



WHEATLEY TRICHROME STAIN

INTENDED USE

Remel Wheatley Trichrome Stain is recommended for use in qualitative procedures for detection and identification of intestinal protozoa, as well as differentiation of these organisms from background material. Trichrome staining is usually performed on PVA-fixed or Schaudinn's solution-preserved fecal specimens.

SUMMARY AND EXPLANATION

The identification of intestinal protozoa requires examination of a permanent stained smear which provides detail and contrast when used with preserved specimens. Trichrome stain was originally developed by Gomori for staining tissue sections and cytological smears.¹ In 1951, Wheatley modified Gomori's technique by adding fixation and dehydration steps resulting in a simple and rapid staining procedure for intestinal amoebas and flagellates.²

PRINCIPLE

Wheatley Trichrome Stain contains two dyes. Chromotrope 2R stains nuclear chromatin, chromatoid bodies, karyosomes, parasite eggs and larvae, bacteria, and ingested erythrocytes red to purple-red. Light green and fast green dyes stain the cytoplasm of preserved cysts, trophozoites, and cellular constituents blue-green. The Trichrome Stain results in excellent contrast and visualization of cellular details that aid in the identification of protozoa.³

REAGENTS (CLASSICAL FORMULA)*

Phosphotungstic Acid (CAS 51312-42-9)	7.0 g
Chromotrope 2R (CAS 4197-07-3)	6.0 g
Light Green SF (CAS 5141-20-8)	1.5 g
Fast Green FCF (CAS 2353-45-9)	1.5 g
Glacial Acetic Acid (CAS 64-19-7)	10.0 ml
Demineralized Water (CAS 7732-18-5)	990.0 ml

*Adjusted as required to meet performance standards.

PRECAUTIONS

CAUTION! May cause eye, skin, and respiratory tract irritation. The toxicological properties of this material have not been fully investigated.

This product is for *In Vitro* diagnostic use and should be used by properly trained individuals. Precautions should be taken against the dangers of microbiological hazards by properly sterilizing specimens, containers, and test materials after use. Directions should be read and followed carefully. Refer to Material Safety Data Sheet for additional information on reagent chemicals.

STORAGE

This product is ready for use and no further preparation is necessary. Store product in its original container at room temperature until used.

PRODUCT DETERIORATION

This product should not be used if (1) the color has changed from a dark-blue to purple liquid, (2) the expiration date has passed, or (3) there are other signs of deterioration.

SPECIMEN COLLECTION, STORAGE, AND TRANSPORT

Specimens should be collected and handled following recommended guidelines.⁴⁻⁶

MATERIALS REQUIRED BUT NOT SUPPLIED

(1) Specimen preservative, fixative, collection containers, (2) Applicator sticks, plain and cotton-tipped, or brush, (3) Disposable glass or plastic pipettes, (4) Incubator, slide warmer, (5) Absorbent paper, paper towels, (6) Coplin jars, staining rack, forceps, (7) Glass microscope slides, coverslips, mounting medium, (8) Microscope with calibrated ocular micrometer, immersion oil, (9) BactiDrop™ Lugol's Iodine Ampules (REF R21528), (10) Ethanol 70% (REF R40135), (11) Acid Ethanol 90% (REF R40134), (12) Ethanol 90% (REF R40132), (13) Xylene-S (REF R40133).

PROCEDURE⁷

Every fecal specimen represents a potential source of infectious material and should be handled accordingly.⁵

1. Preparation of Smear: Stool specimens preserved in PVA should be allowed to fix at least 30 minutes. Fresh specimens received in the laboratory should be mixed with PVA (1 part feces to 3 parts fixative) and allowed to fix for 30 minutes.
2. Thoroughly mix the specimen and the PVA. Pour a small amount of the mixture onto a paper towel to absorb excess fixative. Allow the fixative to soak into the paper towel for 3 minutes before preparing slides.
3. With an applicator stick, pipette, or brush transfer some of the stool material from the paper towel to 2 clean glass slides. Spread the mixture to the edges of the slide so the specimen will adhere to the slide during staining. The amount of material applied to the slide should be thin enough that newsprint can be read through the smear.
4. Allow the slides to dry for an hour at 35-37°C or overnight at room temperature. Smears may also be heat-fixed on a slide warmer at 60°C until dry (about 4 minutes).

Note: Specimens preserved in non-mercury-based fixatives do not require the iodine-alcohol step and the alcohol rinse (steps 5-8). If a non-mercury-based fixative is used proceed to step 9, otherwise, proceed with step 5.

