remel
TRICHROME STAIN SET

INTENDED USE
Remel Trichrome Stain Set is a complete set of reagents recommended for use in the Wheatley Trichrome Stain for detection and identification of intestinal protozoa.

SUMMARY AND EXPLANATION
A diagnosis of intestinal parasitic infections caused by protozoan organisms is confirmed by identification of trophozoites and cysts in fecal specimens. Because smaller protozoans often go undetected in direct wet mount and concentration methods, the identification of intestinal protozoa depends on examination of a permanent stained smear. The Trichrome stain provides excellent detail and contrast 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 addition of fixation and dehydration steps resulting in a simple and rapid staining procedure for intestinal amoebas and flagellates.[2]

PRINCIPLE
Chromotrope 2R has an affinity for chromatin material. Nuclear chromatin, chromatoid bodies, karyosomes, parasite eggs and larvae, bacteria, and ingested erythrocytes stain 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)*
Wheatley Trichrome Stain:
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) ..... 1000.0 ml

Xylene S:
D-Limonene (CAS 5989-27-5)
Butylated Hydroxyanisole (CAS 25013-16-5)

Ethanol 90%
Ethanol 90% (CAS 64-17-5)
Methanol 4.5% (CAS 67-56-1)
Isopropol 5.5% (CAS 67-63-0)

Ethanol 70% (CAS 64-17-5)

Acid Ethanol 90%:
Ethanol 90%(CAS 64-17-5) ..... 995.5 ml
Acetic Acid 0.5%(CAS 64-19-7) ..... 4.5 ml

Lugol's Iodine Ampules:
Lugol's Iodine (CAS 7553-56-2)

*Adjusted as required to meet performance standards.

PRECAUTIONS
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.

Wheatley Trichrome Stain: CAUTION! May cause eye, skin, and respiratory tract irritation. The toxicological properties of this material have not been fully investigated. **Xylene S: WARNING**! Flammable liquid and vapor. May cause allergic skin reaction. Causes eye and skin irritation. May cause respiratory tract irritation. Marine pollutant. **Ethanol 90%: DANGER! POISON!** Flammable liquid and vapor. May be fatal or cause blindness if swallowed. Harmful if swallowed, inhaled, or absorbed through the skin. Vapor harmful. Causes eye, skin, and respiratory tract irritation. This substance has caused adverse reproductive and fetal effects in humans. May cause central nervous system depression. May cause liver, kidney, and heart damage. Cannot be made nonpoisonous. **Ethanol 70%: WARNING!** Causes severe eye irritation. Flammable liquid and vapor. Causes respiratory tract irritation. This substance has caused adverse reproductive and fetal effects in humans. May cause central nervous system depression. May cause liver, kidney, and heart damage. Causes moderate skin irritation. **Acid Ethanol 90%: DANGER! POISON!** Causes severe eye irritation. Vapor harmful. Causes respiratory tract irritation. Flammable liquid and vapor. May be fatal or cause blindness if swallowed. This substance has caused adverse reproductive and fetal effects in humans. May be absorbed through intact skin. May cause central nervous system depression. May form explosive peroxides. May cause liver, kidney, and heart damage. Cannot be made nonpoisonous. **Lugol's Iodine Ampules: CAUTION!** May cause allergic skin reaction. May cause eye, skin, and respiratory tract irritation. May cause reproductive and fetal effects.

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, (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 SUPPLIED
Wheatley Trichrome Stain (1 x 250 ml)
Ethanol 70% (4 x 250 ml)
Lugol's Iodine Ampules (5 x 0.75 ml)
Ethanol 90% (2 x 250 ml)
Acid Ethanol 90% (1 x 250 ml)
Xylene S (2 x 250 ml)
Instructions For Use (IFU)

MATERIALS REQUIRED BUT NOT SUPPLIED
(1) Specimen preservative, fixative, collection containers, (2) Applicator sticks, swabs, (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.

PROCEDURE[7]
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).

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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 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 vary depending on the fixative used. Typical staining reactions with Trichrome Stain are as follows:

1. The 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 individual components of Trichrome Stain Set have been tested in combination 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.[5,6] If positive specimens are not available, use smears of feces containing leukocytes or epithelial cells to verify stain results.

LIMITATIONS

1. Results obtained depend on proper and adequate specimen collection and fixation. Improperly fixed specimens will result in protozoan forms that are non-staining or predominantly red.[6,8]
2. Inadequately dried smears/slides 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 may be mistaken for human parasites and urine may destroy motile organisms.[7]
4. Oily materials, such as mineral oil, create refractile droplets that make examination difficult.[7,8]
5. *Entamoeba coli* cysts are difficult to fix and may be difficult to identify on a 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 result in better morphology.[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 Ethanol-Iodine solution becomes saturated or the slide is not left in the solution long enough. Change Ethanol-Iodine solution often.[8]
8. Prolonged destaining in Acid Ethanol 90% (more than 3 seconds) may result in poor differentiation of cell structures.[6-8]
9. Periodically, the staining strength of the Trichrome Stain can be restored by removing the lid and allowing the Ethanol 70%, 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.[8]
11. *Cryptosporidium parvum* may or may not be seen on a trichrome-stained smear (acid-fast stains are recommended).[8]
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.[8]
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 a lack of contrast. Change all stain solutions periodically to reduce carryover.[6,7]

BIBLIOGRAPHY

1. Gomori, G. 1950. Am. J. Clin. Pathol. 20:661-664.
2. Wheatley, W.B. 1951. Am. J. Clin. Pathol. 21:990-991.
3. Melvin, D. and M. Brooke. 1982. Laboratory Procedures for the Diagnosis of Intestinal Parasites. 3rd ed. U.S. Dept. of H.H.S. and CDC. Atlanta, GA.
4. Forbes, B.A., D.F. Sahm, and A.S. Weissfeld. 2007. Bailey and Scott's Diagnostic Microbiology. 12th ed. Mosby Elsevier, St. Louis, MO.
5. Murray, P.R., E.J. Baron, J.H. Jorgensen, M.L. Landry, and M.A. Pfaller. 2007. Manual of Clinical Microbiology. 9th ed. ASM Press, Washington, D.C.
6. Isenberg, H.D. 2004. Clinical Microbiology Procedures Handbook. 2nd ed. ASM Press, Washington, D.C.
7. Clinical and Laboratory Standards Institute (CLSI). 2005. Procedures for the Recovery and Identification of Parasites from the Intestinal Tract; Approved Guideline, 2nd ed. M28-A2. CLSI, Wayne, PA.
8. Garcia, L.S. 2001. Diagnostic Medical Parasitology. 4th ed. ASM Press, Washington, D.C.

PACKAGING

REF R40217, Trichrome Stain Set ..... 1 Set

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)

Manufactured for Remel Inc.
CAS (Chemical Abstracts Service Registry No.)

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