Lillie's Oil Red O Method For Neutral Lipids

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Abstract

This paper presents a review of Lillie's oil red O method for neutral lipids. Special emphasis was given to the stability of the staining solution, which was found to have much greater stability than originally reported by Lillie. In the described modification of his method, the working oil red O solution, which contains dextrin, was allowed to set for at least 24 hr before use, allowing undissolved dye to settle to the bottom of the solution. This eliminated the red dye precipitate that sometimes forms on tissue sections stained according to the original procedure. Interpretation of the staining results is difficult when this precipitate occurs. Staining results obtained with this modified oil red O method were consistent and reliable. (*The J Histotechnol* 22:309, 1999)

Key words: dextrin, dye precipitate, Sudan dyes

Introduction

One of the most widely used dyes for staining fat is oil red O. This dye stains fat more intensely than Sudan III and Sudan IV, which are similar in chemical structure. Sudan III contains no methyl groups, Sudan IV contains 2 methyl groups, and oil red O contains 4 methyl groups, all of them in the diazo group of the classification of dyes. These dyes are insoluble in aqueous solutions but soluble in ethanol, isopropanol, propylene glycol, and other organic solvents.

Lillie's supersaturated isopropanol method using oil red O appears to be the most popular method for staining neutral fats (1). The dye is also used to demonstrate fat in Luna's propylene glycol method (2). In Lillie's original method, the stock oil red O solution is diluted with distilled water and filtered prior to use; but even after filtration, dye precipitate remains in the solution and sometimes on tissue sections. The precipitate looks similar to fat droplets, making interpretation of the staining results difficult. This is especially true when looking for fat in parathyroid sections. Catalano and Lillie found that this precipitate could be eliminated by the addition of dextrin to the oil red O solution (3). They also observed that dextrin containing solutions hold substantially more oil red O than those without dextrin. They found that the dextrin containing solutions filtered after 10 days contained 2½ times more dye than solutions did when filtered after 1 hr. They did not recommend using the dextrin containing solution after 10 days. This is unfortunate in that we have found that the solution remains stable for at least 1 yr and that there is little, if any, decrease in the intensity of fat staining with the old solution. Also, the stock oil red O solution has excellent stability; at least 2 yr.

In Lillie's method the working oil red O solution is filtered shortly after it has been prepared and used soon afterwards. His method is found in several text books and manuals on histotechnology. Unfortunately, nearly all of them exclude the use of dextrin in preparing the working oil red O solution, leading to undesirable red precipitate (4–13).

Materials and Methods

Fixation

10% buffered neutral formalin

Sections

Unfixed cryostat sections cut at 6–10 μm

Solutions

1. Oil red O stock solution: Dissolve 0.5 gm oil red O (Aldrich, Milwaukee, WI), CI 26125 in 100 ml isopropyl alcohol.
2. 1% Dextrin: Dissolve 1.0 gm dextrin in 100 ml distilled water (DW).
3. Oil red O working solution: To 60 ml stock oil red O solution, add 40 ml 1% dextrin. Allow the solution to set for at least 24 hr. Filter through Whatman #4 filter paper before use.
4. 60% Isopropyl alcohol: To 60 ml isopropyl alcohol, add 40 ml of DW.
5. Modified Lillie-Mayer hematoxylin: Dissolve 5 gm hematoxylin (Fisher Scientific, Pittsburgh, PA), CI 75290 and 50 gm of aluminum ammonium sulfate in 800 ml of DW. Add 200 ml of glycerol and 0.3 gm sodium iodate to the solution. Before use, add 3 ml acetic acid to 100 ml solution. This increases the precision and selectivity of the nuclear stain. Filter before use.
6. 0.3% Sodium borate; Dissolve 0.3 gm sodium borate in 100 ml DW.
7. Glycerol jelly: Dissolve 10 gm gelatin in 60 ml of DW with the aid of heat. Add 70 ml glycerol and 1.0 ml of 90% liquid phenol. This mounting medium is stable for several years at room temperature.

Staining Procedure

1. Mount cryostat-cut sections on clean slides, and fix in 10% buffered neutral formalin for 5 min.
2. Rinse in 4 changes of DW.
3. Dip 6 times in 60% isopropyl alcohol.
4. Stain in working oil red O solution for 10–20 min.
5. Dip 6 times in 60% isopropyl alcohol.
6. Rinse in 4 changes of DW.
7. Counterstain with acidified Lillie-Mayer hematoxylin for 1 min. Other acidified hematoxylins may be used.
8. Rinse in 3 changes of DW.
9. Blue by placing sections in 0.3% sodium borate for 10 sec.
10. Rinse in 4 changes of DW.
11. Mount with glycerol jelly or other aqueous mounting medium.

