VOLU-SOL
BUSINESS OF CHEMICALS

TRICHROME STAIN

WHEATLEY AND PARASITOLOGY KIT COMPONENTS:

PARASITOLOGY STARTER KIT (VXF-019) **REFILL** (VXR-010)

SCHAUDINN'S FIXATIVE:	CARBOL XYLENE:	ETHANOL 70%:	ETHANOL 95%:
VXM-016 (16 oz.)	VXZ-016 (16 oz.)	VXB-032 (32 oz.)	VXC-032 (32 oz.)
VXM-032 (32 oz.)	VXZ-032 (32 oz.)	VXB-128 (128 oz.)	VXC-128 (128 oz.)
IODINE ALCOHOL:	**ACID ETHANOL 90%:**	**TRICHROME STAIN:**	**XYLENE:**
VXI-016 (16 oz.)	VXA-016 (16 oz.)	VXT-008 (8 oz.)	VXX-016 (16 oz.)
VXI-032 (32 oz.)	VXA-032 (32 oz.)	VXT-016 (16 oz.)	VXX-032 (32 oz.)
VXI-128 (128 oz.)	VXA-128 (128 oz.)	VXT-032 (32 oz.)	

PVA MODIFIED FIXATIVE: VPM-016 (16 oz.)/ VPM-032 (32 oz.)/ VPM-128 (128 oz.)

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INTENDED USE: For use in the rapid staining and differentiation of intestinal parasites. According to the Gomori procedure it can be used to counterstain histologic sections.

SUMMARY AND PRINCIPLES: Claude Masson introduced the first trichrome stain in 1929. It offered a simple, quick alternative to the tissue stains previously used. In 1949, George Gomori developed a single solution trichrome stain as a counterstain for hematoxylin stained tissue sections and cytological smears. Peter Wheatley's observation of E. Histolytica stained with chromotrope 2R in 1951 yielded the Wheatley methodology. The modification of traditional fixation and dehydration procedures, combined with trichrome stain, resulted in a procedure which stains amoeba and flagellates rapidly and easily. Volu-Sol's Trichrome Stain is recommended for use according to the Wheatley procedure for parasites, as well as the Gomori procedure for tissues. Volu-Sol's PVA and Schaudinn's Modified Fixatives are copper based and do not contain mercuric chloride. This modification eliminates the iodine-alcohol step typically used in the trichrome stain Kit.

SPECIMEN PREPARATION: **WHEATLY STAINING PROCEDURE FOR FECAL PARASITES:** Fresh fecal specimens should be collected in a clean, dry container and prepared as soon as possible. If a specimen cannot be prepared immediately, it is suggested that it be fixed in PVA modified fixative. Make a thin smear of fresh fecal material on a clean, glass slide. The specimen may be emulsified in a drop of saline if necessary. If a PVA-fixed specimen is used, allow the smear to air-dry overnight and proceed to the staining procedure (heating the slide may cause distortion of the organisms). While smear is still wet, place the slide into a coplin jar containing Schaudinn's Modified Solution for one hour. ***NOTE:** The fixing time may be shortened to 5 minutes if Shaudinn's Modified Solution is heated to 50°C. Schaudinn's Modified fixed slides must not dry before staining.* **GOMORI TRICHROME STAINING PROCEDURE FOR TISSUE AND SMEARS:** Fix smears or spread preparation in alcohol (alcohol-ether) or imbed tissue block in paraffin and section at 3-5 microns. ***NOTE:** Change all solutions periodically to prevent "watering down" and/or carryover. Weakened trichrome stain may be strengthened by exposing to air overnight, or replenishing with fresh stain.*

PRINCIPLES OF THE PROCEDURE: WHEATLEY PROCEDURE: PVA fixed fecal smears are passed through alcohol solutions and stained to produce a permanent slide for observation and identification of parasites. After mounting, slides may be stored indefinitely. **GOMORI PROCEDURE:** Paraffin imbedded specimens are sectioned; deparaffinized and hydrated in distilled water; stained with hematoxylin and counterstained with trichrome stain. After mounting, store slides indefinitely. ***NOTE: SCHAUDINN'S MODIFIED SOLUTION:** On the day of use, add 1/10 volume of glacial acetic acid to 9/10 volume of Schaudinn's modified solution. Stable for only one day. All slide immersion requires occasional gentle agitation.**

PROCEDURE: **WHEATLEY'S PROCEDURE FOR INTESTINAL PARASITES**: Set up coplin jars for the following solutions: **1) ETHANOL, 70%:** Immerse slides for 3-5 minutes. **2) ETHANOL, 95%:** Immerse slides in second solution for 3-5 minutes. **3) TRICHROME STAIN:** Immerse slides for 6-8 minutes. **4) ACID ALCOHOL DECOLORIZER:** Transfer to the next solution when stain runs from smear. Two quick dips. Carryover of stain and/or decolorizing solution can cause cloudiness on slides and/or lack of contrast. **5) ETHANOL, 95%:** Dip slides twice. **6) ETHANOL, 95%:** Immerse slides for 10 minutes. **7) CARBOL XYLENE:** Immerse sides for 3 minutes. **8) XYLENE:** Immerse slides for 3 minutes. Mount smear with coverslip, using a mounting medium of choice. Slides may be screened for parasites as soon as dry. **GOMORI PROCEDURE FOR TISSUE:** Bring smears or sections to water. Stain slides with hematoxylin modified for 5 minutes. Remove excess stain by washing slides in gentle running water. Stain slides with trichrome stain for 5-10 minutes. Wash slides with several quick dips in acetic acid, 0.2%. Dehydrate and clear, using alcohols and xylene. Mount and coverslip.

EXPECTED RESULTS: **FECAL SMEARS:** Background material and artifacts will stain green. Bacterial and red blood cells usually stain red, while yeast, vegetable fibers, etc. stain green. Organisms and cysts stain blue-green with a purplish tinge. Karyosomes of the nuclei, chromatic material will appear red-to-purple. Helminth eggs and larvae also stain red-to-purple. **TISSUE SECTIONS OR SMEARS:** Connective tissue stains green, muscle and cytoplasm red, and nuclei blue.

STORAGE AND EXPIRATION: Store reagents at room temperature (70-77.9 °F/ 20-25.5 °C). Maximum intended shelf life is printed on the label. If necessary to interrupt staining procedure, slides may be stored in ethanol 70% without adverse effects.

REFERENCES:

1. Lillie R.D., 1965 Histopathology Techniques and Practical Histochemistry. McGraw Hill 3rd Ed.
2. Gomori, G., 1950 A Rapid one step Trichrome method: Am J. of Clin. Path. Vol. 20: 661-664