IRON HEMATOXYLIN STAIN

- For in vitro use only - Catalogue No. SI70 & 71

Our Iron Hematoxylin Stain is intended to be used for the preparation of permanent stained slide for the detection and quantification of intestinal amoebae and protozoa.

The use of Iron Hematoxylin Stain has been widely documented and provided most of the original morphological description of intestinal protozoa found in humans. Numerous modifications to the Iron Hematoxylin staining procedure have been made throughout history. Our current protocol is a modification described by Scholten. The procedure uses alcoholic iodine to remove mercuric chloride form Schaudinn's and PVA-fixed smears; alcohol washes to remove residual iodine, a working hematoxylin stain that contains the mordant; picric acid for destaining; and various concentrations of alcohol and xylene to dehydrate and clear the smear.

Formula per Litre

SI70 Iron Hematoxylin Mordant

Ferrous ammonium sulfate	10.0 g
Ferric ammonium sulfate	10.0 g
Hydrochloric acid	10 mL
Water	990 mL

SI71 Iron Hematoxylin Stain

Hematoxylin	10.0 g
Ethanol	1000 mL

Recommended Procedure

PVA and Schaudinn Procedure

1. Prior to use, make a working solution by mixing equal parts of the Iron Hematoxylin Mordant and the Iron Hematoxylin Stain (This working solution should be prepared fresh weekly)
2. For fresh solid fecal specimens, prepare two slides with applicator sticks and without drying place them into Schaudinn's fixative and allow slides to fix for a minimum of 30 minutes. The amount of stool smeared should be thin enough so that newsprint can be read through the smear.
3. If the specimen is liquid, place 3 or 4 drops of PVA on a slide and mix several drops of the fecal material with the PVA. Allow slides to dry in an incubator for several hours or overnight at room temperature.
4. If the specimen is already preserved in PVA then allow the specimen to fix for 30 minutes prior to proceeding. Mix the contents of the vial using an applicator stick. Pour some of the PVA-stool mixture onto a paper towel and allow it to stand for 3 minutes to absorb the PVA. With an applicator stick smear some of the material on the paper towel onto 2 slides and allow them to dry in incubator for several hours or overnight at room temperature. If desired, preserved specimens may be centrifuged and the sediment maybe also used for the slide preparation.
5. For the above prepared slides, place slide in 70% ethyl alcohol for 5 minutes.
6. Place slide in 70% ethanol-iodine solution for 5 to 10 minutes (removes mercuric chloride).
7. Place slide in 70% ethyl alcohol for 5 minutes.
8. Wash slide in running tap water for 10 minutes.
9. Place slide in Iron Hematoxylin working solution for 10 minutes.
10. Wash slide in running tap water for 1 minute.
11. Place slide in picric acid working solution for 10 minutes.
12. Wash slide in running tap water for 10 minutes.
13. Place slide in 70% ethyl alcohol plus

ammonia for 10 minutes.
14. Place slide in 95% ethyl alcohol for 10 minutes.

15. Places slide in two changes of 100% ethyl alcohol for 5 minutes each.
16. Place slide in two changes of xylene for 5 minutes each.
17. Add permount to the stained area and cover with a coverslip.
18. Examine slides microscopically with the 100X objective.

SAF Procedure

1. Prior to use, make a working solution by mixing equal parts of the Iron-hematoxylin mordant and the stain (This working solution should be prepared fresh weekly)
2. Prepare a smear by mixing 1 drop of Mayer's albumin with sediment from the SAF preserved specimen.
3. Allow slide to air dry at room temperature until smear is dry and opaque.
4. Place slide in 70% alcohol for 5 minutes.
5. Wash in container of tap water for 2 minutes.
6. Place slide in Kinyoun stain for 5 minutes.
7. Wash slide in running tap water for 1 minute.
8. Place slide in Kinyoun decolorizer for 4 minutes.
9. Wash slide in running tap water for 1 minute.
10. Place slide in Iron Hematoxylin working solution for 8 minutes.
11. Wash slide in distilled or deionized water in container for 1 minute.
12. Place slide in picric acid working solution for 3 to 5 minutes.
13. Wash slide in running tap water for 10 minutes.
14. Place slide in 70% alcohol plus ammonia for 3 minutes.
15. Place slide in 95% ethyl alcohol for 5 minutes.
16. Place slide in 100% ethyl alcohol for 5 minutes.
17. Place slide in two changes xylene or xylene substitute for 5 minutes.
18. Add permount to the stained area and cover with a coverslip.
19. Examine slides microscopically with the 100X objective.

Interpretation of Results

When stained correctly, organisms will appear bluish or grayish with black nuclear structures. Chromatid bodies of amoebae cysts and inclusions in the cytoplasm of trophozoites, such as yeast and bacterial cells, will stain black or dark blue. Red blood cells will appear pale yellow or green in color. The background material will normally appear a pale blue-gray color.

- *The working should be prepared fresh on a weekly basis. A quick reliability test can be performed by adding a few drops of stain to alkaline tap water. If the mixture turns blue then the working solution is working; if the solution turns brown then a new working solution should be made*

- *Incomplete removal of mercuric chloride for Schaudinn's and PVA-fixed smears may result in highly refractive granules that make visualization of organisms more difficult. In such instances change the 70% alcohol-iodine solution.*

- *This stain is not recommended for the detection or visualization of helminth eggs and larvae. Helminth eggs and larvae and Isospora belli oocysts are best seen in wet preparations*

Quality Control

Internal quality control of the working solution of Iron Hematoxylin Stain must be performed regularly on known reference organisms to ensure the performance of the stain. Include a QC slide when you use a new lot of reagents, or when you add new reagents after cleaning staining dishes.

Storage and Shelf life

Both our Iron Hematoxylin Stain and Mordant should be stored at room temperature and protected from light. Under these conditions it has a shelf life of 52 weeks from the date of manufacture. The diluted working solution of Iron Hematoxylin Stain has a shelf life of approximately 1 week from the date of preparation.

References

1. Spenser FM, Monroe LS. The color atlas of intestinal parasites, 2nd ed. Springfield: Charles C Thomas, 1976.
2. Yang J, Scholten. Am J Clin Path, 67:300-4. 1977.
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4. Murray PR, Baron E, Pfaller M, Tenover F, Yolken. Manual of clinical microbiology, 7th ed. Washington: ASM, 1999.
5. Garcia LS. Diagnostic Medical Parasitology, 4th ed. Washingtion, DC: ASM, 2001.

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