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DEPARTMENT: ANATOMY

COURSE CODE: ANA304

1. If Nissl stain is used to demonstrate RNA/DNA in neurons of CNS, what staining technique is used for identifying the same in peripheral neurons?
2. 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.
3. The Acridine Orange Staining Procedure can be used to stain the RNA/DNA.

**PRINCIPLE**

Acridine orange, a vital stain, will intercalate with nucleic acid, changing the dye’s optical characteristics so that it will fluoresce bright orange under ultraviolet light. All nucleic acid–containing cells will fluoresce orange. Acridine orange is a metachromatic stain and under appropriate conditions, RNA will stain orange and DNA will stain green.

**METHOD**

1. Properly prepare and fix the smear prior to staining.

2. Flood slide with acridine orange stain (available from various commercial suppliers). Allow stain to remain on surface of slide for 2 minutes without drying.

3. Rinse with tap water and allow moisture to drain from slide and air-dry.

4. Examine the slide using fluorescent microscopy.

2)

Luxal blue can be used to detect demyelination in CNS but not in PNS. A suitable stain that can be used is the Sudan Black B stain which is a slightly basic dye and will combine with acidic groups in compound lipids, thus staining phospholipids also.

PROCEDURE:

1. Pick-up frozen sections on clean glass slides if fresh, albuminized slides if fixed.

2. Fix slides in 10% formalin if fresh.

3. Wash well with tap, rinse in distilled, drain off excess water.

4. Propylene glycol, two changes, 5 minutes each.

5. Sudan Black, 7 minutes, agitate.

6. 85% Propylene glycol, 3 minutes.

7. Rinse in distilled water.

8. Nuclear Fast Red, 3 minutes.

9. Wash in water.

10. Wash in tap water, rinse in distilled.

11. Mount with aqueous mounting media, Glycerin Jelly.

RESULTS:

Fat: Blue-Black

Nuclei: Red

The low presence of blue black in the slide shows a low amount of phospholipids. As myelin sheath are lipids this stain can be used to detect the demyelination.