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**MATRIC NUMBER: 17/MHS03/012**

**DEPARTMENT: ANATOMY**

**COURSE: HISTOCHEMISTRY (ANA 304)**

**ASSIGNMENT**

1. What staining technique is used for identifying DNA/RNA in peripheral neurons?

**Ans- Methyl green-pyronin**

1. Is Luxol Fast Blue Stain also used to detect demyelination in the PNS? Whatever your answer is, explain the procedure involved in the demonstration of demyelination in the PNS.

**Answer**

Luxol Fast Blue Stain is not used to detect demyelination in the PNS. Adam’s OTAN method can be used to detect demyelination. The abbreviation stands for osmium tetroxide and alpha-naphthylamine. The technique is applied to cryostat sections, ideally after calcium-formaldehyde fixation.

**Solutions Needed**

A. Osmium Tetroxide-Potassium Chlorate. This is made up as required and used only once.

Osmium tetroxide, 2% stock solution in water: 5 mL Potassium chlorate (KClO3), 1% stock solution in water: 30 mL Water: 5 mL

B. Saturated -Naphthylamine Solution. Dissolve a few crystal of -naphthylamine in 40 mL of water at 40°C. Filter. This solution is used at 37°C

**Procedure**

1. Treat the sections with osmium tetroxide-potassium chlorate (Solution A) overnight at room temperature, in a tightly closed glass container.

2. Wash the sections for 10 min in water (3 changes with occasional agitation).

3. Treat the sections with saturated -naphthylamine solution (B) for 20 min at 37°C.

4. Wash the sections for 5 min in water (3 changes with occasional agitation).

5. Apply coverslips, using an aqueous mounting medium.

**Result**

Normal myelin is brownish–orange. Degenerating myelin (late products only) is black. Fat, if present in the tissue, is also blackened.