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**CONGO RED - PUTCHLER'S MODIFICATION - AMYLOID**

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**PURPOSE:** To demonstrate amyloid deposits in tissue sections.

**PRINCIPLE:** Amyloid is homogeneous and eosinophilic, the deposits are extracellular and may become sufficiently large enough to cause damage to surrounding tissues. when stained with the Congo Red Stain the amyloid, with the aide of polarizing lenses, will birefringe an apple green color. under the microscope.

**CONTROL:** Known amyloidosis containing tissue.

**FIXATION:** Carnoy's and absolute alcohol are recommended, 10% NBF or Bouin's.

**TECHNIQUE:** Cut paraffin sections at 10 microns.

**EQUIPMENT:** Rinse glassware in DI water. Coplin jars, funnel and glass wool, graduated cylinder, 1mm pipette, and microwave.

**REAGENTS:**

**Mayer's Hematoxylin:**

|   |           |
|---|-----------|
| Hematoxylin                               | 1.0 gm    |
| Distilled water                           | 1000.0 ml |
| Sodium iodate                             | 0.2 gm    |
| Ammonium or Potassium<br>aluminum sulfate | 50.0 gm   |
| Citric acid                               | 1.0 gm    |
| Chloral hydrate                           | 50.0 gm   |

**1% Sodium Hydroxide**

|                  |          |
|------------------|----------|
| Sodium hydroxide | 1.0 gm   |
| Distilled water  | 100.0 ml |

Mix well. Label, date and initial.  
Store in the refrigerator, stable for  
3 months. Discard if a precipitate  
forms.

**CAUTION:** Corrosive, irritant.

Dissolve the hematoxylin in water,  
heat to boiling, boil for 5 minutes.  
Remove from heat, add sodium  
iodate, wait 10 minutes to ripen.  
Add the remainder of the chemicals  
in the order given, dissolving each  
one before adding the next. Label,  
initial, stable for 1 year.

**Caution:** Avoid contact and inhalation.

## CARBOHYDRATES

### CONGO RED

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**Sodium Chloride,  
Stock Solution, Saturated:**  
Sodium chloride            30.0 gm  
Distilled water            200.0 ml

Mix well, then add:

100% alcohol            800.0 ml

Label, date and initial. Solution is stable for 1 year.

**CAUTION: Flammable.**

#### **Working Solution:**

Stock sodium chloride    50.0 ml  
1% sodium hydroxide    0.5 ml

Make immediately before use, discard.

**Congo Red  
Stock Solution, Saturated:**  
Congo red stain            1.0 gm  
Stock sodium chloride    500.0 ml

Mix well. Date and initial. Solution is stable for 6 months.

**CAUTION: Flammable, avoid contact and inhalation.**

#### **Working Solution:**

Stock Congo red solution   50.0 ml  
1% sodium hydroxide    0.5 ml

Filter through glass wool, solution should be a clear, not cloudy orange/red. Use immediately, discard after use.

**SAFETY:** Wear gloves, goggles and lab coat. Work in well ventilated area, wear particle mask when preparing dye solutions.

Sodium Hydroxide; severe irritant to skin and eyes. Corrosive to skin and eyes. Can cause burns.

Congo Red: Developmental abnormalities were produced in mouse fetuses after chronic feeding studies. Causes vascular changes in humans after ingestion.

#### **PROCEDURE:**

1. Deparaffinize and hydrate to water.
2. Mayer's Hematoxylin for 10 minutes.
3. Wash in tap water until blue.
4. \*Working sodium chloride solution, microwave, 20 power, 45 seconds. Allow slides to sit in solution for 2-5 minutes.
5. Place directly into Working Congo red solution, microwave, 20 power, for 45 seconds. Allow slides to sit in solution for 2-5 minutes.
6. Dehydrate rapidly in absolute alcohol, 10 dips, 3 changes.
7. Clear in xylene, coverslip with Permount.

\*Conventional Method:

4. Working sodium chloride solution, room temperature, 20 minutes.
5. Place directly into Working Congo red solution for 1 hour.

**RESULTS:**

|         |             |
|---------|-------------|
| Amyloid | red to pink |
| Nuclei  | blue        |

**NOTES:**

1. Sections must be cut at 8 to 10 microns for birefringence.
2. Solution must be filtered through glass wool, not paper filters for birefringence to occur.
3. Tissue fixed in solutions other than formalin may display false positive birefringence.

**REFERENCES:**

Stochem JH, Cytochem, 10:355-364

Sheehan D, Hrapchak B, Theory and practice of Histotechnology, 2nd Ed, 1980, pp 175-178, Battelle Press, Ohio

Crookham, J, Dapson, R, Hazardous Chemicals in the Histopathology Laboratory, 2nd ED, 1991, ANATECH LTD

Prepared: \_\_\_\_\_ By: \_\_\_\_\_

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## PROCEDURE CARD

### AMYLOID - CONGO RED - PUTCHLER'S MODIFICATION

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**CONTROL:** Known amyloidosis containing tissue.

