INTENDED USE

Acid Fast Stain is for the demonstration of Mycobacterium tuberculosis in tissue sections and smears. Acid Fast Stain reagents are for “In Vitro Diagnostic Use”. Carbol-fuchsin acid fast stain is used to demonstrate Mycobacterium tuberculosis in tissue sections and on smears. This procedure uses pararosaniline dye in a phenol solution. Phenol appears to combine the pararosaniline dye within the acid fast bacilli. Dimethyl sulfoxide (DMSO) is added to the solution to accelerate the infiltration of dye. When stain is applied, all cells including hard to stain acid fast, are colored red. The next step is to decolorize all tissue components except the acid fast bacilli. This procedure incorporates the decolorizer with the counterstain in the malachite green solution, it eliminates problems of over decolorization and inconsistent counterstaining. Included is an acid fast stain technique for tissue paraffin sections using rapid staining in a microwave oven.

REAGENTS

CARBOL-FUCHSIN SOLUTION, Catalog No. HT801 (HT8018-250ML; HT80116-500ML) Paraarosaniline (certified), 0.85%; phenol, 2.5%; glycerol, 5%; DMSO, 5%; and ethyl alcohol, 5%, in deionized water.

MALACHITE GREEN SOLUTION, Catalog No. HT802 (HT8028-250ML; HT80216-500ML) Malachite green oxalate (certified), 1.5%; acetic acid, 10%; and glycerol, 17%, in deionized water. Malachite Green Solution contains both counterstain and differentiator.

STORAGE AND STABILITY:

Store Carbol-Fuchsin Solution and Malachite Green Solution at room temperature (18–26°C). Reagents are stable until expiration dates shown on the labels.

PREPARATION:

Carbol-Fuchsin Solution and Malachite Green Solution are ready to use. Malachite Green Solution should be used once and properly discarded.

PRECAUTIONS:

Normal precautions exercised in handling laboratory reagents should be followed. Dispose of waste observing all local, state, provincial or national regulations. Refer to Material Safety Data Sheet and product labeling for any updated risk, hazard or safety information.

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.

Tissue: Any well fixed paraffin section cut 5–6 microns or frozen section.

Smears: Smear a direct or concentrated specimen on clean unused slide. Air dry and fix smears by flaming or a 30-second immersion in absolute alcohol.

SPECIAL MATERIALS REQUIRED BUT NOT PROVIDED:

Positive control slides, such as Acid Fast Tissue-Trol™ Control Slides, Catalog Nos. A2299 or TTR001, should be included in each run.

Xylene or xylene substitute

OPTIONAL REAGENTS/INSTRUMENTS:

Absolute Alcohol
Copol jars
Microwave oven

NOTES:

It is recommended that gloves be worn when performing these procedures. If methylene blue is used as a counterstain, do not over-stain with methylene blue. Overstaining with methylene blue can mask any organism present. If the slides are overstained, the slides should be placed in acid alcohol to remove the methylene blue counterstain. Wash in water and then repeat the counterstaining step. DO NOT dehydrate through graded alcohols after staining. Alcohol dehydration may result in over-differentiation of tissue sections.

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:

TISSUE PARAFFIN SECTIONS – MANUAL:

1. Deparaffinize slides and hydrate to deionized water.
2. Stain in Carbol-Fuchsin Solution for 30–45 minutes.
3. Wash in deionized water to remove excess stain.
4. Differentiate and counterstain in Malachite Green Solution for 2 minutes.
5. Place slides in 40 ml Malachite Green Solution for 1 minute at room temperature.
6. Rinse in tap water and let air dry, or blot completely dry.
7. Dip in xylene and coverslip.

MICROWAVE PROCEDURE:

1. Deparaffinize slides and hydrate to deionized water.
2. Place slides in 40 ml Carbol-Fuchsin Solution contained in a plastic Coplin jar. Loosely cover jar with lid or use vented lids.
3. Microwave on 400 watts for 3 seconds. Stir with beral pipette or applicator stick. Let incubate for 10 minutes.
4. Wash in deionized water to remove excess stain.

SMEARS AND FROZEN SECTIONS – MANUAL:

1. Place slide in Carbol-Fuchsin Solution for 3 minutes.
2. Wash in tap water.
3. Counterstain and differentiate in Malachite Green Solution for 1–2 minutes.
4. Rinse in tap water and let air dry, or blot completely dry.
5. Dip in xylene and coverslip.

PERFORMANCE CHARACTERISTICS

Acid fast bacilli – Red
Background – Green

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

REFERENCES


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