PARASITOLOGY STAINS

PRODUCT:

Bottle:[a]

Hematoxylin Stain Stock, item no. R5940, R5942
Hematoxylin Mordant Stock, item no. R5945, R5947
Lugols Iodine, item no. R5966, R5965
Trichrome Stain, Wheatley, item no. R5991, R5992

[a]see catalog for ordering options

PURPOSE:

Lugols Iodine stain is used in parallel with saline mounts when examining feces or other materials for intestinal protozoa. The Trichrome Stain and Hematoxylin Stain is used in staining a permanent mount of preserved fecal material for the detection and identification of intestinal amoebae and protozoa.

PRINCIPLE:

Currently, the most sensitive procedure for detecting and identifying protozoan trophozoites present in fecal specimens is the permanently stained smear procedure.[4,9] Protozoan cysts can also be detected and identified by this procedure. If all processing procedures are done correctly, the morphology of the trophozoites and cysts is usually seen. The Heidenhain iron hematoxylin stain procedure is considered the best for definitive morphological determinations of intestinal parasites. However, the procedure is complicated and tedious to perform, making it impossible for routine use by most clinical laboratories. Several modifications have been developed to simplify and expedite this procedure while maintaining its excellent staining qualities. One modification, the Scholten modification, works well with Schaudinn's polyvinyl alcohol (PVA) and sodium acetate, acetic acid, formalin (SAF) fixatives.[8,10] The procedure uses alcoholic iodine to remove mercuric chloride from 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, 100% alcohol and xylene to dehydrate and clear the smear.

The Trichrome Stain was originally developed by Gomori[3] and adapted by Wheatley[11] for the examination of intestinal protozoa on a permanent smear. In this stain, the background remains green, and the protozoa cytoplasm stains blue-green to purple with red or purple-red nuclei and inclusions. Helminth eggs and larvae stain dark red to purple, but may be distorted and difficult to identify.

Lugols Iodine is often used in wet mounts of concentrated fecal material. It functions to differentiate parasitic cysts from host white blood cells. Cysts will absorb the iodine and appear light brown in color. Intracellular structures are more easily distinguished. Glycogen vacuoles from *Iodamoeba butschlii* are particularly visible, staining brown to black.

FORMULAS:

Approximate ingredients per liter.

(1) Hematoxylin Stain, Stock:

Hematoxylin Powder	10.0 g
100% Ethyl Alcohol	1000.0 ml

(2) Hematoxylin Mordant, Stock:

Ferrous Ammonium Sulfate	10.0 g
Ferric Ammonium Sulfate	10.0
Hydrochloric Acid	10.0 ml
Water, Deionized	990.0

(3) Lugols Iodine:

Water, Deionized	1000.0 ml
Iodine	50.0 g
Potassium Iodide	100.0

(4) Trichrome Stain:

Water, Deionized	1000.0 ml
Acetic Acid	10.0
Chromotrope 2R	6.0 g
Light Green SF Yellow	1.5
Fast Green FCF	1.5
Phosphotungstic Acid	7.0

PRECAUTIONS:*

For in vitro diagnostic use. Observe all safety precautions consistent with the hazard(s) stated on the product label and/or Material Safety Data Sheet. Trichrome Stain is considered carcinogenic; avoid all contact.

Storage: For Lugols Iodine, upon receipt store at 2-30°C in the dark. For Trichrome and Hematoxylin Stains, upon receipt, store at 10-30°C away from direct light. Stains should not be used if there are signs of deterioration or if the expiration date has passed.

Limitations, Hematoxylin Stain: Except for the staining and destaining steps, the timing of the other steps in the procedure is not as critical. If short immersion times are used, however, slides must be frequently or continually agitated while immersed. Longer exposure times often allow the staining schedule to fit in with the overall work schedule of the laboratory.

The hematoxylin stain working solution (equal quantities of stock stain and mordant) should be used for approximately one week. Its reliability can be tested by adding a few drops of stain to a few drops of alkaline tap water. The rapid development of a blue or blue-black color indicates the stain solution is working. The development of a brownish color indicates the stain solution should be replaced with a freshly made working solution.

Too thick a smear will obscure parasites, while too thin of smear may spread the parasites out, making it difficult to find them.