**TECHNIQUE:** Cut paraffin sections at 10 microns.

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- \* Conventional Method:
4. Working sodium chloride solution, room temperature, 20 minutes.
  5. Place directly into Working Congo red solution for 1 hour.

#### RESULTS:

Amyloid red to pink

Nuclei blue

#### Mayer's Hematoxylin:

|  |           |
|--|-----------|
| Hematoxylin                            | 1.0 gm    |
| Distilled water                        | 1000.0 ml |
| Sodium iodate                          | 0.2 gm    |
| Ammonium or Potassium aluminum sulfate | 50.0 gm   |
| Citric acid                            | 1.0 gm    |
| Chloral hydrate                        | 50.0 gm   |

Dissolve the hematoxylin in water, heat to boiling, boil for 5 minutes. Remove from heat, add sodium iodate, wait 10 minutes to ripen. Add the remainder of the chemicals in the order given, dissolving each one before adding the next. Label, initial, stable for 1 year.

#### 1% Sodium Hydroxide

|                  |          |
|------------------|----------|
| Sodium hydroxide | 1.0 gm   |
| Distilled water  | 100.0 ml |

Mix well. Label, date and initial. Store in the refrigerator, stable for 3 months. Discard if a precipitate forms.

**CAUTION: Corrosive.**

#### Sodium Chloride

##### Stock Solution, Saturate:

|                     |          |
|---------------------|----------|
| Sodium chloride     | 30.0 gm  |
| Distilled water     | 200.0 ml |
| Mix well, then add: |          |
| 100% alcohol        | 800.0 ml |

Label, date and initial. Solution is stable for 1 year.

**CAUTION: Flammable.**

##### Working Solution:

|                       |         |
|-----------------------|---------|
| Stock sodium chloride | 50.0 ml |
| 1% sodium hydroxide   | 0.5 ml  |

Make immediately before use, discard.

#### Congo Red

##### Stock Solution, Saturated:

|                       |          |
|-----------------------|----------|
| Congo red stain       | 1.0 gm   |
| Stock sodium chloride | 500.0 ml |

Mix well. Date and initial. Solution is stable for 6 months.

**CAUTION: Flammable**

##### Working Solution:

|                          |         |
|--------------------------|---------|
| Stock Congo red solution | 50.0 ml |
| 1% sodium hydroxide      | 0.5 ml  |

Filter through glass wool, solution should be a clear, not cloudy orange/red. Use immediately, discard after use.

**CONGO RED**

**STOCK SOLUTION, SATURATED:**

Congo red stain                    1.0 gm  
Stock sodium chloride    500.0 ml

Mix well. Date and initial. Solution is stable for 6 months.

**CAUTION:** Flammable, avoid contact and inhalation.

**WORKING SOLUTION:**

Stock Congo red solution    50.0 ml  
1% sodium hydroxide        0.5 ml

Filter through glass wool, solution should be a clear, not cloudy orange/red. Use immediately, discard after use.

DATE: \_\_\_\_\_

TECH: \_\_\_\_\_

EXPIRATION: \_\_\_\_\_

**SODIUM CHLORIDE**

**STOCK SOLUTION, SATURATED:**

Sodium chloride                    30.0 gm  
Distilled water                    200.0 ml

Mix well, then add:

100% alcohol                    800.0 ml

Label, date and initial. Solution is stable for 1 year.

**CAUTION:** Flammable.

**WORKING SOLUTION:**

Stock sodium chloride        50.0 ml  
1% sodium hydroxide        0.5 ml

Make immediately before use, discard.

DATE: \_\_\_\_\_

TECH: \_\_\_\_\_

EXPIRATION: \_\_\_\_\_

**MAYER'S HEMATOXYLIN:**

|   |           |
|---|-----------|
| Hematoxylin                               | 1.0 gm    |
| Distilled water                           | 1000.0 ml |
| Sodium iodate                             | 0.2 gm    |
| Ammonium or Potassium<br>aluminum sulfate | 50.0 gm   |
| Citric acid                               | 1.0 gm    |
| Chloral hydrate                           | 50.0 gm   |

Dissolve the hematoxylin in water, heat to boiling, boil for 5 minutes. Remove from heat, add sodium iodate, wait 10 minutes to ripen. Add the remainder of the chemicals in the order given, dissolving each one before adding the next. Label, initial, stable for 1 year.

**SAFETY/PPE:** Wear gloves, goggles and lab coat. Work in well ventilated area or under hood.

**Caution: avoid contact and inhalation.**

DATE: \_\_\_\_\_

TECH: \_\_\_\_\_

EXPIRATION: \_\_\_\_\_

**1% SODIUM HYDROXIDE**

|                  |          |
|------------------|----------|
| Sodium hydroxide | 1.0 gm   |
| Distilled water  | 100.0 ml |

Mix well. Label, date and initial. Store in the refrigerator, stable for 3 months. Discard if a precipitate forms.

**CAUTION:** Corrosive.

**SAFETY/PPE:** Wear gloves, goggles and lab coat.

DATE: \_\_\_\_\_

TECH: \_\_\_\_\_

EXPIRATION: \_\_\_\_\_