I. PRINCIPLE:

This technique is suggested as an alternative to methods employing benzidine and its derivatives which are carcinogens. This method is based on the oxidation of the indicator 3-amino-9-ethylcarbazole to an insoluble red-orange reaction product in the presence of hydrogen peroxide and myeloperoxidase.

II. SPECIMEN:

Coverslips or 3x1 slides using patient's peripheral blood (EDTA) or a bone marrow sample.

III. REAGENTS:

A. 3-amino-9-ethylcarbazole (in cabinet in staining area-protect from light)
B. Dimethyl Sulfoxide (A.R.)-in staining cabinet
C. Hydrogen Peroxide 0.3% (A.R.) (1 ml 3% H_2O_2 in 9 ml H_20)
D. Glacial acetic acid (A.R.)-in acid cabinet
E. Hematoxylin C.I. #75290
F. Sodium acetate-$3H_2O$ (A.R.)
G. Sodium iodate (A.R.)
H. Aluminum potassium sulfate- 12 H_2O
I. Sodium phosphate- dibasic anhydrous (Na_2HPO_4) (A.R.)
J. Potassium phosphate- monobasic (KH_2PO_4) (A.R.)
K. Absolute acetone- reagent grade
L. Formaldehyde- 37% (A.R.)

Solutions:

1. Fixative- buffered formalin acetone-prepare when depleted or when past expiration date. Combine the following per procedure:

Dibasic sodium phosphate	0.2 g.
Monobasic potassium phosphate	1.0 g.
distilled water	300 ml.
Acetone	450 ml.
Formaldehyde	250 ml.

Dissolve sodium phosphate and potassium phosphate in distilled water. Add acetone (do not use plastic funnel to deliver acetone) and mix. Add formaldehyde

and mix thoroughly. Adjust to pH 6.6 with 5% NaOH. Check after each drop, mix well. Store at room temperature, stable for a year.

2. Buffer- 0.02 M acetate buffer pH 5.0-5.2

   a. 0.02 M acetic acid: 1.16 ml. Glacial acetic acid diluted to 1 liter with distilled water.

   b. 0.02 M sodium acetate: 2.27 gm. Sodium acetat-$3H_2O$ diluted to 1 liter with distilled water

   Mix 176 ml of (a) and 800 ml of (b). Adjust pH to 5.0- 5.1, use 5% NaOH, or 5% Acetic Acid as needed. Store in the refrigerator (warm to room temperature before use) Stable 6 months.

3. Mayer's Hematoxylin

   Add 1 g. Hematoxylin to 500 ml. Distilled water. Heat just to boiling and add another 500 ml of water. Then add 0.2 g. of sodium iodate and 50 g. of aluminum potassium sulfate. Shake well and filter. Store in brown bottle at room temperature.

IV. QUALITY CONTROL:

Coverslips or 3x1 slides of a normal EDTA sample with monocytes >10, lymphocytes >18 and a WBC count of >6,000.

V. PROCEDURE:

A. Fix in buffered formalin acetone for 15 seconds at room temperature. In a coplin jar or on staining rack.
B. Wash gently in running tap water.
C. Combine in a dry flask:

1. Solution of 6 ml **dimethyl sulfoxide** added to red top tube containing premeasured 10 mg. **3-amino-9 ethylcarbazole**.
2. 50 ml of **0.02 M acetate buffer (pH 5.0-5.2)**
3. 0.4 ml of **0.3% hydrogen peroxide solution made fresh as follows : 1 ml 3% H_2O_2 and 9 ml H_2O**

Mix and filter onto coverslips or slides in a Coplin jar or on staining rack.

D. Incubate in above mixture for 2.5 minutes at room temperature.
E. Wash gently in running tap water.
F. Counterstain with Mayer's hematoxylin for 8 minutes.
G. Wash in tap water and air dry.
H. Mount in **glycerin**.

VI. RESULTS:

Peroxidase activity is represented by red-brown granular deposits in the cytoplasm of the granulocytes and monocytes only. Strongest activity is observed in eosinophils.

Results are examined and reported by an attending physician and resident.

VII. REFERENCES:

Graham, R.C> Jr., Lundholm, U. & Karnovsky, M.J., Cytochemical demonstration of peroxidase activity with 3 - amino-9-ethylcarbazole. J.Histochem. Cytochem. 13:150-152, 1965

Schaefer, H.E. & Fischer, R. Peroxidase detection in smear preparations and tissue sections after decalcification and paraffin embedding Klin. Wschr. 46: 1228-1230. 1968

Yam, L.T., Li, C.Y. & Crosby, W.H. Cytochemical identification of monocytes and granulocytes. Amer. J. Clin. Path. 55: 283-290. 1971.

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.