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INTENDED USE
Wheatley's modification of trichrome staining uses trichrome stain in a rapid, simple procedure for the staining of intestinal protozoa preserved in fixative.

SUMMARY
Intestinal parasitic infections are diagnosed by recovery and identification of protozoa trophozoites and/or cysts or helminth eggs and/or juveniles. The detection and correct identification of intestinal protozoa is frequently dependent on the examination of a permanently stained smear, as smaller protozoa are often missed during direct smear and concentration methods. Trichrome stain has been used since 1929 as a histological stain for muscle tissue. in 1949, Gomori developed a shortened, more rapid method for trichrome staining of histologic and cytologic sections. In 1951, Wheatley modified the Gomori procedure and, using trichrome stain, developed a rapid staining procedure for intestinal amoeba and flagellates.

FOR IN VITRO DIAGNOSTIC USE ONLY

PRECAUTIONS
This product should be used by properly trained individuals. Precautions should be taken against the dangers of microbiological hazards by properly sterilizing specimens, containers, and media after their use. Directions should be read and followed carefully. Refer to Safety Data Sheets for additional information.

STABILITY AND STORAGE
Store the product in the original container at room temperature (15–30ºC). Avoid extremes of temperature and light. Keep all containers tightly closed when not in use.

USER QUALITY CONTROL
A positive parasite smear should be processed with each batch of slides or per the regulatory agency used by your laboratory to verify the quality of the staining reagents and the technique of the procedure. The product should not be used if the color has changed, the expiration date has passed, or there are other signs of deterioration.

SPECIMEN COLLECTION AND PREPARATION
Permanent smears may be prepared from fresh or preserved fecal material. Specimens should be collected and handled following recommended guidelines.

Preparation of Smears for Staining Specimens Preserved in PVA (Zn) Fixative:
1. Place 1-2 drops of PVA (Zn)-fixed specimen on a clean glass slide. Lay or hold the slide flat with the specimen side up. Using an applicator stick, gently and evenly spread the sample over the slide. Using a chopping motion, spread the specimen out to create thick and thin areas.
2. Lay the slide flat with the film up. Allow to dry at room temperature for 3-24 hours.
3. Slides should be completely dry before staining.

Preparation of Smears for Staining Specimens Preserved in PROTO-FIX® Fixative:
PROTO-FIX contains no PVA and as such may require the use of a coated slide, such as a CELL-BOND® Slide (#0003257), to improve the adhesion of the specimen to the slide during staining procedures.
1. Transfer 1-2 drops of the PROTO-FIX fixed specimen to a CELL-BOND slide. Lay or hold the slide flat with the specimen side up. Using an applicator stick, gently and evenly spread the sample over the slide. Using a chopping motion, spread the specimen out to create thick and thin areas.
2. Allow the slide to remain flat for 1 to 2 minutes. Fecal smears can be slightly wet when staining begins. If there is still excessive liquid on the slide, stand it in a drying rack at a 45º angle to allow any excess liquid to drain. If fecal smears are still wet after 15 minutes, they may be air-dried with a cool fan. (Do not use heat to dry the fecal smear slides.) When the excess liquid stops draining, carefully wipe away any excess liquid on the edges of the slide and proceed with staining.

PROCEDURE
Materials Provided: Trichrome Staining Reagents.
Materials Not Provided: Mounting medium, coverslips, immersion oil, microscope slides, absorbent paper, Coplin jars.

Staining Procedure for PVA(Zn)

REAGENT	TIMING
70% Ethanol	3-5 minutes. Remove and drain off excess liquid by touching edge of slide to absorbent material
70% Ethanol	3-5 minutes. Remove and drain off excess liquid by touching edge of slide to absorbent material
Trichrome Stain	10 minutes. Remove and drain off excess liquid by touching edge of slide to absorbent material
Acid-Ethanol, 90%	1-3 seconds. Remove and drain off excess liquid by touching edge of slide to absorbent material
95% or 100% Ethanol*	5-10 seconds. Remove and drain off excess liquid by touching edge of slide to absorbent material
95% or 100% Ethanol*	3 minutes. Remove and drain off excess liquid by touching edge of slide to absorbent material
95% or 100% Ethanol*	3 minutes. Remove and drain off excess liquid by touching edge of slide to absorbent material
PRO-CLEAR™ or Xylene	10 minutes. Remove and drain off excess liquid by touching edge of slide to absorbent material
PRO-CLEAR™ or Xylene	10 minutes. Remove and drain off excess liquid by touching edge of slide to absorbent material
Coverslip and examine with an oil immersion lens	

NOTE: Staining time can vary depending on the intensity desired for the final stain result.
*** NOTE:** 100% Ethanol-based reagent alcohol.

Staining Procedure for PROTO-FIX

REAGENT	TIMING
70% Ethanol	1.5 minutes. Remove and drain off excess liquid by touching edge of slide to absorbent material
70% Ethanol	1.5 minutes. Remove and drain off excess liquid by touching edge of slide to absorbent material
Trichrome Stain	13 minutes. Remove and drain off excess liquid by touching edge of slide to absorbent material
Acid-Ethanol, 90%	1-3 seconds. Remove and drain off excess liquid by touching edge of slide to absorbent material
95% or 100% Ethanol	5-10 seconds. Remove and drain off excess liquid by touching edge of slide to absorbent material
95% or 100% Ethanol	1 minute. Remove and drain off excess liquid by touching edge of slide to absorbent material
95% or 100% Ethanol	1 minute. Remove and drain off excess liquid by touching edge of slide to absorbent material
PRO-CLEAR™ or Xylene	3 minutes. Remove and drain off excess liquid by touching edge of slide to absorbent material
Coverslip and examine with an oil immersion lens	

NOTE: Staining time can vary depending on the intensity desired for the final stain result.
NOTE: The Xylene substitute AmeriClear® does not yield appropriate stain results and should not be used.