Smears should be thoroughly dehydrated before mounting. The defined strengths of the dehydrating solutions must be maintained and the smears left in the solutions for the appropriate period of time.

The staining of a slide is not a highly technical procedure; however, the interpretation of the stained smear does require expertise obtainable by expert training and continual practice.

Smears may not stain as well with the modified hematoxylin stain procedures as they do with the original, longer method, but satisfactory results are achievable for routine diagnostic purposes.

Smears stained with trichrome can be restained with hematoxylin if certain procedures are followed.[4]

Preserved specimens cannot be used for culturing purposes.

Once staining has begun, the smears should NOT be allowed to dry until mounted and cover-slipped.

Limitations, Lugols Iodine: Lugols Iodine should always be used in conjunction with saline preparations since iodine paralyzes the motility of parasitic organisms and may obscure helminth eggs.

With few exceptions, organisms should not be identified on the basis of the wet mount alone. Permanent-stained smears should be examined to confirm the identification of the suspected organisms.

Dientamoeba fragilis trophozoites can be seen only in permanent stains.

A weak iodine solution is recommended; if the solution is too strong it may obscure the organisms. Iodine eventually lightens in color and loses its staining strength; observe and follow the expiration date of the staining reagent for best results.

Limitations, Trichrome Stain:
Good results in the final stage of dehydration (alcohol) or clearing (xylene) depend on the use of fresh reagents. It is recommended that these solutions be changed weekly or more often if large numbers of slides (10-50) are stained daily.

Stock solutions and stain containers should have well-fitting lids to prevent evaporation and absorption of moisture in the air. An exception is the Trichrome Stain; a weakened stain can be revitalized by exposure to air overnight.

If the xylene solutions appear cloudy after the slides have been transferred from the 95% alcohol, there is water in the solution. Transfer the slides back to the alcohol and immediately change the xylene solution.

Fecal specimens fixed with Formalin or MIF are not appropriate to this staining procedure.

Delayed or inadequate fixation of specimens results in staining cysts red or degenerated organisms staining pale green.

If crystalline structures, which are probably mercuric chloride, are seen in a PVA smear, the iodine in the iodine-alcohol solution may have been too weak to remove the mercuric chloride or the smear may not have been exposed long enough to the solution. If the iodine concentrations or exposure time is correct and crystals are seen in a smear, they are probably in the specimen and another specimen needs to be collected.

If the stained smear appears washed out, it is probably due to over-decolorization. This can be caused by too long an exposure to either the acid alcohol, the alcohol wash after the destain has become acidic, or the xylene. It may also be due to overuse of the stains.

PROCEDURE:*

Specimen Collection: Information on specimen collection and transport is found in standard reference material on the subject. In general, for fresh samples, specimens should be delivered to the laboratory without delay. Consult appropriate references for the details of collection and preservation.[1,5]

Method of Use, Hematoxylin Stain:

Specimens preserved in Schaudinn's or PVA fixatives:

Resuspend the preserved specimen, mix well, and strain through fine gauze into a centrifuge tube. Do not strain diarrheic specimens or those containing obvious amounts of mucous. Centrifuge the suspension for one minute at 2000 rpm. Decant the supernatant. Add 15ml of 0.85% saline to the tube and suspend the sediment. Centrifuge as in step 2, above. Decant supernatant, drain well but avoid losing any sediment. Place one to two drops on the slide. Using an applicator stick, spread the drop out on the slide using a "dabbing" motion. DO NOT STREAK THE MATERIAL as for fresh stools or blood smears. The resulting smear should vary in thickness. Allow the resulting smear to dry at room temperature until opaque, which may take 10 minutes or longer. The prepared slides are placed into the following reagents in the sequence listed for the times indicated:

Alcoholic iodine	10+ minutes (for removal of mercuric chloride)
95% ethyl alcohol	10+ minutes
70% ethyl alcohol	5+ minutes

Specimens Preserved in SAF fixative:

