I. PRINCIPLE:

Sudan Black B stains lipids in cells. Neutrophils stain the most strikingly, followed by monocytes with a sprinkling of black granules. Eosinophilic granules stain as black circles as only the membrane of the granules stain.

II. SPECIMEN:

Coverslips or 3x1 slides using patient peripheral blood (EDTA) or bone marrow samples.

III. REAGENTS:

A. 40% formalin

B. Stock buffer solution
1. 91 ml of liquid phenol -88%- (A.R.)
2. 150 ml absolute ethanol (200 proof)
3. 1.5 gm Na_2HPO_4. 12 H_2O dissolved first in 500 ml distilled water
   Mix phenol and ethanol then add phosphate and water mixture.

C. Stock Sudan solution
1. 1.50 gm Sudan Black B (Fisher CI #26150)
2. Dissolve in 500 ml absolute ethanol
3. Dissolve completely by shaking continually two hours.

D. Working incubation mixture-**make fresh**

For 3x1 slides:
1. 20 ml stock buffer
2. 30 ml stock Sudan solution
3. Filter into coplin jar over slides or onto slides on staining rack.

For coverslips:
1. 8 ml stock buffer
2. 12 ml stock Sudan solution
3. Filter over coverslips in coplin jar.

IV. QUALITY CONTROL:

A. Normal patient EDTA coverslip sample with Monocytes >10 and Lymphocytes > 18 with a WBC count >6,000.

V. PROCEDURE:

A. Fix in Formalin Vapor for 10 minutes.

B. Stain fixed smear in Sudan (working incubation mixture) for 10 minutes.

C. Wash slide well with 95 % ethanol (95 ml absolute ethanol with 5 ml DI water).

D. Wash with tap water.

E. Counterstain with 1:10 Giemsa using Hematek buffer for 20 minutes.
(Mix in a red top tube 1ml bottled Giemsa stain with 9 ml of Hematek buffer)
Pour over coverslips in coplin jar or onto slides in coplin jar or on staining rack:
If using large slides in coplin jar adjust volume as follows: **5ml Giemsa with 45ml Hematek buffer.**

F. Wash with tap water and air dry.

G. Mount coverslips with permount, coverslip 3x1 slides with permount.

VII. RESULTS:

Results are evaluated by an attending physician and resident.

VIII. REFERENCES:

1. Sheehan, H.L., Storey, GW: An improved Method of Staining Leukocyte Granule with Sudan Black B, Journal of Pathology 59:336-337. 1947

2. Baillif, RN, Kinbrough, R.T.: Studies on Leukocyte Granules after Staining with Sudan Black B Nag-Grimwald Giemsa, J. Lab. Clin. Med. 32, p. 155-166.

IX. HISTORY:

H-1 This procedure was written by P. Morris on 08-05-97.
H-2 This procedure was revised by Susan Richardson on 5/24/12.