**ETHANOL 70%**

**INTENDED USE**
Remel Ethanol 70% reagent is recommended for use in qualitative procedures in performing 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. 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.

**PRINCIPLE**
The Wheatley Trichrome stain produces well-stained smears from fresh and preserved material. Ethanol 70% provides for dehydration of the specimen material during the staining process.

**REAGENTS (CLASSICAL FORMULA)**
- Ethanol 62.3-64% (CAS 64-17-5)
- Methanol 2.8-3.85% (CAS 67-56-1)
- Isopropanol 2.8-4.2% (CAS 67-63-0)
- Lugol’s Iodine

*Adjusted as required to meet performances standards.

**PRECAUTIONS**
**WARNING!** May cause irritation to skin, eyes, and respiratory tract. Flammable, keep away from heat, sparks and flame. Avoid breathing vapor and eye/skin contact.

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.

**STORAGE**
This product is ready for use and no further preparation is necessary. Store product in its original container at room temperature (20-25°C) until used.

**PRODUCT DETERIORATION**
This product should not be used if (1) the color has changed from a clear 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.

**MATERIALS REQUIRED BUT NOT SUPPLIED**
- Specimen preservative, fixative, collection containers, Applicator sticks, plain and cotton-tipped, Disposable glass or plastic pipettes, Incubator, slide warmer, Absorbent paper, paper towels, Coplin jars, staining rack, forceps, Glass microscope slides, coverslips, mounting medium, Microscope with calibrated ocular micrometer, immersion oil, Lugol’s Iodine Ampules (REF R21528), Wheatley Trichrome Stain (REF R40025), Acid Ethanol 90% (REF R40134), Ethanol 90% (REF R40132), Xylene-S (REF R40133).

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

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 slide on absorbent paper between all solutions.
6. Slides prepared from fresh specimens should be immersed in Ethanol-lodine for 1 minute. PVA-preserved, air-dried smears should be immersed in Ethanol-lodine for 5-10 minutes. (To prepare Ethanol-lodine 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 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. 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 yeasts vary in staining reactions.
4. Background material stains green.
QUALITY CONTROL
All lot numbers of Ethanol 70% 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. 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 and bacterial morphology that are non-staining or predominantly red.7
2. Smears that are inadequately dried on the slide may flake off or peel. Slides used for staining should be free of grease.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.6,8
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.8
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.7
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.7,8
8. Prolonged destaining in the Acid Ethanol 90% (more than 3 seconds) may result in poor differentiation.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.7
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.7
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.7,5
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

BIBLIOGRAPHY

PACKAGING
REF R40135, Ethanol 70% .................................................250 ml/Bottle
REF R2470110, Ethanol 70% .............................................6/Pack

Symbol Legend

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CAS (Chemical Abstracts Service Registry No.)

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