

WHEATLEY'S TRICHROME METHOD FOR INTESTINAL PROTOZOA

PURPOSE:	For In Vitro Diagnostic Use: Intended for the qualitative demonstration of fungi and Pneumocystis Carinii in tissue.
PRINCIPLE:	Smears are either fixed in PVA or Schaudinn's. Iodine is used to remove the mercury from the tissue. An acid dye is applied to the section which binds all acidophilic elements including both collagen and muscle. The Phosphotungstic Acid then displaces the acid dye from the collagen. Collagen is more permeable than cytoplasm and thus the large phosphotungstic molecule cannot displace the acid dye from the cytoplasm. It also acts as a mordant for the Light Green to bind to collagen.
CONTROL:	Fixed smear known positive for protozoa <i>Control Slides can be purchased from Histology Control Systems. See inside back cover, Item# cs027.</i>
SPECIMEN PREPARATION:	Formalin fixed, paraffin embedded sections cut at 6 micrometers
SOLUTIONS:	<ol style="list-style-type: none"> 1. Iodine Alcohol For Trichrome Item# s2030 2. Acid Alcohol Trichrome Stain Item# s2031 3. Carbol Xylene For Intestinal Protozoa Trichrome Item# s2032 4. Wheatley's Trichrome Stain Item# s1910 <p><i>Solutions can be purchased separately from Poly Scientific.</i></p>
NOTES:	Schaudinn's Fixative not included.
REFERENCE:	Wheatley, W.B., 1951. "A Rapid Staining Procedure for Intestinal Amoeba and Flagellates". <u>Amer. J. of Clin. Path.</u> Vol. 21: pp. 990-991.

STAINING PROCEDURE:

Staining technique with fresh specimen:

Note: DO NOT let smear dry.

1. Schaudinn's Fixative for 5 minutes at 50°C or 1 hour at room temperature. Iodine Alcohol for 3 minutes.
2. 70% Alcohol, 2 changes, 3 minutes each.
3. Wheatley's Trichrome stain for 2-8 minutes.
4. Decolorize in Acid Alcohol 10-20 seconds (do not leave in over 20 seconds).
5. Alcohol 95%, 2 changes.
6. 100% Alcohol or Carbol Xylene, 3 minutes.
7. Xylene for 1-3 minutes.
8. Mount with Poly Mount (Item# s2153) or any other acceptable mounting medium.

Staining technique with PVA films:

1. Iodine Alcohol for 10-20 minutes.
2. 70% Alcohol, 2 changes, 3-5 minutes each.
3. Wheatley's Trichrome Stain for 6-8 minutes.
4. Acid Alcohol for 10-20 seconds.
5. 95% Alcohol rinse to remove acid.
6. 95% Alcohol for 5 minutes.
7. Carbol Xylene for 5-10 minutes.
8. Xylene for 10 minutes.
9. Mount with Poly Mount (Item# s2153) or any other acceptable mounting medium.

RESULTS:

Cytoplasm of Cysts & TrophozoitesBlue-Green Tinged with Purple

Nuclear Chromatin, Chromatoid Bodies Ingested Red Cells and Bacteria.....Red or Purplish Red

Background MaterialGreen

Poly Scientific R&D Corp.

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