

Toluidine Blue for Mast Cells

Purpose

The demonstration of mast cells in tissue. Mast cells will stain metachromatically with toluidine blue; that is, they will stain a different color from the dye solution and the rest of the tissue. The control section should show mast cells stained red-purple (metachromatic staining) The color shift, called metachromasia, generally is attributed to the cationic or basic dye and is somewhat dependent on pH, dye concentration, and temperature. Blue or violet dyes will show a red color shift, and red dyes will show a yellow color shift with metachromatic tissue elements.

Fixative

10% Neutral buffered formalin is preferred.

Equipment

Coplin jars, Erlenmeyer flask, graduated cylinder

Technique

Cut paraffin sections at 4-5 microns

Quality Control

A section containing mast cells must be used as a control

Reagents

Toluidine Blue Solution

Toluidine blue	.1 g
ddH ₂ O	100 mL

Procedure

1. Deparaffinize sections and hydrate to ddH₂O
2. Stain sections in toluidine blue solution for 10 minutes
3. Rinse in distilled water
4. quickly dehydrate through 95% and absolute alcohols
5. clear in xylene and mount with synthetic resin.

Results

Mast cells – deep violet

Background – blue

Notes

1. alcohol cannot be used for dehydration in most metachromatic staining procedures, but metachromasia of mast cell granules is stable and will not be lost after alcoholic dehydration.
2. A rapid screening method for mast cells is to simply apply a light methylene blue stain as if you were counterstaining an acid-fast stain. The mast cells are very well demonstrated.
3. Mast cells will also be stained orange-red by the methyl green-pyronin stain .