

## **PAS/MY STAINING PROTOCOL FWRI HISTOLOGY**

Note: Prior to staining, slides must be baked for at least two at a minimum temperature of 70° C to inhibit detachment of sections during the staining procedure.

### **STAINING PROCEDURE**

1. Immerse in 1% Periodic Acid for 15 minutes.
2. Rinse thoroughly in 3 changes of DI.
3. Drain slides thoroughly to minimize water transfer to Schiff's reagent.
4. Immerse in Schiff's reagent for 15 minutes.
5. Wash in running tap water for 10 minutes.
6. Stain in Weigert's Hematoxylin, working solution, for 6 minutes.
7. Drain slides.
8. Differentiate in 70% Acid-Ethanol, pH 2.5, two changes, three quick dips each.
9. Differentiate in 70% Acid-DI, pH 2.5, two changes, three quick dips each.
10. Wash in running tap water for 15 minutes. Drain excess water from slides.
11. Stain with Metanil Yellow, working solution, for two minutes.
12. Drain slides.
13. Rinse in DI, three changes, two seconds each.
14. Differentiate and dehydrate in 95% ethanol, two changes, two seconds each.
15. Dehydrate in 100% ethanol, three changes, two seconds each.
16. Completely dehydrate in acetone, two changes, two seconds each.
17. Clear in Histoclear, two changes, one minute each.
18. Transfer to fresh Histoclear and coverslip with mounting medium.

## **RESULTS**

**Nuclei** – deep blue-violet

**Chromatin** – blue-black

**Nucleoli** – blue-grey to yellow-grey

**Nuclei of primary oocytes** – pale yellow-grey

**Chromatin nucleoli of primary oocytes** – pale yellow-grey

**Cytoplasm of pre-vitellogenic oocytes** – deep blue violet

**Cytoplasm of other cell types** – varying shades of yellow or yellow-tan

**Collagen** – magenta

**Muscle** – bright yellow

**PAS reactions** – Reaction intensities will vary; however, the following substances will be PAS-positive (magenta): glycogen, neutral mucosubstances, basement membranes, collagen fibers, fungal cell walls, and glycoproteins such as yolk vesicles and phospholipids.