Wheatley's Trichrome Stain

Intended Use

The Wheatley's Trichrome Stain Set is a complete set of reagents for use in performing the Wheatley's Trichrome Stain procedure on PVA & Schaudinn's fixed fecal specimens for the identification of intestinal protozoa.

Purpose & Explanation of Test

The Trichrome stain has been used since 1929 as a histological stain for muscle tissue. In 1949, Gomori developed a shortened and 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. The contrast of the blue-green, purplish-tinged or red-stained organisms with the green background material is a considerable aid to locating organisms when compared with iron-hematoxylin. 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 with only the direct smear and concentration methods.

Safety Measures

All reagents are for in vitro diagnostic use only.

2. 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.

3. When utilizing PVA fixed specimens the slides must be absolutely dry. Accelerating the drying process by warming may distort some organisms.

4. When utilizing Schaudinn's fixed specimens the slides must not be allowed to dry at any time until finally mounted.

5. If necessary to interrupt the staining procedure the slides may be stored for long periods of time in the last ethanol bath prior to staining. (Smear Preparation: Step No. D).

6. The purpose of the iodine alcohol (Staining: Step No. A) is to remove the mercuric chloride in Schaudinn's fixative and PVA fixative. Many problems experienced in the trichrome method are directly attributable to saturation of this solution; change it frequently.

7. The purpose of the first alcohol rinse after decolorizing is to halt decolorization. Since carryover of the acid alcohol will occur, change this solution frequently

Expiry/Storage:

Shelf life of the Wheatley's Trichrome Stain is indicated on the outer package label. Store the product in the original bottles at room temperature (15-30ºC). Use Coplin jars for staining. Avoid extremes of temperature and light. Keep all bottles/jars tightly closed when not in use.

Stool Preparation

1. Prepare the fresh stool sample. Place small samples of stool on clean sterile microscopic slides using applicator sticks. Making very thin smears of the sample.
2. Preparation Smears for Specimens Preserved in Schaudinn's fixative: While the smear is still wet, Immediately place the slide in a Coplin jar containing Schaudinn's fixative for 5 minutes at 50ºC or 1-2 hours at room temperature.
3. Preparation Smears for Specimens Preserved in PVA Fixative: Place 1-2 drops of PVA 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 then, using a chopping motion, spread the specimen out to create thick and thin areas. Lay the slide flat with the film up. Allow to dry at room temperature or 37°C for 3-24 hours. Slides should be completely dry before staining.

Staining the Smears

1. PVA & Schaudinn's fixed smear preparation is placed in Solution B (Iodine-Alcohol) for 10 minutes.
2. For other samples with different fixatives (SAF) without mercuric chloride, omit the iodine step.

Remaining steps for all the fixatives will be the same as mentioned below:

3. Place the slide in 70% Ethanol for 5 minutes.
4. Place the slide in 70% Ethanol for 3 minutes.
5. Place the slide in Trichrome stain for 10 minutes.
6. Place the slide in Solution C (Acid-Alcohol) for 1-3 seconds and drain the rack immediately and proceed to the next step. Do not allow the slide to remain in this solution.
7. Dip the smeared slide in 95% Ethanol, this is the rinsing step.
8. Again, place the slide in (2nd) 95% Ethanol for 3 minutes.
9. Place the slide in 100% Ethanol for 3 minutes.
10. Place the slide in Solution D (Carbol-Xylene) 3 minutes
11. Place it in Xylene for 5-10 minutes.
12. Mount the smear with a cover slip using a mounting medium (Canada Balsam/DPX).

Results/Observations

- Observe protozoan trophozoites and cysts.
- The cytoplasm of the protozoan trophozoites stains blue-green or light purple.
- The cysts appear more purple.
- The presence of yeast and human cells such as Red blood cells, Polymorphonuclear cells (PMNs), and Macrophages can be identified and stained red in color.
- The nuclei and inclusion bodies also stain red with a tinged purple.
- The background stains green showing a great contrast with that of protozoans.
- Glycogen molecules are dissolved by the stain solvents and they appear as clear as the organism.

Limitations of Wheatley's Trichrome Stain

- Helminth eggs and larvae cannot be stained permanently by the Wheatley Trichrome Stain.
- To identify and examine protozoans, the stain requires high magnification and under oil immersion, some morphologies of the protozoans can be lost or missed.
- Some protozoans cannot be identified using the Wheatley's Trichrome stain such as Cryptosporidium parvum and Cyclospora cayetanensis.
- Microsporidia spores cannot be seen using the Wheatley's Trichrome Stained smear.

Trouble Shooting

The Wheatley's Trichrome method is a remarkably trouble-free procedure when used exactly as directed. Occasionally, as with any procedure, problems may arise. These may be divided into four categories:

1. Problems related to incomplete fixation of the smear resulting from failure to thoroughly emulsify the fecal specimen in the fixative.
    a) Degenerate forms staining a pale green.
    b) Unsatisfactory staining of cytoplasm and/or nucleus. Be sure to thoroughly mix the specimen in the fixative.
2. Failure to observe the smear carefully while decolorizing (Staining: Step No. F).
    a) Lack of contrast between organisms and background material of cytoplasm and nucleus. Remove the slide from the acid-ethanol and proceed to the ethanol rinse as soon as the color begins to run from the smear.
3. Saturation of the iodine-alcohol when using fixatives containing mercuric chloride:

a) Obscuration of the smear by dark crystals. Use the Solution B (Iodine-Alcohol solution) exactly as directed and change it frequently.

4. Carryover of staining/decolorizing solutions from one jar to the next:
   a) Smear too green or lack of contrast.
   b) Smear is “cloudy”. Lack of contrast usually results from carryover of the acid-alcohol to the ethanol rinses or failure to observe the slide while decolorizing. After repeated use the stain solution may be “watered down” by carryover of 70% ethanol. “Cloudy” smears are caused by incomplete dehydration after staining and decolorization.
   c) Solution D (Carbol-Xylene) or absolute ethanol and xylene are dehydrating agents in this procedure. Change all solutions periodically to avoid problems.