**AFE BABALOLA UNIVERSITY**

**COLLEGE OF HEALTH SCIENCES**

**FACULTY OF BASIC MEDICAL SCIENCES**

**DEPARTMENT OF HUMAN ANATOMY AND CELL BIOLOGY**

**ASSIGNMENT**

**BY**

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**ON**

**ANA304**

**GENERAL HISTOCHEMISTRY**

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1. **Nissl stain is used to demonstrate RNA/DNA in the neurons of CNS, what staining technique is used for identifying the same in Peripherial neurons?**

Granules of Nissl substance are found in the cell body and correspond to rough endoplasmic reticulum Nissl substance, also called tigroid substance and chromidal
substance, refers to basophilic material in the cytoplasm of the neuron.
Ultrastructurally, this material can be identified as large aggregates of
rough endoplasmic reticulum, with the RNA content providing the basis
for demonstration by special light microscopic techniques

They are basophilic due to the associated nucleic acid. Many basic dyes (e.g. neutral red, methylene blue, azur, pyronin, thionin, toluidine blue and cresyl fast violet) stain Nissl substance. Nissl substance are found both in PNS and CNS.

**CRESYL ECHT VIOLET METHOD**
*Purpose*
Identification of neurons in tissue sections, or demonstration of the loss of Nissl substance (chromatolysis). This loss occurs when the axons are transected, injured, or destroyed. This is a reversible change in response to axonal injury and is apparently related to the need for the cell to increase protein synthesis as the cell attempts to regenerate a new axon. When the need for increased protein synthesis is ended, the Niss! substance will return to normal. However, if the axon is injured very close to the cell body, the neuron may just disappear.

*Principle*

Neurons contain Niss! substance, which is primarily composed of rough endoplasmic reticulum, with the amount, form, and distribution varying in different types of neurons. Because of the RNA content, Niss! substance is very basophilic and will be very sharply stained with basic aniline dyes. By varying the pH and the degree of differentiation, both Niss! substance and nuclei, or only Niss) substance, may be demonstrated.

*Fixative*
10% neutral-buffered formalin

*Procedure*

1. Deparaffinize sections, and hydrate to distilled water.

2. Stain for 3-5 minutes in cresyl echt violet solution.

3. Rinse in 2 changes of distilled water.

4. Place sections in 95% alcohol for 30 seconds.

5. Transfer sections to absolute alcohol for 30 seconds.

6. Place in xylene for 1 minute.

7. Place in balsam-xylene mixture for 2 minutes.

8. Differentiate in absolute alcohol, 2 changes for 10-30

seconds each. Check the sections microscopically.

9. Take through several changes of xylene.

10. Steps 7 through 9 probably will have to be repeated several

times. When differentiation is complete, the background

should be colorless, with nuclei and Niss! substance well

demonstrated.

11. Mount sections with synthetic resin.

*Results*

• Niss! substance Blue to purple

• Nuclei Blue to purple

• Background Colorless

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.**

Luxol fast blue is usually used to detect myelin fibres in Peripheral Nervous System (PNS), however, it can also be used to detect demyelination in PNS by testing for the present of Myelin fibres in PNS, if there is reduction in Myelin fibres in the PNS, it indicates present of demyelination in PNS, also if the test for myelin is negative it indicates complete demyelination, while if the test for myelin is positive it means there is no demyelination.

**LUXOL FAST BLUE METHOD**

*Purpose*

Demonstration of myelin in tissue sections. When an axon degenerates, the myelin covering breaks down into simpler lipids that will be removed eventually.

*Principle*

Luxol fast blue, like alcian blue, is of the sulfonated copper phthalocyanine type, but it is alcohol-soluble, whereas alcian blue is water-soluble. Staining is caused by lipoproteins, and the mechanism is that of an acid-base reaction with salt formation; the base of the lipoprotein replaces the base of the dye.

*Fixative*

10% neutral-buffered formalin

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 *Quality Control*

A section of spinal cord or medulla provides a good control.

*Reagents*

Luxol Fast Blue, 0.1% Solution

Luxol fast blue MBSN 0.1 g

Alcohol, 95% alcohol lOOmL

Dissolve dye in alcohol, then add:

Acetic acid, 10% 0.5 mL

The solution is stable

*Procedure*

1. Deparaffinize sections, and hydrate to 95% alcohol.

2. Place slides in Luxol fast blue solution, and leave overnight

at 56°C to 58°C. The container should be tightly capped as

this alcoholic solution will evaporate readily.

3. Rinse sections in 95% alcohol to remove excess stain.

4. Rinse in distilled water.

5. Begin the differentiation by immersing the slides in

lithium carbonate solution for 10-20 seconds.

6. Continue the differentiation in 70% alcohol solution

until gray and white matter can be distinguished. Do not

over differentiate.

7. Wash the sections in distilled water.

8. Finish the differentiation by rinsing briefly in lithium

carbonate solution and then putting through several

changes of 70% alcohol solution until the greenish blue of

the white matter contrasts sharply with the colorless gray

matter.

9. Rinse thoroughly in distilled water.

IO. Dehydrate in several changes of 95% and absolute alcohols.

11. Clear in xylene, and mount with synthetic resin.



*Results*

• Myelin Blue to blue-green

• Background Colorless

**CLINICAL APPLICATIONS**

***Cresyl Echt Violet Staining – Diagnostic Application***

Cresyl echt violet is used to demonstrate the presence (or Loss) of Nissl in neurons. The loss of Nissl substance, Chromatolysis, occurs near the nucleus first and can then disappear altogether. This is an indication that neurons have been injured or damaged in some way.

***Luxol Fast Blue (LFB) Staining – Diagnostic Applications***

Demyelination diseases, such as Multiple Sclerosis (MS), damage the myelin sheath that protects nerve fibres (Loss of the myelin sheath is known as demyelination).

While there are several stains that can be used to demonstrate myelin, all with different coloration, Luxol Fast Blue (LFB) has been most useful in laboratories. In additional to demonstrating myelin, it also differentiates between white and gray matter of the brain. Tissue sections that have been stained with LFB can be counterstain to show additional cellular structures.