Results

Neutral lipids are red or orange-red and nuclei are blue. See Figure 1.

Discussion

The presence of dextrin in the working oil red O solution stabilizes it and greatly extends its shelf life. I have found that the addition of dextrin to other fat strains (Sudan III, Sudan IV, Sudan black B) also extends the shelf life of the solutions, and eliminates undesirable precipitates.

Oil red O, Sudan III, Sudan IV, and Sudan black B are certified by the Biological Stain Commission. The staining solutions of these dyes containing dextrin are used to stain frozen sections of skin, fatty liver, and avocado. The avocado makes an excellent fat control because it contains both acidic and neutral fats. Avocado is also used to test Nile blue A for certification using Lillie's method for staining fats, in which neutral lipids (triglycerides, cholesterol, steroids) stain red to pink and acidic lipids (fatty acids, phospholipids) stain blue (14).

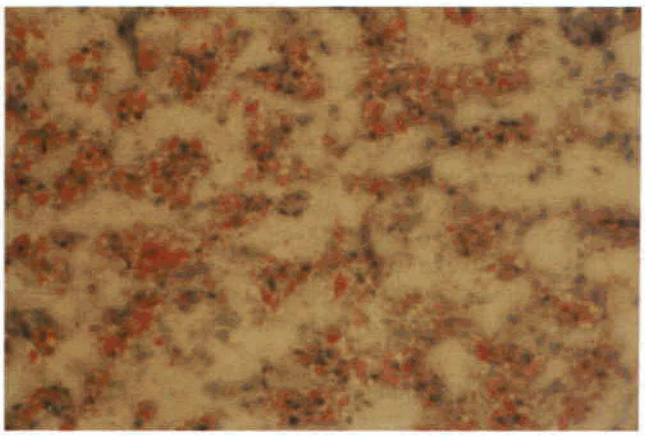

Figure 1. An unfixed cryostat-cut 10 μm liver section, showing extensive fatty degeneration of modified Lillie's oil red O method. The working oil red O solution used to stain the section was 6 weeks old and was prepared with a stock oil red O solution that was more than 1 yr old. Original magnification ×250.

Oil red O is insoluble in water and has varying degrees of solubility in organic solvents. Like the other Sudan dyes discussed, it stains tissue lipids by an absorption process. The staining mechanism with Sudan dyes is based on the greater solubility of the dye in lipids than in the dye solvents. This is a physical method of staining, not a chemical method such as anionic and cationic dye binding. The dye absorption is dependent on the dye concentration, the molecular weight of the dye, and the solvent; it decreases with temperature. Tissue lipids of high melting point (cholesterol and its esters) will not stain with Sudan dyes unless they are heated to their melting points.

Besides isopropyl alcohol, other solvents that have been used for dissolving Sudan dyes are carbowax 400 (a low molecular weight fraction of polyethylene glycol, liquid at room temperature), propylene glycol, acetone, triethyl phosphate, and ethyl alcohol. The most often used solvents are isopropyl alcohol or propylene glycol because the staining solution is easier to prepare. In a study conducted by members of the Biological Stain Commission, the staining results of oil red O with the Lillie-Ashburn isopropyl alcohol method were compared with those obtained with Herxheimer alcohol-acetone and the Feldman-Dapson triethylphosphate oil red O methods. The results with the Lillie-Ashburn method were found to be superior to those obtained with the other methods (15,16).

Paraffin processed tissue sections cannot be used for staining neutral lipids with any of the oil red O or Sudan dye methods because the lipids have been dissolved in the organic solvents used to process the tissues. Aqueous mounting media, such as glycerol jelly, must be used because the organic solvents in which synthetic resins are dissolved will remove the lipids. The lipids in the section are relatively liquid and mobile, so care should be taken that little or no pressure is placed on the coverslip when mounting or the lipid may be displaced. If air bubbles are present in the section, the coverslip is removed by placing the slide in warm (not hot) distilled water, and coverslipping again. When glycerol jelly is used for mounting, care must be taken not to overheat it as this may melt the lipids and cause them to be displaced.

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