**UW Medicine - Pathology**

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Bennhold's Congo Red Technique for Demonstration of Amyloid Procedure

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| Adopted Date: 09/05/07  Review Date: 09/03/10  Revision Date: 04/05/11 |

PURPOSE

To identify the method for performing the special stain of Bennhold's Congo Red Technique for demonstration of amyloid.

PROCEDURE

**Fixation:**

Results are best with Carnoy's fluid or absolute alcohol; 10% buffered neutral formalin and Bouin's fluid may be used.

**Sectioning:**

Cut paraffin sections at 10 microns.

**Solutions:**

(Use Type II deionized water for all solution preparation.)

**Congo Red**

Richard Allan Scientific. Ready made.

*Working:*

**Congo Red Solution** 50 ml

**Sodium Hydroxide** 10 drops

Keep stock solution refrigerated. Discard working solution at end of the day.

**Harris Hematoxylin**

Richard Allan Scientific. Ready made.

**Bluing Reagent**

Richard Allan Scientific. Ready made.

**Modified Mayer’s Hematoxylin**

Richard Allan Scientific. Ready made.

**Procedure:**

Use control slide.

1. De-paraffinize and hydrate to distilled water.
2. Stain in working Alkaline Congo Red for 40 minutes
3. Rinse in distilled water for 1 minute.
4. Place slides in Mayer Hematoxylin for 4 minutes.
5. Rinse in distilled water for 1 minute.
6. Stain in bluing solution for 1 minute.
7. Rinse in distilled water for 1 minute.
8. Dehydrate in 2 changes of 100% alcohol, 1 minute each
9. Clear in 3 changes of xylene, 1 minue each.

**Results:**

Amyloid red to pink-red

Nuclei blue

Elastic fibers lighter red

Other tissue elements unstained

**Comments:**

The working solutions of saturated sodium chloride in 80% Alcohol and saturated Congo Red are not stable. Congo red deteriorates in alkaline media. After standing

for one hour or longer, staining of amyloid is diminished and coloration of background increases.

Staining is more intense in alcohol-fixed tissues than in formalin-fixed tissues, but even in the latter small deposits of amyloid are visible.

Sections are cut at 10 microns since small amounts of amyloid are more easily demonstrated at this thickness.

The congo red dye is a linear molecule. This configuration permits hydrogen bonding of the azo and amine groups of the dye to similarly spaced carbohydrate hydroxyl radicals of the amyloid. The pretreatment with alkali aids in releasing native internal hydrogen bonding between adjacent polysaccharide chains resulting in more potential sites available for dye binding. Amyloid is also birefringent after staining with Congo Red.

REFERENCES

Sheehan, Dezna C. and Hrapchak, B.B.: *Theory and Practice of Histotechnology,*  The C.V.Mosby Co., 1973, pg. 99-100.

Written By: Director Approval:

(Signature and Date) (Signature and Date)

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Histology Supervisor