
AURAMINE-RHODAMINE FLUORESCENCE - ACID FAST BACTERIA

PURPOSE: To demonstrate acid fast bacteria, mycobacterium tuberculosis.

PRINCIPLE: Both dyes are basic dyes that fluoresce at short wavelengths.

CONTROL: Tissue containing acid-fast organisms. Millipore™-filtered water only should be used in the floatation bath, in stain preparation, and procedure.

FIXATIVE: 10% formalin

TECHNIQUE: Cut paraffin sections 4-5m

EQUIPMENT: Coplin jars (all glassware must be rinsed with DI water) microwave oven, fluorescence microscope.

REAGENTS:

Auramine-Rhodamine Solution:

Auramine O	10.5 gm
Rhodamine B	5.25 gm
Glycerol	525.0 ml
Phenol	70.0 ml
Millipore™ water	350.0 ml

Rinse all glassware in distilled water. Place the solution in a 60°C oven overnight, mix on stir plate. Filter amount needed prior to use, discard after use. Label with initials and date, solution is stable for 1 year.

CAUTION: Possible carcinogen.

0.5% Acid Alcohol:

Hydrochloric acid	5.0 ml
70% alcohol	995.0 ml

Mix well, label with date and initials. Solution is stable for 1 year.

CAUTION: Flammable, corrosive.

0.5% Potassium Permanganate:

Potassium permanganate	0.25 gm
Distilled water	50.0 ml

Make fresh, discard after use.

CAUTION: Corrosive.

MICROORGANISMS

AURAMINE-RHODAMINE

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SAFETY: Wear gloves, lab coat and goggles. Work in a well ventilated area, preferable under a hood. Avoid contact and inhalation of chemicals.

Auramine O: possible carcinogen to humans. Animal studies; causes tumors, neoplastic

Rhodamine B: possible carcinogen, equivocal tumorigenic.

Phenol: toxic by ingestion, inhalation and skin absorption. Readily absorbed through skin, causing increased heart rate, convulsions and death. Will burn eyes and skin, analgesic action may cause loss of pain. Target organ effects in digestive, nervous and urinary systems.

Hydrochloric acid: strong irritant to skin, eyes and respiratory system. Target organ effects via inhalation on skin, respiratory, reproductive and fetal systems.

Potassium permanganate: skin and eye irritant, ingestion will lead to severe gastrointestinal distress. Oxidant.

PROCEDURE:

1. Deparaffinize and bring to distilled water.
2. *Auramine-rhodamine solution, microwave 80 power, 45 seconds, allow to stand 5 minutes.
3. Differentiate sections in acid alcohol until colorless.
4. Wash in running tap water.
5. Potassium permanganate, 2 minutes.
6. Rinse in distilled water.
7. Dehydrate, clear, and coverslip.

* Conventional Method: 60°C oven for 1 hour.

RESULTS:

Acid-fast organisms: reddish-yellow fluorescence

Background: black

REFERENCES:

Carson F, Histotechnology: A Self-Instructional Text,1990,pp192-193,
ASCP, ILL

Crookham,J, Dapson,R, Hazardous Chemicals in the Histopathology
Laboratory, 2nd ED, 1991, Anatech

Prepared:_____By: _____

Approved:_____By:_____

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PROCEDURE CARD

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SAFETY: Wear gloves, lab coat and goggles. Work in a well ventilated area, preferable under a hood.

Auramine and Rhodamine: carcinogen.

Open hot solutions within exhaust hood. Phenol will burn the skin wear nitrile gloves.

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TECH: _____

DATE: _____

EXPIRATION: _____

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