M.Sc ZOOLOGY

SEMESTER 2

PAPER CC7

GENERAL PRINCIPLE OF HISTO-CHEMICAL DEMONSTRATION OF LIPIDS

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HISTOCHEMISTRY

Histochemistry is an important technique that is used for the visualization of biological structures. As such, it is concerned with the identification and distribution of various chemical components of tissues through the use of stains, indicators as well as microscopy.

Essentially, identification and distribution of chemical constituents of tissues is achieved through the exploitation of unique chemical environments in cells, heterologous expression techniques as well as enzymatic activities.

LIPIDS STAINING

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

- Explain the principle of lipid stain
- Describe various reagents used for lipid stains
- Describe the procedure of lipid staining.

LIPID STAIN

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

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

PRECAUTIONS

- 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.