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Section: <b>Mycology Bench Manual</b>	Subject Title: <b>Acid-Fast Stain for Nocardia (Modified Kinyoun)</b>	
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## **ACID-FAST STAIN FOR NOCARDIA (MODIFIED KINYOUN)**

### **Principle**

*Nocardia* species possess the unique characteristic of resisting decolorization with acid alcohol.

### **Reagents**

1. Carbol-fuchsin
 

Basic fuchsin solution	(3 g basic fuchsin in 100 mL 95% ethyl alcohol)
	10 mL
Phenol 5% aqueous	90 mL
  
2. Decolourizer (1% sulfuric acid)
 

H <sub>2</sub> SO <sub>4</sub> (concentrated)	1 mL
Distilled water	99 mL
  
3. Methylene blue
 

Methylene blue	0.3 g
Distilled water	100 mL

### **Staining Procedure**

1. Fix the smear by gentle heating.
2. Flood the smear with Carbol fuchsin solution.
3. Allow the slide to stand for 5 minutes.
4. Wash the smear with tap water.
5. Decolorize the smear with 1% sulfuric acid until no more colour appears in the washing (approx. 1 min.).
6. Rinse with tap water.
7. Counterstain with methylene blue for approximately 1 minute.
8. Rinse with tap water and air dry.

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### **Interpretation**

The filaments of *Nocardia* species and *Rhodococcus* appear red-stained against a blue background.

### **Quality Control**

A positive control slide of *Nocardia* species is stained simultaneously with the clinical specimens.

### **References**

1. Murray, PA. et al. Manual of Clinical Microbiology, 7<sup>th</sup> edition, 1999 ASM Press, Washington, D.C.