

## **A-111      WARTHIN-STARRY METHOD FOR SPIROCHETES AND DONOVAN BODIES**

**FIXATION:**    **10% Buffered Neutral Formalin** (F-113). Avoid chromate fixatives.

**TECHQUE:**    Cut paraffin at 6 microns

**STAINING PROCEDURE:**    Use control slides.

1. Deparaffinize and hydrate to triple distilled water.
2. Impregnate with **Silver Nitrate, 1%** (A-111-1), heated in a flotation bath to 43°C for 30 minutes. (Use paraffin-coated forceps throughout).
3. Prepare developer solution:
  - 1.5ml **Silver Nitrate, 2%** (A-111-2).
  - 3.75ml Gelatin **Solution, 5%** (A-111-5).
  - 2.0ml Hydroquinone **Solution, 0.15%** (A-111-6).Place solutions in 50ml flasks a flotation bath @ 54°C for 15-30 minutes.  
Combine solutions in order in a small beaker making certain solutions are mixed well. Prepare immediately before use.
4. Support sections on glass rods and flood with developer solution. Develop until sections are light brown or yellow. Check control under microscope: Spirochetes should be black and the background light brown or yellow.
5. Wash quickly and thoroughly in 54°C tap water. Rinse in distilled water.
6. Dehydrate in two changes of 95% Alcohol, Absolute Alcohol.
7. Clear in **Xylene** (C-120) two changes.
8. Mount with **Permout** (M-18).

**RESULTS:**

Spirochetes, Donovan Bodies	black
Background	yellow to light brown

**REFERENCES:**

AFIP, Manual of Histological Staining Methods, 3<sup>rd</sup>. ed., Ed. L. Luna: New York: McGraw Hill Publications, c. 1968, p. 238.

Kerr, D.A.: Amer. J. Clin. Path. Tech. Suppl., 8:64, 1938