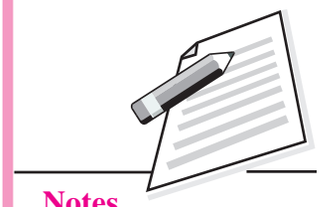

13

LIPID STAIN

13.1 INTRODUCTION

The Oil Red O (ORO) stain can identify neutral lipids and fatty acids in smears and tissues. Fresh smears or cryostat sections of tissue are necessary because fixatives containing alcohols, or routine tissue processing with clearing, will remove lipids. The ORO is a rapid and simple stain.

OBJECTIVES

After reading this lesson, you will be able to:

- explain the principle of lipid stain
- describe various reagents used for lipid stains
- describe the procedure of lipid staining.

13.2 LIPID STAIN

Aim: To demonstrate intracellular lipid in tissue sections.

Principle: The dye being more soluble in the lipid to be demonstrated than in the vehicular solvent. The polyazo group of dyes includes the oil red series, the sudan red series and sudan blacks. All these dyes are interchangeable and may be substituted.

Sudan series - Sudan III

- Sudan IV

- Sudan black

Control - Lipid positive section

Notes

Reagents

1. Oil Red O stock solution -

Oil Red O	0.5gm
Isopropanol	100ml

   Dissolve the dye in isopropanol using gentle heat in water bath.

2. Oil Red O working solution

Stock Oil Red O solution	30ml
Distilled water	20ml

   Dilute the stock solution with distilled water and keep it for 10 minutes, filter and cover it immediately.

3. Glycerine Jelly Mounting medium

Gelatin	10gm
Distilled water	60ml
Glycerol	70ml
Phenol	0.25gm

   Dissolve the gelatin in distilled water using sufficient heat to melt the gelatin, add glycerol and phenol. Mix well and transfer to a small capped bottle and refrigerate.

Procedure

- Fix timer in formalin, wash with running tap water for 5 to 10 minutes.
- Cut frozen section of 8 to 10 micron thickness and air dry.
- Rinse with 60% isopropanol.
- Stain with freshly prepared Oil Red O working solution for 15 minutes.
- Rinse with 60% isopropanol.
- Few dips in Alum hematoxylin to stain nuclei.
- Rinse with distilled water.
- Mount in water or glycerine jelly.

Result

- Lipid red
- Nuclei blue

Note

- Use cryostat sections of 8 to 10 micron thickness or formalin fixed smears.
- working Oil Red O solution should be freshly prepared from stock solution and kept in close container.
- Never take the sections through clearing solvent prior to mounting as this will remove the lipid to be demonstrated.
- Frozen sections should be used to stain neutral triglycerides.
- Lipoproteins may be demonstrated on paraffin sections.
- Alcohol fixation removes most lipids.

INTEXT QUESTIONS 13.1

1. Dyes used in lipid stain is .....
2. ..... is used to stain nuclei
3. Lipid is demonstrated by ..... colour
4. Nuclei is demonstrated by ..... colour
5. Lipoproteins may be demonstrated on ..... section
6. ..... fixation removes lipids

WHAT HAVE YOU LEARNT

- Lipid stain is used to demonstrate intracellular lipid in tissue sections
- Polyazo group of dyes like oil red series, sudan red series and sudan blacks are the dye used for demonstrating lipids in tissue section
- The frozen section should be cut 8 to 10 micron thickness
- Lipids appear as red and nuclei appear blue
- Lipoproteins may be demonstrated on paraffin sections
- Alcohol fixation removes most lipids

TERMINAL QUESTIONS

1. What is principle of lipid stain?
2. Name three dyes used to demonstrate lipid in tissue sections.
3. What precautions should be observed during lipid staining on tissue sections?
4. What should be the thickness of sections for lipid staining?
5. What is the mounting media used in lipid staining?

ANSWERS TO INTEXT QUESTIONS

13.1

1. Sudan series
2. Alum hematoxylin
3. Red
4. Blue
5. Paraffin
6. Alcohol