EXPECTED RESULTS
Typical staining reactions using the Wheatley's Trichrome Stain method with adequately fixed and stained specimens are as follows:
1. The nuclear chromatin, chromatoid bodies, ingested erythrocytes and bacteria are purple to red-violet.
2. The cytoplasm of trophozoites and cysts stains blue-green tinted with purple when using PVA (Zn) and blue tinted with purple for PROTO-FIX specimens.
3. Background material and artifacts stain blue-green to purple.
4. Helminth ova and larvae stain red to purple, but may be more easily identified in a concentrated wet mount.
5. Macrophages, leukocytes, and yeasts show variable staining from green to blue to purple or red.
6. Special staining procedures are necessary for the identification of Cryptosporidium, Cyclospora, and Microsporidia.

LIMITATION OF PROCEDURES
Wheatley's Trichrome Stain method is a trouble-free procedure when used as directed. Any problems that occur may be one of the following:

Problem: Poor contrast of the chromatin material.
Cause: Over-decolorizing.
Solution: Decolorizing (acid ethanol) requires only a very brief contact, followed by an immediate dip in ethanol.

Problem: Poor staining of the cytoplasm and the nucleus. Degenerate forms that stain weakly.
Cause: Parasitic and cellular elements have degenerated because of improper fixation.
Solution: To ensure proper fixation, specimens must be placed in a fixative solution immediately after passage. The proportion of specimen to fixative (1:3) must be observed, be thoroughly mixed, and have sufficient time for fixation.

Problem: Stained preparation is "cloudy" with poor contrast of cellular detail.
Cause: Carryover of solutions from one step to another. Excessive staining (more than 30–40 slides) will weaken or dilute the stain.
Solution: Change all solutions regularly to avoid staining clarity problems.

Problem: Inadequate material for microscopic examination.
Cause: Smear is too thin.
Solution: Smears should be moderately thick. If necessary, concentrate the fixed specimen by centrifugation. Pour off excess specimen fixative to provide thicker material for smears.

Problem: Persistent staining failure after considering all of the above.
Cause: Faulty technique and/or contaminated reagents.
Solution: Discard the entire stain series. Try again with new staining solutions.

BIBLIOGRAPHY
1. Gomori, G., 1950. "A Rapid One Step Trichrome Method". Am J Clin Pathol., 20:661-664.
2. Lennette, E.H. et al., 1980. Manual of Clinical Microbiology. ASM, Washington, D.C., Third Edition, pp 1021-1022.
3. Melvin, D. and M. Brooke, 1980. Laboratory Procedures for the Diagnosis of Intestinal Parasites. U.S. Department of H.E.W., CDC Atlanta, GA., pp 123-124.
4. Wheatley, W., 1951. "A Rapid Staining Procedure for Intestinal Amoeba and Flagellates." Am J Clin Pathol. 21:990-991.

CONTACT
CalibreScientific US, Inc.. offers a complete line of reagents, stains, QC1™ Quality Control Slides, O&P collection and transport sets, and concentration systems for parasitology specimen processing. For Technical Assistance email Technical@AlphaTecSystems.com and for Customer Service email Sales@AlphaTecSystems.com, or call [+1] 800.221.6058 or [+1] 360.260.2779 between 8 am and 4 pm Monday through Friday, Pacific Time.

WARRANTY

TRADEMARKS

PRODUCT CODES
0003125 Wheatley's Trichrome Stain Set, 10 x 50 ml
0003303 100% Ethanol, 1 L
0003336 PRO-CLEAR, 1 L
0003342 Xylene, 500 ml
0003350 90% Acid Ethanol, 475 ml
0003351 Trichrome Stain (Wheatley's), 500 ml
0003356 95% Ethanol, 1 L
0003359 70% Ethanol, 1 L
0004213 95% Ethanol, 3.875 L
0004216 70% Ethanol, 3.875 L
0004404 100% Ethanol, 3.875 L
0004406 90% Acid Ethanol, 1 L

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GLOSSARY OF SYMBOLS

LOT — Batch code / Numéro de lot / Número de Lote / Numero di lotto / Lot Nummer / Lotnummer / Lotnummer / Šaržna številka / Número de lote

REF — Catalog number / Référence du catalogue / Número de catálogo / Numero di catalogo / Katalognummer / Catalog nummer / Het aantal van de catalogus / Kataloška številka / Número de catálogo

IVD — In vitro diagnostic medical device / Pour usage diagnostique in vitro / Para uso diagnóstico in vitro solamente / Solo per uso diagnostico in vitro / Nur zur Verwendung als in vitro-Diagnostikum / Alleen voor in vitro diagnostisch gebruik / För invitrodiagnostik enbart / Samo za invitro diagnostiko / Apenas para uso em diagnóstico in vitro

EC REP — Authorized representative in the European Community / Représentant européen autorisé / Representante Europeo Autorizado / Rappresentante europeo autorizzato / Autorisierter Europäischer Repräsentant / Germachtigde Europese vertegenwoordiger / Auktoriserad europeisk representant / Pooblaščen evropski predstavnik / Representante Europeu Autorizado

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