

F-374 VERHOFFS VAN GIESON

Fixation: Any well-fixed tissue – **10% Buffered Neutral Formalin**(F-113) or Zenker's (F-155a) is preferred.

Sections: 5 microns.

Procedure:

1. Hydrate slides to distilled water.
2. Stain in Verhoffs solution for 1 hour. Tissue should be completely black.

*To prepare working Verhoeff's solution, add in order the following reagents – prepare fresh!

Alcoholic Hematoxylin, 5% (F-374-1) 20 ml

Ferric Chloride, 10% (F-374-2) 8 ml

Weigert's Iodine Solution (F-374-3) 8 ml

Mix well, solution should be jet black. Use immediately.

At the conclusion of staining time, pour off the Verhoeff mixture into a container and save it until after the differentiation process has been properly completed. If it should prove necessary, the saved solution may be used.

3. Rinse in tap water with 2 or 3 changes.
4. Differentiate in **Ferric Chloride, 2%** (F-374-4). Agitate slides gently during the process. Stop differentiation with several changes of tap water and check microscopically for black elastic fiber staining and gray background. Repeat Ferric Chloride, 2% treatment and tap water rinses as necessary for adequate demonstration. Kidney and myometrium are good controls. If the elastic fiber staining is too pale, restain in the saved Verhoeffs solution for 30 minutes and then proceed with the differentiation process.

Note: It is better to slightly underdifferentiate the tissue, since the subsequent Van Geison counterstain can extract the elastic stain somewhat.

5. Wash slides in tap water.
6. Treat with **Sodium Thiosulfate, 5%** (F-374-5) for 1 minute. Discard solution.
7. Wash in running tap water for 5 minutes.
8. Counterstain in **Van Giesons Solution** (F-374-6) for 3 to 5 minutes.
9. Dehydrate, clear in Xylene and coverslip, using a synthetic mounting medium.

Stain Results:

Elastic Fibers	Blue-black to black Fine elastic fibrils may not be stained with this method
Nuclei	Blue to black
Collagen	Red
Other tissue elements	yellow

References:

Sheehan, Dezna C., Hrapchack, Barbara B. Theory and Practice of Histotechnology, 2nd ed. The C.V. Mosby Company, St. Louis, p. 196-197., c. 1980