FA Rhodamine Counterstain

FA Rhodamine Counterstain is used as a counterstain in the Fluorescent Acid Fast staining procedure.

**Composition**

**Ingredients**

- Potassium permanganate: 5.000 gms
- Distilled water: 100.000 ml

**Formula adjusted, standardized to suit performance parameters**

**Directions**

1. Prepare heat fixed smears.
2. Flood the smear with auramine O-rhodamine B stain and heat to 60°C for 10 min; or 37°C for 15 min; or 25°C for 20 min.
3. Rinse briefly in tap water.
4. Decolorize with 0.5% HCl in 70% ethanol for 2-3 min, and then rinse thoroughly.
5. Flood the smear with S041 (FA Rhodamine counterstain) for at least 2 min, but not longer than 4 min.
6. Rinse, blot dry, and examine by fluorescent microscopy. Use a 25X to 45X objective and a 10X eyepiece. Confirm the cellular nature of fluorescent areas using the 100X oil immersion objective.

**Principle And Interpretation**

The Acid fast staining technique is useful for identification of the tubercle bacillus, other Mycobacteria, and Nocardia, which depends on the chemical composition of the bacterial cell wall. Because of the difficulty in staining these organisms with ordinary dyes, basic dyes in the presence of controlled amounts of acid are used. Generally, heat must be applied during the staining procedure. The phenolated fluorescent dye is used to stain acid fast bacilli. The advantage of a fluorescent procedure is that the acid-fast organisms appear as bright fluorescence against a dark background. Mycobacteria decolourizer is used for decolorizing the non-acid fast bacteria or other cellular matter; while Potassium permanganate is used as counter stain. Potassium permanganate apparently serves as a gentle decolorizer and results in less fluorescence in the background material.(1)

**Quality Control**

**Appearance**

Reddish violet coloured solution.

**Clarity**

Clear without any particles.

**Microscopic Examination**

Acid fast staining is carried out and staining characteristic of organism is observed under fluorescent microscope by using oil immersion lens.

**Results**

Acid fast organism shows fluorescence where as non-acid fast organisms and background remain non-fluorescent or show slight fluorescence.

**Storage and Shelf Life**

Store below 30°C in tightly closed container and away from bright light. Use before expiry date on label.

**Reference**

1. Staining Procedures 4th edition: Edited by George Clark

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**Disclaimer**

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