REAGENTS

SODIUM HYDROXIDE SOLUTION, Catalog No. HT1021-100 ml 3% aqueous solution

POTASSIUM PERMANGANATE SOLUTION, Catalog No. HT1022-100 ml 1% aqueous solution

OXALIC ACID SOLUTION, Catalog No. HT1023-100 ml 1% aqueous solution

FERRIC AMMONIUM SULFATE SOLUTION, Catalog No. HT1024-500 ml 2.5% aqueous solution

SILVER NITRATE SOLUTION, Catalog No. HT1025-50 ml 10% aqueous solution

GOLD CHLORIDE SOLUTION, Catalog No. HT1026-100 ml 0.2% aqueous solution

SODIUM THIOSULFATE SOLUTION, Catalog No. HT1027-500 ml 5% aqueous solution

STORAGE AND STABILITY:

Store unopened Reticulum Stain kit in refrigerator (2–8°C). After opening, Sodium Hydroxide, Potassium Permanganate, Oxalic Acid, Ferric Ammonium Sulfate and Sodium Thiosulfate Solutions may be stored in the refrigerator or at room temperature (2–26°C). Store Silver Nitrate and Gold Chloride Solutions in refrigerator (2–4°C). Reagents are stable until the expiration dates shown on the labels.

Silver Nitrate Working Solution should be used once and then discarded.

In dry form ammoniacal salts may present an explosive hazard. Do not allow ammoniacal silver solution to dry out. Store used ammoniacal silver solution in plastic bottles – do not store in glass. Wallington recommended the inactivation of the ammoniacal silver solution by the addition of dilute hydrochloric acid or sodium chloride solution.

DETERMINATION:

Potassium Permanganate Solution should be purple. Solution may be used several times, but should be discarded if it turns brown.

PREPARATION:

To prepare AMMONIACAL SILVER NITRATE SOLUTION:

1. Pipet 5 ml Silver Nitrate Solution in an Erlenmeyer flask.
2. In a hood, while shaking or swirling the flask continuously, add concentrated ammonium hydroxide, drop by drop, until the precipitate it formed is completely dissolved. Do not add excess ammonium hydroxide.
3. Add 5 ml Sodium Hydroxide Solution to the flask. Solution will turn black and precipitate will form. Continuously swirl the flask and add concentrated ammonium hydroxide, drop by drop, until the precipitate just dissolves. At this stage the solution should not be completely clear.

NOTE: If no cloudiness remains, add Silver Nitrate Solution drop by drop, until one drop causes the solution to become permanently cloudy. Only a faint cloudiness is desirable.

4. Dilute the resulting solution to 50 ml with distilled or deionized water. Filter into a chemically clean Coplin jar. Use once and then discard.

5. Wash slides in several changes of distilled water.

6. Sensitize sections in Ferric Ammonium Sulfate Solution for 2 minutes.

7. Wash slides in several changes of distilled water.

8. Impregnate sections with Ammoniacal Silver Nitrate Solution for 2 minutes.

9. Rinse slides well with distilled water.

10. Reduce sections for 2 minutes in 10% Formalin Solution.

11. Wash slides in tap water for 3 minutes.

12. Tone sections in Gold Chloride Solution for 10 minutes.

13. Rinse slides in distilled water.

14. Place slides in Sodium Thiosulfate Solution for 1 minute.

15. Wash slides in tap water for 2 minutes.

16. Counterstain, if desired, with Nuclear Fast Red for 3–5 minutes or an Eosin Y Solution for 1–2 minutes. Generally, all sections except those from liver are counterstained. Wash well in water.

17. Dehydrate in two changes each of 95% ethanol and absolute ethanol.

18. Clear in xylene and mount with synthetic resin.

PROCEDURE

SPECIMEN COLLECTION:

It is recommended that specimen collection be carried out in accordance with CLSI document M29-A3. 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.

Fix specimen in 10% neutral buffered formalin, process and embed in paraffin. Cut paraffin sections at 4 to 5 microns. Incorporate appropriate controls.

SPECIAL MATERIALS REQUIRED, BUT NOT PROVIDED:

Positive control slides, such as Sigma Reticulum TISSUE-TROL™ slides, Catalog Nos. R478B or TTR010, should be included in each run

Counterstain (optional) Eosin Y solution or Nuclear Fast Red Solution, Catalog No. N3020, nuclear fast red 0.1% in 5% aluminum sulfate

Ammonium Hydroxide Solution, concentrated

Ethanol, absolute

Formalin Solution, 10%

Reagent Alcohol

Xylene or xylene substitute

Forceps, plastic or paraffin coated

Coplin jars, chemically clean

NOTE:

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:

1. Deparaffinize sections and hydrate to distilled water.

2. Oxidize sections in Potassium Permanganate Solution for 5 minutes.

3. Rinse slides in tap water for 2 minutes.

4. Wash in Oxalic Acid Solution for 2 minutes or until sections are colorless.

5. Washing slides in tap water for 2 minutes.

6. Sensitize sections in Ferric Ammonium Sulfate Solution for 15 minutes.

7. Wash slides in several changes of distilled water.

8. Impregnate sections with Ammoniacal Silver Nitrate Solution for 2 minutes.

9. Rinse slides well with distilled water.

10. Reduce sections for 2 minutes in 10% Formalin Solution.

11. Wash slides in tap water for 3 minutes.

12. Tone sections in Gold Chloride Solution for 10 minutes.

13. Rinse slides in distilled water.

14. Place slides in Sodium Thiosulfate Solution for 1 minute.

15. Wash slides in tap water for 2 minutes.

16. Counterstain, if desired, with Nuclear Fast Red Solution for 3–5 minutes or an Eosin Y Solution for 1–2 minutes. Generally, all sections except those from liver are counterstained. Wash well in water.

17. Dehydrate in two changes each of 95% ethanol and absolute ethanol.

18. Clear in xylene and mount with synthetic resin.

PERFORMANCE CHARACTERISTICS

Reticulum – Black
Background – Pink to rose (if counterstained with Nuclear Fast Red)

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

REFERENCES


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