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TERM PAPER

1. If Nissl stain is used to demonstrate RNA/DNA in the 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

ANSWERS

1. A technique used in identifying DNA/RNA in neurons of PNS is **Propidium iodide staining**. It is a fluorescent intercalating agent that can be used to stain cells and nucleic acids. **PI** binds to DNA by intercalating between the bases with little or no sequence preference. **PI** also binds to RNA, necessitating treatment with nucleases to distinguish between RNA and DNA staining in peripheral neurons.

   Reagents:

   - 95% Ethanol at -20°C
   - Propidium Iodide stock 1mg/mL in PBS (Sigma P4170)
   - RNase A Stock 1mg/mL in PBS (Sigma R5000)

   Staining Solutions:

   - 900µl 1x PBS + 2mM $MgCl_2$
   - 50µl PI Stock Solution
   - 50µl RNase Stock Solution
   - 1ml total staining volume for ~$2x10^6$ cells

Procedure:

- Wash cells in PBS to remove all traces of serum. Spin and aspirate supernatant.
- Adjust cell concentration to $2x10^6$ cells/100 l in PBS.
- Add 900μl of 95% ethanol, dropwise, to the cells while vortexing gently. 4. Store cells at 4°C to fix. Fix cells overnight, or ideally for 24 hours.
- If the core will be processing your cells, you can stop the procedure and bring them at this stage. If you will be running them yourself, follow the rest of the protocol.
- Spin cells in ethanol to pellet. Aspirate ethanol and wash once with PBS.
- Spin cells again and aspirate most of the PBS, leaving a small amount to resuspend cells in.
- Add 1ml of staining solution to each sample ($2x10^6$ cells) and incubate in the dark at 37°C for 20 minutes.
- After incubation store cells on ice and analyze within a few hours.

2. Yes, Luxol Fast Blue staining can also be used in the detection of demyelination in PNS. However, it is more commonly used in the detection of demyelination in the CNS. LFB is a myelin sheath stain that stains phospholipids (the main constituents of the myelin sheath around nerve processes) blue. In demyelinating diseases where the myelin sheath is broken down, the distribution of lesions can be clearly identified. The myelin, including phospholipids, will be stained blue to green, and the neurons will be stained violet.

Fixation: 10% formalin.

Section: Paraffin sections at 5-10 um. Frozen sections at 20-30um.

Solutions and Reagents:

0.1% Luxol fast blue solution:

Luxol fast blue, MBS ---------- 0.1 gm
Ethyl Alcohol, 95% ---------- 100 ml
Glacial acetic acid ---------- 0.5 ml

0.1% Cresyl echt violet solution:

Cresyl echt violet (cresyl fast violet) ------- 0.1 gm
Distilled water ---------- 100 ml
Add 10 drops of glacial acetic acid just before use and filter.

0.05% Lithium carbonate solution:

Lithium carbonate ---------- 0.05 gm
Distilled water ---------- 100 ml

Procedure:

- Deparaffinize and hydrate to 95% ethyl alcohol (Frozen/vibratome sections may need to have de-fat step: place sections directly into 1:1 alcohol/chloroform for a few hours/overnight and then hydrate back 95% ethyl alcohol).

- Leave in luxol fast blue solution in 56 C oven overnight (for frozen sections, not longer than 16 hours).
- Rinse off excess stain with 95% ethyl alcohol.
- Rinse in distilled water.
- Differentiate the slides in the lithium carbonate solution for 30 seconds.
- Continue differentiation in the 70% ethyl alcohol for 30 seconds.
- Rinse in distilled water.
- Check microscopically to see if gray matter is clear and white matter sharply defined.
- Repeat the differentiation steps (step 5-7) if necessary.
- When differentiation is complete, place in distilled water.
- Counterstain in the cresyl violet solution for 30-40 seconds.
- Rinse in distilled water (don't rinse in 70% alcohol which will clear off fast blue staining).
- Differentiate the slides in 95% ethyl alcohol for 5 minutes (check microscopically).
- 100% alcohol 2x5 min.
- Xylene 2x5 min.
- Mount with resinous medium.

Results:

Myelin, including phospholipids ---------- blue to green
Neurons ---------- pink to violet