5. Place slides in Ethanol 70% for 5 minutes. (This step can be eliminated for PVA air-dried smears.) Drain excess liquid from the slide on absorbent paper between all solutions.
6. Slides prepared from fresh specimens should be immersed in Ethanol-Iodine for 1 minute. PVA-preserved, air-dried smears should be immersed in Ethanol-Iodine for 5-10 minutes. (To prepare Ethanol-Iodine mixture, add enough iodine to Ethanol 70% to make a dark concentrated solution; strong tea or amber colored in appearance).
7. Place slides in Ethanol 70% for 5 minutes. Drain excess liquid.
8. Place slides in a second jar of Ethanol 70% for 3 minutes.
9. Place slides in Wheatley Trichrome Stain for 10 minutes.
10. Place slides in Acid Ethanol 90% for 1-3 seconds. Immediately proceed to the next step. Do not allow the slides to remain in contact with this solution longer than 3 seconds.
11. Dip slides several times in Ethanol 90%.
12. Place slides in two changes of Ethanol 90% for 3 minutes each.
13. Place slides in two changes of Xylene-S for 5-10 minutes each.
14. Apply mounting medium to the smear and cover with a No. 1 thickness coverslip.
15. Allow the smear to dry overnight at room temperature or for 1 hour at 35-37°C.
16. Examine the slide microscopically, using the oil immersion objective for nuclear detail. At least 200-300 oil immersion fields should be examined.

INTERPRETATION

Staining characteristics may vary depending on the fixative that is used. Typical staining reactions with Trichrome Stain are listed below.

1. Nuclear chromatin, chromatoid bodies, ingested erythrocytes, and bacteria stain red to purple-red.
2. Cytoplasm stains blue-green with a faint purple tinge.
3. Macrophages, leukocytes, and yeast cells vary in staining reactions.
4. Background material stains green.

QUALITY CONTROL

All lot numbers of Wheatley Trichrome Stain have been tested and found to be acceptable. Testing of positive and negative controls should be performed in accordance with established laboratory quality control procedures. If aberrant quality control results are noted, patient results should not be reported.

It is recommended that positive control slides be tested prior to the use of new lot numbers of permanent stain and at least weekly thereafter.⁶ If positive specimens are not available, use stained smears of feces containing leukocytes or epithelial cells to verify stain results.

LIMITATIONS

1. Results obtained will largely depend on proper and adequate specimen collection and fixation. Improperly fixed specimens will result in protozoan forms that are non-staining or predominantly red.⁵
2. Inadequately dried smears may peel or wash off during staining. Allow slides to dry thoroughly before staining.^{6,7}
3. Specimens should not be contaminated with water or urine. Water may contain free-living organisms that can be mistaken for human parasites and urine may destroy motile organisms.⁸
4. Oily materials, such as mineral oil, create refractile droplets that make examination difficult.^{7,8}
5. *Entamoeba coli* cysts are difficult to fix properly and may be difficult to identify on the stained slide. For this reason, it is possible to have fixatives that meet quality control criteria and yet do not always yield good morphology for this organism. A longer fixation time (60 minutes) may produce better morphology after staining.⁷
6. Inadequate removal of iodine by Ethanol 70% may result in a smear that is predominantly green. To avoid this, lengthen the timing of steps 6 and 7 or change Ethanol 70% more frequently.⁸
7. The appearance of dark crystalline materials (mercuric chloride crystals) occurs when the alcohol-iodine solution becomes saturated or the slide is not left in the solution long enough. Change alcohol-iodine solution often.⁸
8. Prolonged destaining in the Acid Ethanol 90% (more than 3 seconds) may result in poor differentiation of internal structures.⁶⁻⁸
9. Periodically, the staining strength of the Trichrome stain can be restored by removing the lid and allowing the 70% alcohol carried over from the preceding jar to evaporate.⁸
10. The trichrome stain is not recommended for staining helminth eggs or larvae. However, if they are present and recognizable they will stain red to purple.⁷
11. *Cryptosporidium parvum* may or may not be seen on a trichrome-stained smear (acid-fast stains are recommended).⁷
12. Helminth eggs and larvae, *Balantidium coli* trophozoites and cysts, *Entamoeba coli* cysts, and *Isospora belli* oocysts are best seen in wet preparations.⁸

13. Carefully drain slides between solutions. Touch the end of the slide (or slide rack) to absorbent paper for two seconds to remove excess fluid before proceeding to the next step.⁷
14. Fecal specimens should never be incubated or frozen prior to examination.³
15. Carryover of solutions from one jar to the next may result in a smear that is cloudy, too green, or has internal structures that lack contrast. Change solutions regularly to avoid carryover from one solution to the next.^{6,7}




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PACKAGING

REF R40025, Wheatley Trichrome Stain250 ml/Bottle

Symbol Legend

REF	Catalog Number
IVD	In Vitro Diagnostic Medical Device
LAB	For Laboratory Use
	Consult Instructions for Use (IFU)
	Temperature Limitation (Storage Temp.)
LOT	Batch Code (Lot Number)
	Use By (Expiration Date)

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