**Congo Red Staining Protocol for Amyloid**

**Description:** This modified Highman's Congo Red stain. It is used for the detection of amyloid on formalin-fixed, paraffin-embedded tissue sections with amyloidosis, and may be used for frozen sections as well. The amyloid deposits will be stained red and the nuclei will be stained blue. The thickness of sections is usually 5 um. But in case of inadequate amyloid deposits, 10um thick sections will be more satisfactory. Congo red stains amyloid in tissue sections.

**Fixation:** 10% Neutral Buffered formalin.

**Section:** paraffin sections at 5 – 10 um (in case inadequate amyloid deposits, use 10 um thick sections).

**Solutions and Reagents:**

0.5% Congo red in 50% alcohol:

 Congo red (Sigma, Cat# C-6277) -------- 0.5 g

 50% Alcohol -------------------------------- 100 ml

1% Sodium Hydroxide:

 Sodium hydroxide -------------------------- 1 g

 Distilled water ------------------------------ 100 ml

Alkaline Alcohol Solution:

 1% Sodium hydroxide ----------------------- 1 ml

 50% alcohol ---------------------------------- 100 ml

**Procedure:**

1. Dewax in Xylene – 2 mins
2. Dewax in Xylene – 2 mins
3. Dewax in Xylene – 2 mins
4. Wash in Absolute Alcohol – 2 mins
5. Wash in Absolute Alcohol – 2 mins
6. Wash in 90% Alcohol – 2 mins
7. Wash in 70% Alcohol – 2 mins
8. Wash in Running Water – 2 mins
9. Stain in congo red solution for 15-20 minutes.
10. Rinse in distilled water.
11. Differentiate quickly (5-10 dips) in alkaline alcohol solution.
12. Rinse in tap water for 1 minute.
13. Counterstain with Gill's hematoxylin for 30 seconds.
14. Rinse in tap water for 2 minutes.
15. Dehydrate through 95% alcohol, 2 changes of 100% alcohol, 3 minutes each.
16. Clear in xylene or xylene substitute, 2 changes, 3 minutes each.
17. Mount with resinous mounting medium.

**Results:**

 Amyloid, elastic fibers, eosinophil granules -------- red

 Nuclei --------------------------------------------------- blue