
INTENDED USE

The Sigma-Aldrich Silver Stain kit is intended for use in the histologic demonstration of fungi, basement membrane and some opportunistic organisms. The ACCUSTAIN® Silver Stain kit is for "In Vitro Diagnostic Use."

Silver methenamine-borate procedures are well documented.^{1,2} Generally, these require elaborate solution preparation prior to test performance. Solution stability is limited and results vary remarkably due to the capricious nature of metal impregnation and photographic development.

Sigma-Aldrich now offers a Silver Stain that incorporates a stable working silver methenamine salt along with buffer, toning reagent and developer. This provides the laboratory a unique package for visualizing fungi, basement membrane and opportunistic organisms such as *Pneumocystis carinii*.³⁻⁵ Included are silver techniques for rapid staining in microwave ovens.⁶⁻⁸

In brief, microbial cell wall and basement membrane polysaccharides are oxidized to aldehydes by treatment with periodic acid. The aldehyde group, at alkaline pH, reduces silver ion to metallic silver. Rinsing with gold salts forms a more stable gold complex and excess silver is removed by a thiosulfate wash.

REAGENTS

PERIODIC ACID SOLUTION, Catalog No. HT100-1
Periodic acid, 1 g/dl, in deionized water.

BORAX SOLUTION, Catalog No. HT100-2
Borax, 5 g/dl, in deionized water.

SILVER METHENAMINE REAGENT, Catalog No. HT100-3
Silver methenamine, 110 mg/vial.

GOLD CHLORIDE SOLUTION, Catalog No. HT100-4
Gold chloride, 200 mg/dl, in deionized water.

SODIUM THIOSULFATE SOLUTION, Catalog No. HT100-5
Sodium thiosulfate, 2 g/dl, in deionized water.

STORAGE AND STABILITY:

Store Periodic Acid Solution, Working Silver Methenamine Reagent, Gold Chloride Solution, and Sodium Thiosulfate Solution refrigerated (2–8°C).

Store Borax Solution at room temperature (18–26°C). NOTE: Borax may form precipitate during refrigeration. Bring to room temperature and redissolve prior to use.

Reagent labels bear expiration date.

PREPARATION:

Prepare Working Silver Methenamine Solution by combining 8 ml Borax Solution, 100 ml deionized water and contents of Silver Methenamine Reagent vial. Mix until dissolved. Use once, then discard.

NOTE: To increase tests per vial to two, Silver Methenamine may be dissolved in 100 ml of deionized water and stored tightly capped. Stable refrigerated (2–8°C) for 1 month. When ready to use, take 50 ml of Silver Methenamine Solution and add 4 ml of Borax Solution. Return unused portion of Silver Methenamine Solution to refrigerator.

Prewarm all reagents to room temperature before use.

PRECAUTIONS:

Normal precautions exercised in handling laboratory reagents should be followed. Dispose of waste observing all local, state, provincial or national regulations. In case of contact with eyes, rinse immediately with plenty of water and seek medical advice. Wear suitable protective clothing, gloves and eye/face protection. Refer to Material Safety Data Sheet for any updated risk, hazard or safety information.

Fungi TISSUE-TROL/Pneumocystis TISSUE-TROL control slides are paraffin embedded human tissue containing fungi/pneumocystis and should be considered potentially infectious.

US Risks and Safety Statements

Periodic Acid Solution is CORROSIVE. Causes burns. In case of contact with eyes, rinse immediately with plenty of water and seek medical advice. Take off immediately all contaminated clothing. Wear suitable protective clothing, gloves and eye/face protection.

Borax Solution. Caution: Substance has not been fully tested. Avoid contact and inhalation.

Silver Methenamine Reagent is CORROSIVE. Harmful if swallowed. Causes burns. Limited evidence of a carcinogenic effect. May cause sensitization by inhalation and skin contact. In case of contact with eyes, rinse immediately with plenty of water and seek medical advice. Wear suitable protective clothing, gloves and eye/face protection. In case of accident or if you feel unwell, seek medical advice immediately (show the label where possible). Target organs: kidneys and eyes.

Gold Chloride Solution is an IRRITANT. Irritating to eyes, respiratory system and skin. In case of contact with eyes, rinse immediately with plenty of water and seek medical advice. Wear suitable protective clothing and gloves.

Sodium Thiosulfate Solution is an IRRITANT. Irritating to eyes and skin. In case of contact with eyes, rinse immediately with plenty of water and seek medical advice. Wear suitable gloves and eye/face protection.

