Microscopy

***Simple Histological and Histochemical Staining Methods* for Freehand Sections**

Toluidine blue O Staining of Hand Sections

- TBO is the best stain for fresh botanical specimens, especially hand sections.
- It is a cationic, polychromatic dye that reacts differently to different chemical components of cells and results in a multicolored specimen.

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- TBO will react with carboxylated polysaccharides such as pectic acid to give a purplish color,

Toluidine blue O Staining of Hand Sections

- TBO give a purplish color, greenish blue or bright blue with polyphenolic substances such as lignin and tannins, and purplish or greenish blue with nucleic acids

TBO: General expected results

- pectin will be red or reddish purple;
- lignin, blue;
- other phenolic compounds, green to blue-green.

TBO: General expected results

- Thin-walled parenchyma will be reddish purple;
- Cells with lignified secondary walls usually appear blue;
- Sieve tubes and companion cells, purple;
- Middle lamella, red to reddish purple;
- Callose and starch, unstained

TBO procedure

- For freehand sections, apply the TBO stain directly to the sections for 1–2 min.
- Gently remove the stain by using a fine tip pipette or a piece of filter paper. Wash the sections by flooding them with water followed by its removal. Repeat until there is no excess stain around the sections. Transfer the sections to slides, add a drop of clean water over the sections, and apply a coverslip. The slide is ready for examination.

Phloroglucinol HCl Test for Lignin

- Lignin is a common constituent in plant cell walls, especially the secondary walls.
- The cinnamaldehyde end groups of lignin appear to react with phloroglucinol-HCl to give a red-violet color

PhloroglucinolHCl

- Different forms of lignin give different staining intensity toward the phloroglucinol stain.
- Because of the ease of staining, this is the most common procedure to test for the presence of lignin in plant cell wall.

PhloroglucinolHCl:
General expected results

- Lignified walls become red.

PhloroglucinolHCl:
General procedure

- Transfer cells, cell clusters, or hand sections directly onto a slide.
- Apply one or two drops of stain over the plant material and stain for at least 2 min.
- Since the staining intensity varies and the color fades gradually, it is advisable to examine the specimen without removing the stain.
- Gently apply a coverslip over the specimen without washing.
- Examine the specimen at once

Starch: IodinePotassium Iodide Test

- The iodine-potassium iodide (IKI) stain is specific for starch.
- The length of the starch molecule determines the color of the reaction
- the shorter the molecule, the more red the color;
- the longer the molecule, the more deep blue the color.

(IKI) stain: General expected results

- Starches will give a deep blue color in a few minutes.
- If the staining intensity is too high, it is advisable to dilute the stain. The color will fade over time.

Iodine Potassium Iodide (IKI) stain:

- Stain sections by applying a few drops of IKI solution directly on the sections.
- Wait for 2 min, rinse sections in water, and transfer the sections to a slide with a brush. Apply a coverslip and examine with a microscope. Small blue-black bodies indicate the presence of starch.

Total Lipid: Sudan Dyes

- The mechanism of staining is based on differential solubility.
- The Sudan dyes are more soluble in apolar solvents.
- As a result, they tend to dissolve more in structures such as the cuticle, lipid droplets, or suberin which are all hydrophobic substances.

Sudan Dyes: General expected results

- For Sudan III and IV, fats and oils will stain orange to red.
- Leaf cuticle, suberized walls of cork cells, and the Casparian strip, if present, will give a positive reaction.
- For Sudan black B, lipidic substances appear gray to black.

Sudan Dyes

1. Apply the Sudan staining solution onto the cells or hand section in a small Petri dish. Stain the material for about 10–30 min.
2. Rinse the section in 50 % ethanol to remove excess stain.
3. Mount the section in 50% glycerol and examine with a compound lightmicroscope.

Clearing for hand sections

- The choice of the most suitable clearing method depends on the types of tissues, their pigmentation, size, and the objective of the experiment.

Clearing chlorophyll

- Methyl cellosolve extracts chlorophyll readily from leaves and can be used as the first step in the clearing process.
- The entire procedure can be carried out in a suitable size glass vial.

Clearing chlorophyll
Procedure

- Treat whole leaves or leaf segments with methyl cellosolve to extract pigments from the specimens at room temperature.
- Use 20 × volume of methyl cellosolve to 1 volume of tissues. Place vials on a rotary mixer to facilitate pigment extraction. Replace methyl cellosolve if necessary.

Procedure

- Once the pigments have been extracted, replace methyl cellosolve with a 5% sodium hydroxide solution and place the vial in a 50 °C oven for 1–2 days.
- The volume of hydroxide solution should be at least 20 × the volume of the leaf tissue.
- Remove the sodium hydroxide solution and gently rinse the specimens with several changes of water. Place specimens into 50% ethanol for 30 min prior to staining.

Maceration for hand sections

- The following procedure is a chemical method that weakens the middle lamella allowing the cells to separate.
- A variety of plant tissues ranging from soft pith tissues to woody xylem samples can be studied using this technique; however, the duration of treatment will vary.

Maceration Fluids

- The fluid is prepared by mixing equal volumes of 10% chromic acid with 10% nitric acid.
- 1 part of a 30% solution of hydrogen peroxide, 4 parts of distilled water, and 5 parts of glacial acetic acid.