up to 24 hours in these solutions with satisfactory results.

Note: Xylene substitutes may be used.

SOLUTIONS NOT PROVIDED:

Schaudin's fixative

Mercuric chloride, saturated aqueous sol.	600 mL
(110g HgCl ₂ per 1000 mL H ₂ O)	
Ethyl alcohol, 95%	300 mL

Immediately before use, add 5 mL of glacial acetic acid per 100 mL of stock solution.

70% Ethanol Plus Iodine

1. Prepare stock solution by adding iodine crystals to 70% alcohol until a dark solution is obtained. (1-2 g/100 mL)
2. To use, dilute the stock solution with 70% alcohol until a dark red-brown or strong tea color is obtained.

90% Ethanol Plus Acetic Acid

90% ethanol	99.5 mL
Acetic acid (glacial)	0.5 mL

RESULTS:

Protozoan trophozoites and cysts will be readily seen. *Entamoeba histolytica* will have a blue-green cytoplasm and cysts will also appear blue-green. *Entamoeba Coli* cysts will appear blue-green with a purple tint. Helminth eggs and larvae (wet smears from concentration are recommended) retain stain and will appear red-purple. Yeast may be identified and will appear green. Human cells such as RBC's will appear red. Karyosomes of nuclei, chromatoid bodies and chromatin material appear red-purple.

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