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QUESTION 1

If Nissal stain is used to demonstrate RNA / DNA in the neurons of CNS. What staining technique is used to identify the same in peripheral neurons?

ANSWER

RNA and DNA can be stained using Acridine orange.

Acridine orange, a vital stain, will intercalate with nucleic acid, changing the dyes optical characteristics so that it will give 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.

Acridine Orange Is Cell-permeable which allows the dye to interact with DNA by intercalation, or RNA via Electrostatic attractions. When bound to DNA, Acridine Orange is very similar spectrally to an organic compound known as Fluorescein

METHODS

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

2. Flood slide with acridine orange stain. 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 microscope

RESULT

Bacteria and yeast will give bright orange against a green fluorescing or dark background of human cells and debris.

APPLICATIONS

1. To differentiate DNA from RNA

2. For identifying engulfed apoptotic cells, because it will fluoresce upon engulfment

3. For differential staining of human cells and prokaryotic cell with a fluorescence microscope. Human cells are stained black to faint green in which bright orange organisms are easily detected.

LIMITATION

1. Cellular debris within a sample such as white blood cells, epithelia cells, and dead bacteria may distort the microscopic image.

2. Acridine orange is a very sensitive stain, and caution should be used when interpreting results.

QUESTION 2a

Is Luxol Fast Blue stain also used to detect demyelination in the PNS?

ANSWER

NO, Luxol Fast Blue stain is commonly used to detect demyelination in the central nervous system (CNS) but cannot discern myelination in the peripheral nervous system (PNS).

QUESTION 2B

Procedure involved in the demonstration of demyelination In the PNS

ANSWER

Swank and Davenport’s Marchi method can be used to detect a degenerating myelin. The technique incorporates improvement that minimize artifacts such as sporadic staining of normal myelinated fibers. Specimen are fixed for 2-4 days in phosphate buffered formaldehyde ( 2-3 weeks for human or other large brain) and cut into slices not more than 3mm thick.

SOLUTION NEEDED

This can be kept for a few months in a suitable bottle .It is used only once. It include; Potassium chlorate 15g, Water 200ml, Osmium tetroxide 0.5g, Formalin (37-40%) 0.5ml and Glacial acetic acid 2.5ml

RESULT

Normal myelin is brownish orange. Degenerating myelin is black.