

## D-251 CUSTER'S METHOD FOR SECTIONS

**Fixation:** Formol Zenker (Helly's) Solution for 4-6 hours.

**Embedding Schedule:**

1. Wash 1 hour in running water.
2. Decalcify overnight if necessary (until bony button is soft to the prick of a needle) in **Formic Acid, 5%** (F-92). Cut off cortical bone and wash gently one hour in running water.
3. Dehydrate in ascending strengths of alcohol, clear and pass into paraffin as usual.

**Sections:** Paraffin at 3 - 4 microns.

**Staining:**

1. Carry through xylene and absolute alcohol into 95% alcohol.
2. Remove the mercuric precipitate (from fixative) by immersing several minutes in **Alcoholic Iodine, 2%**, (D-251-6), then in 80% alcohol, tap water, **Sodium Thiosulfate, 5%** (D-251-7) and three changes of distilled water.
3. Place vertically in the working staining solution for 15- 16 hours. To prepare working staining solution, mix, just before use:

<b>Eosin Y, 0.1%, Aqueous</b> (D-251-1)	20 ml
<b>Azure II, 0.1%, Aqueous</b> (D-251-2)	10 ml
Distilled Water	80 ml

Filter. If the tissue is very bloody, it is advisable to use only 5 ml of **Eosin Y, 0.1%, Aqueous** (D-251-1).

4. Pass through two changes of 95% alcohol, controlling differentiation by examination with a low power microscope. Wash in two changes of absolute alcohol
5. Clear in two changes of Xylene (C-120) and mount in Canada Balsam (M-6).

**Results:**

Erythrocytes	Orange
Cytoplasm of lymphocytes and blastocytes	Blue
Nuclei	Deep blue to violet-blue
Must cell granules	Violet to reddish purple
Cartilage	A more reddish purple
Bone matrix	Pink

**References:**

Clark, G.: *Staining Procedures*, Williams and Wilkins Co., Baltimore, 3<sup>rd</sup> Ed., c. 1973, p. 132.

Kolmer, J.A. & Boerner, F., *Approved Laboratory Technic*, 3<sup>rd</sup> Ed., Appleton-Century Co., Inc. New York, 1941, pp. 833-4.

Custer, R.P., *Am. J. M. Sc.*, N.S. 185, pp. 617-24, 1933.