Reagent Alcohol is FLAMMABLE and an IRRITANT. Irritating to eyes, respiratory system and skin. Keep container tightly closed. Keep away from sources of ignition - no smoking. In case of contact with eyes, rinse immediately with plenty of water and seek medical advice. Wear suitable protective clothing. Target organs: Nerves and liver.

Xylene is FLAMMABLE and HARMFUL. Possible risk of impaired fertility. May cause harm to the unborn child. Harmful by inhalation and in contact with skin. Irritating to respiratory system and skin. Risk of serious damage to eyes. Keep away from sources of ignition - no smoking. In case of contact with eyes, rinse immediately with plenty of water and seek medical advice. Wear suitable protective clothing, gloves and eye/face protection. In case of accident or if you feel unwell, seek medical advice immediately (show the label where possible).

10% Neutral Buffered Formalin is HARMFUL. Harmful by inhalation, in contact with skin and if swallowed. Harmful: possible risk of irreversible effects through inhalation, in contact with skin and if swallowed. May cause sensitization by inhalation and skin contact. Do not breathe vapor. Wear suitable protective clothing and gloves. In case of accident or if you feel unwell, seek medical advice immediately (show the label where possible).

EU Risks and Safety Statements

Periodic Acid Solution is CORROSIVE. Causes burns. In case of contact with eyes, rinse immediately with plenty of water and seek medical advice. Take off immediately all contaminated clothing. Wear suitable protective clothing, gloves and eye/face protection.

Borax Solution. Avoid contact with skin and eyes.

Silver Methenamine Reagent is CORROSIVE. Harmful if swallowed. Causes burns. Limited evidence of a carcinogenic effect. May cause sensitization by inhalation and skin contact. In case of contact with eyes, rinse immediately with plenty of water and seek medical advice. Wear suitable protective clothing, gloves and eye/face protection. In case of accident or if you feel unwell, seek medical advice immediately (show the label where possible).

Gold Chloride Solution is an IRRITANT. Irritating to eyes, respiratory system and skin. In case of contact with eyes, rinse immediately with plenty of water and seek medical advice. Wear suitable protective clothing and gloves.

Sodium Thiosulfate Solution. Avoid contact with skin and eyes.

Reagent Alcohol is HIGHLY FLAMMABLE and an IRRITANT. Highly flammable. Irritating to eyes, respiratory system and skin. Keep container tightly closed. Keep away from sources of ignition - no smoking. In case of contact with eyes, rinse immediately with plenty of water and seek medical advice. Wear suitable protective clothing.

Xylene is HARMFUL. Flammable. Harmful by inhalation and in contact with skin. Irritating to skin. Avoid contact with eyes.

10% Neutral Buffered Formalin is HARMFUL. Harmful by inhalation, in contact with skin and if swallowed. Limited evidence of a carcinogenic effect. May cause sensitization by skin contact. Wear suitable protective clothing and gloves.

PROCEDURE

SPECIMEN COLLECTION:

It is recommended that specimen collection be carried out in accordance with NCCLS document M29-A2. No known test method can offer complete assurance that blood samples or tissue will not transmit infection. Therefore, all blood derivatives or tissue specimens should be considered potentially infectious.

Fungus and Opportunistic Organisms such as *P. carinii*: Fix in 10% neutral buffered formalin or Bouin's Solution, embed in paraffin and cut 5 micron sections.¹

Basement Membrane: Fix in 10% neutral buffered formalin, Bouin's or Zenker's Solution and cut paraffin sections 1–4 microns.¹

SPECIAL MATERIALS REQUIRED BUT NOT PROVIDED:

Plastic forceps or paraffin coated metal forceps

Positive control slides, such as Sigma Fungi TISSUE-TROL, Catalog No. F 0766 or

Pneumocystis TISSUE-TROL slides, Catalog No. P 2418, should be included in each run

FOR STANDARD PROCEDURE ONLY:

Water bath, 62°C

FOR MICROWAVE PROCEDURE ONLY:

Plastic Coplin jars with lids

Accumate™ H2100 Microwave Oven, Catalog No. A 9084, (110 volt) or Catalog No. A 9209, (220 volt)

NOTES:

1. Staining dishes should be washed in SIGMACLEAN™ Liquid Laboratory Cleaning Concentrate, Catalog No. S 4107, then bleach solution, rinsed well in tap water and then in at least 6 changes of deionized water.
2. Appropriate control slides, such as: Fungi TISSUE-TROL, Catalog No. F 0766 or Pneumocystis TISSUE-TROL, Catalog No. P 2418, should be included in each run.
3. If the Sigma-Aldrich H2100 Microwave Oven is used, please see the Owner's Manual for instructions.
4. The data obtained from this procedure serves only as an aid to diagnosis and should be reviewed in conjunction with other clinical diagnostic tests or information.

