

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:

Eosin Y, 0.1%, Aqueous (D-251-1)	20 ml
Azure II, 0.1%, Aqueous (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, 3rd Ed., c. 1973, p. 132.
Kolmer, J.A. & Boerner, F., Approved Laboratory Technic, 3rd 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.