Resuspend the preserved specimen and add 2-3ml of specimen to 15ml of 0.85% saline. Mix well and strain through fine gauze into a centrifuge tube. Do not strain diarrheic specimens or those containing obvious amounts of mucous. Centrifuge the saline suspension for one minute at 2000 rpm. Decant the supernatant. Add 15ml of 0.85% saline to the tube and resuspend the sediment. Centrifuge as in Step 2, above. Repeat this step 2-3 times. After the last centrifugation, decant the supernatant. The final sediment volume should be 0.5-1.0 ml. Place one drop Mayer's albumin (equal amounts of glycerine and fresh egg white) on the slide and one drop of sediment. Using the applicator stick, mix and spread the drops out on the slide using a "dabbling"motion. DO NOT STREAK THE MATERIAL as for fresh stools or blood smears. The resulting smear should vary in thickness. Allow the smear to dry at room temperature until opaque. Do not heat to accelerate the drying process. After drying, the prepared smear is placed in 70% ethyl alcohol for 10 minutes. It is then ready for staining. The alcoholic iodine and 95% ethyl alcohol rinses are not necessary because there is no mercuric chloride present in SAF fixative.

NOTE: The remaining sediment in the centrifuge tube can be used in the formalin-ethyl acetate or ether concentration procedure.

STAINING PROCEDURE, Hematoxylin Stain

General Procedure: A working solution of hematoxylin stain is prepared by mixing equal quantities of stock stain with stock mordant. The prepared smears are placed in the following reagents in the sequence and for the times indicated (see "Limitations"). A working solution of hematoxylin stain is prepared by mixing equal quantities of stock stain with stock mordant. Smears should not be allowed to dry during the staining procedure until mounted and cover-slipped. The times listed are for the staining of several smears at the same time.

	Reagent	Time
1.	Tap water, running	2 minutes
2.	Hematoxylin Stain, working solution	10 minutes
3.	Water, D.I. or distilled	1 minute
4.	Picric acid, working solution	10 minutes
5.	Tap water, running	10 minutes
6.	70% alcohol + ammonia	10 minutes
7.	95% ethyl alcohol	10 minutes
8.	100% ethyl alcohol	5 minutes
9.	100% ethyl alcohol	5 minutes
10.	Xylene*	10 minutes
11.	Xylene*	10 minutes

Modified Staining Procedure: This procedure is modified to allow for the staining of acid-fast organisms, such as *Cryptosporidium, Iospora,* and *Sarcocystis.*[5]

	Reagent	Time
1.	Tap water, running	2 minutes
2.	Kinyoun Stain (carbol fuchsin)	5 minutes
3.	Tap water, running	1 minute
4.	Kinyoun Decolorizer (acid alcohol)	4 minutes
5.	Tap water, running	1 minute
6.	Hematoxylin Stain, working solution	8 minutes
7.	Water (D.I. or distilled) rinse	1 minute
8.	Picric acid, working solution	3 minutes
9.	Tap water, running	10 minutes
10.	70% alcohol + ammonia	5+ minutes
11.	95% ethyl alcohol	5+ minutes
12.	100% ethyl alcohol	2 minutes
13.	100% ethyl alcohol	2 minutes
14.	Xylene substitute	3+ minutes
15.	Xylene substitute	1+ minute

Mount stains immediately in Permount or other suitable mounting medium and examine microscopically.
*may be replaced with commercial substitutes.

Method of Use, Lugols Iodine: Prior to use, dilute the Lugols iodine 1:5 with deionized water. This solution should be made fresh daily. Place two small drops of concentrated specimen on a clean, glass slide. To one drop, add a drop of saline. To the other drop, add a drop of Lugols Iodine. Coverslip each drop and examine for intestinal protozoa. Examine the saline mount first; confirm results with the preparation stained with Lugols Iodine.

Method of Use, Trichrome Stain:

Unpreserved specimens with Schaudinn fixative:

To prepare a thin smear, place a drop of physiological saline on a clean microscope slide (1 x 3 in). With an applicator stick, place a small representative portion of the specimen in the drop of saline, and mix the two. Spread the solution into a film by rolling the applicator stock along the surface. Albumin may be used to fix the specimen to the slide. Immerse the smear in the following solutions for the duration indicated. CAUTION: DO NOT ALLOW SMEARS TO DRY OUT.

Unpreserved specimens with PVA fixative:

On a clean (1 x 3-in) slide, thoroughly mix 1 drop of unfixed specimen with a drop of PVA fixative. Spread specimen as described below. Allow the smear to dry, preferably overnight, before it is stained.