PROCEDURE:

STANDARD PROCEDURE:

1. Prepare Working Silver Methenamine Solution as described in "Preparation" section and place in 62°C water bath.

NOTE: Do not allow Working Silver Methenamine Solution to come into contact with metals. Check solution to be sure it is at 62°C.

2. Deparaffinize tissue sections and rehydrate to deionized water.
3. Place rehydrated sections in Periodic Acid Solution, Catalog No. HT100-1, for **5 minutes** for fungi and opportunistic organisms or **11 minutes** for basement membrane.
4. Remove slides and rinse in 6 changes of deionized water.
5. **For Demonstration of Fungi and Opportunistic Organisms**, place slides in prewarmed Working Silver Methenamine Solution. Examine microscopically after **20 minutes**. Fungi and opportunistic organisms should be dark brown against a pale yellow background.

For Demonstration of Basement Membrane, place slides in prewarmed Working Silver Methenamine Solution. Examine microscopically after **30 minutes**. Basement membrane should be black against a dark yellow-brown background.

NOTE: While incubating sections at 62°C, place a Coplin jar containing deionized water in 62°C water bath. Use this water for rinsing prior to evaluation in step 5. Examine sections microscopically for adequate staining at stated intervals. Return slides to warm Working Silver Methenamine Solution until desired intensity is achieved.

6. Remove slides from Working Silver Methenamine Solution after adequate structural staining is achieved and rinse 6 times in room temperature deionized water.
7. Tone sections in Gold Chloride Solution, Catalog No. HT100-4, for **30 seconds**.
8. Rinse in room temperature deionized water 6 times.
9. Place sections in Sodium Thiosulfate Solution, Catalog No. HT100-5, for **2 minutes**.
10. Wash well in running tap water.
11. Counterstain according to personal preference with Tartrazine Solution, Fast Green FCF, Light Green SF Yellowish, Harris Hematoxylin Solution or Eosin Y Solution.
12. Dehydrate in alcohol, clear in xylene, mount and examine microscopically.

ACCUMATE™ H2100 MICROWAVE PROCEDURE:

1. Deparaffinize slides and hydrate to deionized water.
2. Place slides in **40 ml** of Periodic Acid Solution, Catalog No. HT100-1, contained in a plastic Coplin jar. Loosely cover Coplin jar with lid before placing in microwave oven, or use Coplin jars with holes drilled into the lids. Microwave on **800 watts for 10 seconds**.

***Periodic Acid Solution may be reused if used at room temperature for **5 minutes** instead of microwaving. ***For basement membranes Periodic Acid Solution Incubation time is **11 minutes at room temperature**.

3. Remove slides from Periodic Acid Solution and rinse in 6 changes of deionized water.
4. Place slides in **40 ml** of Working Silver Methenamine Solution contained in a plastic Coplin jar.
5. a. **For Fungal Organisms** microwave on **600 watts for 35 seconds**. Gently mix solution with a beral pipette or applicator stick. Let incubate for **2-3 minutes**. After no longer than **2 to 3 minutes**, place slides in warm deionized water and check development microscopically. If organisms are not sufficiently developed, return slides to Working Silver Methenamine Solution and let sit for **30 seconds to 1 minute**.

NOTE: Suggested developing times for the following fungal organisms are as follows:

Blastomyces	2 minutes
Pneumocystis	2-3 minutes
Aspergillus	2-3 minutes

- b. **For Basement Membrane** microwave on **600 watts for 35 seconds**. Gently agitate solution. Let slides incubate for **5 minutes**. Microwave a second time at **600 watts for 10 seconds**. Let slides develop for 2 minutes. Check slides microscopically. If slides are not sufficiently developed, repeat last step until desired tone is achieved.
6. Rinse slides in 6 changes of deionized water.
7. Tone slides in Gold Chloride Solution, Catalog No. HT100-4, for **30 seconds at room temperature**.
8. Rinse slides in 6 changes of deionized water.
9. Place slides in Sodium Thiosulfate Solution, Catalog No. HT100-5, for **2 minutes at room temperature**.
10. Wash well in running tap water.
11. Counterstain according to personal preference with Fast Green FCF, Light Green SF Yellowish, Tartrazine or Hematoxylin or Eosin Y Solutions.
12. Dehydrate in alcohol, clear in xylene, mount and examine microscopically.

PERFORMANCE CHARACTERISTICS

Fungi	Purplish-brown to black
P. carinii	Purplish-brown to black
Basement Membrane	Black
Background	Dependent upon counterstain

If observed results vary from expected results, please contact Sigma-Aldrich Technical Service for assistance.

REFERENCES

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