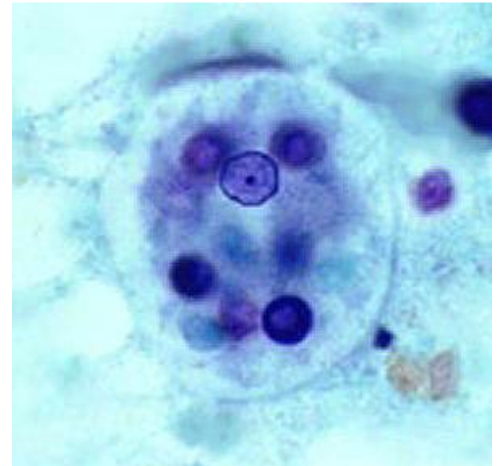
Trichrome Staining Tips

Eleven ways to make a better slide

By Lynne S. Garcia



Entamoeba histolytica

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Diagnostic Medical Parasitology, 5th ed, 2007, ASM Press.

Practical Guide to Diagnostic Parasitology, 1999, ASM Press.

Editor-in-Chief, Clinical Microbiology Procedures Handbook

Editor-in-Chief of Clinical Laboratory Management in the 21st Century, 2004, ASM Press

Reviewer: 9 journals

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1.

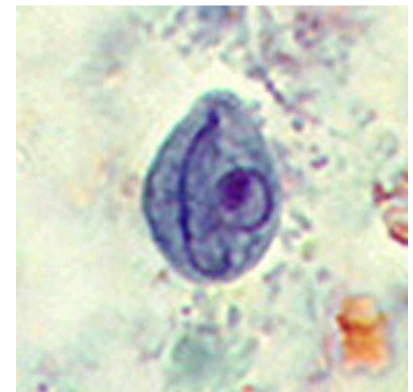
The single most important step in the preparation of a well stained fecal smear is good fixation. If this has not been done, the protozoa may be distorted or shrunk, may not be stained, or may exhibit an overall pink or red color with poor internal morphology.

2.

Slides should always be drained between solutions. Touch the end of the slide to a paper towel for two seconds to remove excess fluid before proceeding to the next step. You can also pull the slide from the fluid, allow the excess fluid to run back into the container, and then move the slide to the next reagent container. This will maintain the staining solution for a longer period. If you are using staining dishes and racks, lift the rack from the fluid, rest the rack against the dish to allow excess fluid to run back into the dish, and then move the rack to the next dish of reagent.

3.

Incomplete removal of mercuric chloride (liquid Schaudinn's fixative and liquid Schaudinn's fixative with PVA) may cause smear to contain highly refractive crystals or granules, which may prevent detection or identification of any organisms present. The 70% ethanol-iodine solution removes the mercury from the slide; the subsequent alcohol rinses then removes the iodine (should be strong-tea color). Thus, when the slide is ready for trichrome staining, both the mercury and iodine have been removed. A few minutes (3-5 min) are usually sufficient to keep the slides in the iodine-alcohol; too long a time in this solution may also adversely affect the staining of the organisms.



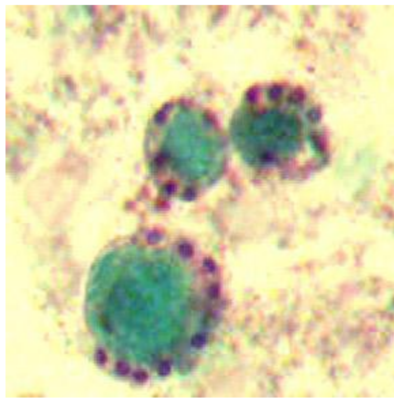
Chilomastix mesnili cyst

4.

When using non-mercury-based fixatives, the iodine-alcohol step (used for the removal of mercury) and the subsequent alcohol rinses can be eliminated from the procedure. The smears for staining can be pre-rinsed with 70% alcohol and then placed in the trichrome stain, or they can be placed directly into the trichrome stain as the first step in the staining protocol.

5.

Smears that are predominantly green may be due to the inadequate removal of iodine by the 70% ethanol (steps 4 and 5). Lengthening the time of these steps or more frequent changing of the 70% ethanol will help.



Blastocystis hominis

6.

To restore weakened trichrome stain, remove the cap and allow the ethanol (carried over on the staining rack from a previous dish) to evaporate. After a few hours, fresh stock stain may be added to restore lost volume. Older, more concentrated stain produces more intense colors and may require slightly longer destaining times (an extra dip). Remember that PVA smears usually require a slightly longer staining time.

7.

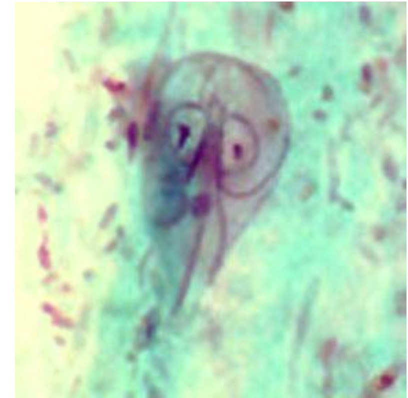
Although the trichrome stain is used essentially as a "progressive" stain (no destaining is necessary), the best results are obtained by using the stain "regressively" (destaining the smears briefly in acidified alcohol). Good differentiation is obtained by destaining for a very short time (two dips only, approximately 2 to 3 seconds); prolonged destaining results in poor differentiation.

8.

It is essential to rinse the smears free of acid to prevent continued destaining. Since 90% alcohol will continue to leach trichrome stain from the smears, it is recommended that after the acid-alcohol is used, the slides be quickly rinsed in 100% alcohol and then dehydrated through two additional changes of 100% alcohol.

9.

In the final stages of dehydration (steps 9 to 11), the 100% ethanol and the xylene (or xylene substitute) should be kept as free from water as possible. Coplin jars must have tight-fitting caps to prevent both evaporation of reagents and absorption of moisture. If the xylene becomes cloudy after addition of slides from the 100% ethanol, return the slides to fresh 100% ethanol and replace the xylene with fresh stock.



Giardia lamblia trophozoite

10.

If the smears peel or flake off, the specimen might have been inadequately dried on the slide (for PVA-fixed specimens), the smear may have been too thick, or the slide may have been greasy (fingerprints). However, slides generally do not have to be cleaned with alcohol prior to use.

11.

If the stain appears unsatisfactory upon the examination and it is not possible to obtain another slide to stain, the slide may be restained. Place the slide in xylene to remove the coverslip, and reverse the dehydration steps, adding 50% ethanol as the last step. Destain the slide in 10% acetic acid from several hours, and wash it thoroughly first in water and then in 50 and 70% ethanol. Place the slide in the trichrome stain for 8 minutes, and complete the staining procedure.

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REFERENCES

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Hardy Diagnostics Trichrome Reagents

Trichrome Stain
Trichrome Stain Starter Kit
Trichrome Blue, Modified for Microsporidia
Ethyl Acetate
Mayer's Albumin
Carbol Xylene
Acid Ethanol
Coplin Jars
Ethanol, 70%
Ethanol, 95%
Iodine, Dobell and O'Connor
Iodine Alcohol
Schaudinn's Fixative
Xylene
Envirene, Xylene Substitute
Cryptosporidium Stain Kit