MODIFIED THIOFLAVIN S - SENILE PLAQUES (AMYLOID)

PURPOSE: To demonstrate neurofibrillary tangles and senile plaques in Alzheimer's diseased brain tissue sections.

PRINCIPLE: 30m thick sections, paraffin or fixed frozen, are not mounted on slides but rather free floating sections. The stained slide is examined using a fluorescence scope.

Senile plaques display clusters of abnormally distended neuronal processes or neurites and are thus called “neuritic”. These plaques may contain well formed amyloid cores with the distended neurites arranged radially around the cores.

CONTROL: Brain tissue with senile plaques

FIXATION: 10% formalin

TECHNIQUE: Cut section 30m, do not mount on slide.

EQUIPMENT: Petri dishes or small beakers, bent glass rod.

REAGENTS:

0.25% Potassium Permanganate:
- Potassium permanganate 0.25 gm
- Distilled water 100.0 ml

Make fresh, discard after use.

CAUTION: Corrosive.

Bleaching Solution:
- Potassium metabisulfite 1.0 gm
- Oxalic acid 1.0 gm
- Distilled water 100.0 ml

Make fresh, discard after use.

CAUTION: Corrosive.

Blocking Solution:
- Sodium hydroxide 1.0 gm
- 30% hydrogen peroxide 3.0 ml
- Distilled water 100.0 ml

Make fresh, discard after use.

CAUTION: Corrosive.

0.25% Acetic Acid Rinse:
- Acetic acid 0.25 ml
- Distilled water 100.0 ml

Make fresh, discard after use.

CAUTION: Corrosive.
NERVE TISSUE
THIOFLAVINE S/ SENILE PLAQUES

Thioflavine S Staining Solution:
Thioflavine S 0.0125 gm
50% ethanol 100.0 ml
Mix fresh, discard after use.

50% Ethanol:
Absolute ethanol 50.0 ml
Distilled water 50.0 ml
CAUTION: Flammable.

Glycerin Jelly:
See Stock Solutions

SAFETY: Wear gloves, goggles and lab coat. Avoid contact and inhalation.

Potassium permanganate: Skin and eye irritant, ingestion will lead to severe gastrointestinal distress. Strong oxidant.

Oxalic acid: can cause severe burns of the eyes, skin or mucous membranes. Toxic by inhalation and ingestion. Target organ effects on kidneys and cardiovascular system, repeated exposure can cause dermatitis. Corrosive.

Hydrogen peroxide; Irritant to skin.

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

PROCEDURE:
1. Deparaffinize sections, through xylene and alcohol, bring to water.
2. Float sections in 0.25% Potassium permanganate solution for 20 minutes.
3. Rinse in distilled water.
4. Float sections in Bleaching solution for 2 minutes.
5. Rinse in distilled water.
6. Float in Blocking solution for 20 minutes.
7. Rinse in distilled water.
8. Float in 0.25% Acetic acid for 5 seconds.
9. Rinse in distilled water.
10. Mount sections on silinated slide, allow to dry, keeping slides flat:
11. Rinse slide with distilled water.
12. Drop on Thioflavin S staining solution, allow to stand 3-5 minutes.
13. Flood slides with two changes of 50% ethanol.
14. Flood slides with two changes of distilled water.
15. Mount with glycerin jelly.
NERVE TISSUE

THIOFLAVINE S/ SENILE PLAQUES

RESULTS:
Senile plaques and neurofibrillary tangles fluorescence background black

REFERENCES:
R. Guntern, C. Bouras, P. R. Hof, and P. G. Vallett, An Improved Thioflavine S Method for Staining Neurofibrillary Tangles and Senile Plaques in Alzheimer’s Disease, 1992

Prepared:__________________________ By: ________________

Approved:________________________ By: ________________

Downloaded from WebPath: Internet Pathology Laboratory
http://www-medlib.med.utah.edu/WebPath/webpath.html
PROCEDURE CARD

MODIFIED THIOFLAVIN S - SENILE PLAQUES (AMYLOID)

CONTROL: Brain tissue with senile plaques

FIXATION: 10% formalin

TECHNIQUE: Cut section 30m, paraffin or fixed frozen, do not mount on slide.

PROCEDURE:
1. Deparaffinize sections, through xylene and alcohol, bring to water.
2. Float sections in 0.25% Potassium permanganate solution for 20 minutes.
3. Rinse in distilled water.
4. Float sections in Bleaching solution for 2 minutes.
5. Rinse in distilled water.
6. Float in Blocking solution for 20 minutes.
7. Rinse in distilled water.
8. Float in 0.25% Acetic acid for 5 seconds.
9. Rinse in distilled water.
10. Mount sections on silinated slide, allow to dry, keeping slides flat:
11. Rinse slide with distilled water.
12. Drop on Thioflavin S staining solution, allow to stand 3-5 minutes.
13. Flood slides with two changes of 50% ethanol.
14. Flood slides with two changes of distilled water.
15. Mount with glycerin jelly.

RESULTS:
Senile plaques and neurofibrillary tangles fluorescence background black

0.25% Potassium Permanganate:
Potassium permanganate 0.25 gm
Distilled water 100.0 ml
Make fresh, discard after use.

CAUTION: Corrosive.

Bleaching Solution:
Potassium metabisulfite 1.0 gm
Oxalic acid 1.0 gm
Distilled water 100.0 ml
Make fresh, discard after use.

CAUTION: Corrosive.

Blocking Solution:
Sodium hydroxide 1.0 gm
30% hydrogen peroxide 3.0 ml
Distilled water 100.0 ml
Make fresh, discard after use.

CAUTION: Corrosive.

0.25% Acetic Acid Rinse:
Acetic acid 0.25 ml
Distilled water 100.0 ml
Make fresh, discard after use.

CAUTION: Corrosive.

Thioflavine S Staining Solution:
Thioflavine S 0.0125 gm
50% ethanol 100.0 ml
Mix fresh, discard after use.

CAUTION: Poison, flammable.

50% Ethanol:
Absolute ethanol 50.0 ml
Distilled water 50.0 ml

CAUTION: Flammable.