Luna’s method for Eosinophils and Erythrocytes

Safety equipment: Work under a hood with lab coat, gloves, and glasses.

1. Hydrate slides to running water ------------------------ 5 minutes
2. Stain in working Hematoxylin-Biebrich scarlet --------- 5 minutes
3. Differentiate in 1% acid alcohol ----------------------- 8 dips
4. Rinse in tap water ----------------------------------- 30 seconds
5. Dip in lithium carbonate – until section turns blue and erythrocytes are bright red
6. Wash in running water ------------------------------- 2 minutes
7. Dehydrate in 95% alcohol----------------------------- 30 seconds
8. Dehydrate in 100% alcohol----------------------------- 1 minute
9. Dehydrate in 100% alcohol----------------------------- 2 minutes
10. Clear in xylene (3) ---------------------------------- 2 minutes (re-use)
11. Clear in xylene (3) ---------------------------------- 5 minutes (re-use)
12. Results:

Eosinophil granules ------------------- Red
Erythrocytes ------------------------- Red
Charcott Leyden Crystals -------------- Red
Background -------------------------- Blue

Remarks – Especially useful for phagocytosis and sometimes demonstrating Negri bodies.
**SOLUTIONS**

**Weigert's Iron Hematoxylin**

Use Weigert's for Masson's Trichrome

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**1% Biebrich Scarlet**

Biebrich Scarlet ------------------------------------------ 1.0gm
DH2O ------------------------------------------ 100.0ml

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**Hematoxylin-Biebrich Scarlet (working)**

Weigert's iron hematoxylin solution (working) ------------------- 45.0ml
Biebrich Scarlet solution ------------------------------------------ 5.0ml

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**1% Acid Alcohol**

Use acid alcohol for H&E

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**0.5% Lithium Carbonate**

Lithium Carbonate ------------------------------------------ 0.5gm
DH2O ------------------------------------------ 100.0ml