

**OLIVE VIEW-UCLA MEDICAL CENTER  
DEPARTMENT OF PATHOLOGY  
POLICY & PROCEDURE**

**NUMBER: 11877  
VERSION: 1**

**SUBJECT/TITLE: SS 011 - KINYOUN ACID FAST STAIN**

**POLICY:** Kinyoun Acid Fast Stain shall be performed to determine the presence of Acid-Fast Mycobacteria in patient tissue samples.

**PURPOSE:** To diagnosis the presence of Acid-Fast Mycobacteria in patient tissue samples.

**DEPARTMENTS:** **PATHOLOGY & LABORATORY SERVICES**

**DEFINITIONS:**

**PROCEDURE:**

**I. Fixative**

Although 10% neutral buffered formalin is preferred, other fixatives except for Carnoy solution, may be utilized.

**II. Equipment**

Coplin jars	Filter Paper	Pipettes
Erlenmeyer flasks	Graduated Cylinders	

**III. Slide Preparation**

Section paraffin tissue blocks at 4-5 microns.

**IV. Quality Control**

Tissue section contain Acid-Fast organisms must be utilized.

**V. Reagents**

**A. Carbol Fuchsin – Kinyoun**

- Commercially prepared – Poly Scientific (S161-8oz)

**B. Decolorizing Solution (1% Acid Alcohol)**

- 99 ml of 70% Alcohol
- 1 ml of Hydrochloric Acid (conc.)

**C. Fast Green – 0.01% Aqueous**

- Commercially prepared – Poly Scientific (s2252-8oz)

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**IV. Staining Procedure**

- A. Deparaffinize and hydrate tissue slides to distilled water. If the tissue was fixed in a “B-5” solution, perform the Removal of Mercury Pigment procedure. (Outlined in HIS 76.)
- B. Stain the slides in Kinyoun Carbol-Fuchsin solution (freshly filtered) for 1 hour at room temperature.
- C. Rinse the slides well in running tap water, then wash them in three (3) changes of distilled water for 1 minute.
- D. Dip the slides in 2 changes of 1% Acid Alcohol until tissue is pale pink.
- E. Wash the slides well in running tap water, then rinse them in distilled water for 1 minute.
- F. Counterstain the slides in the Fast-Green solution for 15 – 30 seconds.
- G. Dehydrate the slides in two changes each of 95% and absolute ethyl alcohol.
- H. Clean the slides in three changes of xylene and mount with synthetic resin.

**VI. Results**

Acid Fast organisms..... Red  
Background.....Green

**Notes:**

- 1. The counterstain is critical in this procedure, as over counterstaining with Fast Green will mask any organisms present. If the section is overstained, take it back to the acid-alcohol to remove the Fast Green, wash with water, and then repeat the counterstaining step.
- 2. If the acid is not washed out of the tissue before the counterstaining step, the tissue will not stain.
- 3. If the sections dry after the Carbol-Fuchsin stain is applied, a compound that is resistant to decolorization will be formed.

References: Freida L. Carson, PhD, HT (ASCP), Histotechnology, A Self-Instructional Text, ASCP Press, ASCP, Chicago, 1997, page 183.	
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