

G-486, LUNA'S METHOD FOR ERYTHROCYTES AND EOSINOPHIL GRANULES

Fixation: 10% Buffered Neutral Formalin (F-113)

Sections: Paraffin @ 6 microns

Procedure:

1. Deparaffinize and hydrate to distilled water.
2. Stain for 5 minutes in Working Hematoxylin-Biebrich Scarlet Solution.

*To prepare Working Hematoxylin-Biebrich Scarlet Solution:

First prepare Hematoxylin Working Solution by mixing:

1. Weigert's Hematoxylin A (G-486-1A).....22.5 ml
2. Weigert's Hematoxylin B (G-486-1B)... ..22.5 ml

Mix solutions well- then add...

3. Biebrich Scarlet, 1% Aqueous (G-486-2)...5 ml

Mix well.

3. Differentiate in Acid Alcohol, 1% (G-486-3) until the desired nuclear detail is achieved (usually 8 dips). Remove Acid Alcohol by rinsing in water.
4. Dip in Lithium Carbonate, 0.5% (G-486-4) until section turns blue and erythrocytes are bright red (usually 5 dips). Wash in running water for 2 minutes.
5. Dehydrate in 95% alcohol, absolute alcohol, and clear in xylene, two changes each.
6. Mount with Permount (M-18).

Stain Results:

Eosinophil granules, erythrocytes and Charcott Leyden crystals	Red
Background	Blue

References:

Luna, L.G., (ed.), Manual of Histologic Staining Methods of the AFIP, 3rd edition,
McGraw-Hill, NY, p1111, c 1968.