I. PRINCIPLE:

Periodic acid oxidizes the glycogen in blood and bone marrow cells to aldehydes, which in turn react with Schiff's reagent (Leuko-fuchsin) and stain red to pink. The counterstain, Mayer's hematoxylin, then stains the nuclei blue.

II. SPECIMEN:

3"x1" slides of patient peripheral blood (EDTA) or bone marrow sample. Coverslips can be processed as well but the larger slides are preferred.

III. REAGENTS:

A. Fixative: 10% formaldehyde in ethanol. Mix 5 ml of 36% formaldehyde with 45 ml of 95% ethanol if staining 3x1 slides and using coplin jars. Mix 1 ml 36% formaldehyde with 9 ml 95% Ethanol if staining coverslips or using the staining rack instead of coplin jars for the large slides. Make fixative fresh each time.

B. Periodic Acid: (H_5IO_6) 1.0%. 5 gm in 500 ml distilled water. Store in refrigerator in a brown bottle (for 1 year).

C. Schiff's reagent: Sigma (395-2-016), check manufacturer's expiration date.

D. Mayer's Hematoxylin: Add 1 g hematoxylin to 500 ml distilled water. Heat to just Boiling and add another 500 ml water. Then add 0.2 g sodium iodate and 50 g aluminum potassium sulfate. Shake well and filter. Store in brown bottle at room temperature. Stable for a year.

IV. QUALITY CONTROL:

Coverslips of normal patient with >10 monocytes, >18 lymphocytes and a WBC>6,000.

V. PROCEDURE:

A. Prepare blood or bone marrow slides or coverslips, dry and fix in 10% formaldehyde in 95% ethanol for 1 minute.

B. Wash in running tap water for 1 minute.

C. Slides are immersed in 1.0% periodic acid at **room temperature** for 5 minutes or covered with the solution while on the staining rack and rinsed in several changes of distilled water.

D. Coverslips or slides are then immersed in Schiff's reagent (**brought to room temperature**)or slides are flooded with the solution while on the staining rack for 15 minutes, then rinsed in running tap water for 5 minutes.

E. Lastly, the coverslips or slides are counterstained with Mayer's hematoxylin for 90 seconds, washed in running tap water for 15-30 seconds, air dried then mounted with permount.

VI. RESULTS:

A. Peripheral Blood

Segs: cytoplasm intensely red or pink, ± granular appearance.
Monocytes: cytoplasm faintly pink, ± fine or coarse granules.
Lymphocytes: ± few small red and pink granules, lymphocytes of patients with lymphoma, Hodgkin's disease, chronic lymphocytic leukemia, mycosis fungoides (Sezary syndrome) or infectious mononucleosis, as well as lymphoblasts in patients with acute lymphocytic leukemia, may contain an increased number of PAS-positive granules of varying size.

B. Bone Marrow

Granulocyte precursors: earliest not stained, but increasing stainability with maturity, myeloblasts in acute granulocytic leukemia are PAS negative.
Megakaryocytes: intensely stained.

RBC precursors: not stained, may stain pink or red or contain red granules in erythroleukemia, refractory anemias (iron deficiency anemia,

thalassemia) and severe acquired hemolytic anemia. These PAS positive cells may be megaloblastoid, whereas the megaloblasts in Vitamin B_{12} or folic acid deficiencies are PAS negative.

VII. REFERENCES:

1. Quaglino, D. and Hayhoe, F.G.J. Observations on the periodic acid Schiff reaction in lymphoproliferative disease. J. Path. and Bact. 78:521, 1959.

2. Quaglino, D. and Hayhoe, F.G.J. P.A.S. positivity in erythroblasts with special reference to DiGuglielmo's disease. Brit. J. Haemat. 6:26, 1959.

3. Mitus, W.J. et al. Cytochemical studies of glycogen content of lymphocytes in lymphocytic proliferations. Blood 13:743, 1958. 10/97

VIII. HISTORY:

H-1 This procedure was written by P. Morris on 08-05-97.
H-2 This procedure was revised by S. Richardson on 4/30/12.