Specimens fixed with PVA, m-PVA, Zinc PVA, or SAF:

Add 1-3 drops of fixed specimen (preserved at least 1 hour) on a clean, glass microscope slide (1 x 3 in). Make sure there is not too little or too much sediment. Spread the material over the center third of the slide by rolling the specimen with an applicator stick, achieving peaks and valleys in the smear. The smear should extend to the top and bottom edges of the slide. Allow the smear to dry completely overnight at 25°C or in an urgent situation at 37°C for 4 hours. Stain the fixed smear by immersing in the following solutions for the durations indicated.

STAINING PROCEDURE, Trichrome Stain

In Schaudinn Fixative:

	Reagent	Time
1.	Schaudinn fixative	1 hour (5 minutes at 50°C)
2.	Iodine Alcohol (approximately 3 ml of iodine to 35 ml of 70% ethanol to a strong tea color)	1 minute
3.	Isopropanol, 70%	1 minute
4.	Isopropanol, 70%	1 minute
5.	Trichrome stain	8 minutes
6.	Acid Alcohol, 90%	5-10 seconds. (Up to 20 seconds for larger trophozoites).
7.	Ethanol, 95%	quick rinse
8.	Ethanol, 95%	5 minutes
9.	Xylene*	3 minutes
10.	Xylene*	1-3 minutes or until refraction at smear-xylene interface stops.

Mount with a coverslip, using Permount, balsam, or other mounting media.

In PVA, m-PVA, Zinc PVA, or SAF:

	Reagent	Time
1.	Iodine Alcohol (approximately 3 ml iodine to 35 ml 70% ethanol to a strong tea color) This step is for PVA-fixed specimens only; skip this step for specimens fixed with m-PVA, SAF, or Zinc PVA.	10-20 minutes
2.	Ethanol, 70%	5 minutes
3.	Ethanol, 70%	5 minutes
4.	Trichrome Stain	8 minutes
5.	Acid Alcohol, 90%	3-10 seconds
6.	Ethanol, 95%	quick rinse
7.	Ethanol, 95%	5 minutes
8.	Xylene*	10 minutes
9.	Xylene*	10 minutes

Mount with coverslip using Permount, balsam, or other mounting medium. Do not excessively destain material. Wash acid-alcohol decolorizer off, in the first 95% ethanol rinse as soon as possible. Change this alcohol frequently to avoid the accumulation of acid in this rinse. Keep the second 95% alcohol and xylene in tightly closed stain jars to prevent hydration.

*Commercially available substitutes, such as Hemo-D, Americlear, or Histoclear may be used.

Interpretation, Hematoxylin Stain: Well-stained organisms will appear bluish or grayish with black nuclear structures. Chromatoid bodies of amoebae cysts and inclusions in the cytoplasm of trophozoites, such as yeast and bacterial cells, will be black or dark blue. Red blood cells will appear a pale yellow/green color. The general background material normally will be a pale blue-gray color. Acid-fast organisms stain red when stained with the modified procedure, above.

Interpretation, Lugols Iodine: Cysts appear light brown in color. Intracellular material stains darker. Glycogen vacuoles stain dark brown to black.

Interpretation, Trichrome Stain: The following is a list of expected reactions for various structures that may be found in a stained smear:

Cysts: Blue-green tinged with purple
Trophozoites: Blue-green tinged with purple
Nuclear chromatin: Red or purplish-red
Chromatoid bodies: Red or purplish-red
Ingested erythrocytes: Red or purplish-red
Ingested bacteria: Red or purplish-red
Yeast, mold: Green (common)
Background material: Green
Eggs, larvae: Red

Materials Required but Not Provided: Standard microbiological supplies and equipment such as applicator sticks, fixative, Permount, and balsam, as well as dehydration and clearing agents, are not provided.

QUALITY CONTROL:

The effectiveness of the stains and the staining techniques is controlled by using prepared smears of known positive specimens. Using control slides, stain simultaneously with test slides. Any inconsistency in the control slides is an indication that the technique was incorrect or that the stains themselves are defective.

User Quality Control: Check for signs of deterioration. Check the performance of the reagents with positive control slides with each test run.

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*For more detailed information, consult appropriate references and/or details in the preface of the PML Technical Manual.

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