VOLU-SOL
BUSINESS OF CHEMICALS

DIP STAIN KIT AND REAGENTS (16/ 128 OZ.)

DIP STAIN KIT VDS-100 (16 oz.)

DIP STAIN I:	DIP STAIN II:	DIP STAIN III:
VDF-016 (16 oz.)	VDE-016 (16 oz.)	VDB-016 (16 oz.)
VDF-128 (128 oz.)	VDE-128 (128 oz.)	VDB-128 (128 oz.)

Order online at **www.volusol.com**

INTENDED USE: For use in differential staining of blood smears, bone marrow, and blood parasites.

SUMMARY AND PRINCIPLES: It is the function of a hematology stain to clearly define individual cells, their nuclear detail, and cytoplasmic structure for microscopic examination. In 1901, Dmitri Romanowsky created a mixture of eosin y and modified methylene blue, known as the Romanowsky Stain. James Wright and William Leishman modified the stain by adding methanol in 1902. In the same year, Gustav Giemsa culminated Wright and Leishman's modifications by adding glycerol in order to synthesize a neutral stain with a greater chemical purity. The classic Wright's Stain or polychrome methylene blue requires the use of a buffer with various salts and controlled pH to perform its differential staining.

SPECIMEN PREPARATION: Capillary or venous blood is acceptable for making blood films. Make blood film immediately if no anticoagulant is employed. Should an anticoagulant be required, ethylenediaminetetraacetic (EDTA) acid, is currently preferred.

PRINCIPLES OF THE PROCEDURE: A neutral stain is a compound dye molecule which consists of both acidic and basic chromophore groups ionically bound in an alcoholic solution. The cytoplasmic structures exhibit differential affinity for the chromophore groups, based on their charged group interactions. Volu-Sol's Dip Stain I is composed of a controlled mixture, in methanol, providing the richness of color that characterizes a Volu-Sol Stain. Alcoholic solutions of compound dyes penetrate cells rapidly but stain poorly, while aqueous solutions of compound dyes stain quite well. The foregoing situation is resolved, if a buffer solution of the correct pH (e.g. Volu-Sol's Dip Stain II and III) dissociates and hydrates the chromophore regions so as to make permeable the stain barrier. Once differential staining has taken place, it becomes a function of the rinse to halt the process, prevent over staining, and to remove any precipitate which may have accumulated during the buffering.

PROCEDURE: Prepare a film of blood or bone marrow on a microscope slide and allow to dry. Prepare three containers (e.g., coplin jars or staining dishes). Fill one container with Volu-Sol's Dip Stain Solution I, the second with Volu-Sol's Dip Stain Solution II, and the third container with Volu-Sol's Dip Stain Solution III. Pour adequate amounts of each of Volu-Sol's Dip Stain Solutions into the coplin jars or staining dishes. Dip slide or rack of slides 5 times for 1 second each dip into solution I. Allow excess to drain into jar or dish and blot edge on absorbent paper. Dip slide or rack of slides 5 times for 1 second each dip into solution II. Allow excess to drain into jar or dish and blot edge on absorbent paper. Dip slide or rack of slides 5 times for 1 second each dip into solution III. Allow excess to drain into jar or dish and blot edge on absorbent paper. Rinse slide or rack of slides by dipping or swishing in distilled or deionized water. Air dry slides or use warm air blower before mounting with oil and reading.
*__NOTE:__ Tone and depth of color may be adjusted as follows **1)** Too blue—decrease 1 dip in solution III. **2)** Too red—decrease 1 dip in solution II. **3)** Too dark—decrease by 1 or 2 dips in both solution II and III. **4)** Too light—increase by 1 or 2 dips in both solution II and III is required. Always dip slide in solution I a minimum of 5 (1 second dips). For bone marrow smears, double the above times.*

EXPECTED RESULTS: The reaction of cytoplasm to staining is subject to many variables. Since the majority of staining occurs during the buffering stage, the variable of greatest magnitude is the resultant pH of the stain/buffer mixture at the cellular surfaces. The overall color of the red blood cells is a guide to stain quality and should be used in adjusting staining times for desired results. **ERYTHROCYTES:** Pink-tan with degrees of chromasia. **WBC'S:** Nuclei with bright, bluish-purple chromatin, light blue nucleoli. **LYMPHOCYTES:** Clear blue cytoplasm, red-purple granules may be present. **MONOCYTES:** Mosaic of pink and blue cytoplasm, azure granules usually present. **NEUTROPHILS:** Light purplish-pinkish or lavender granules in cytoplasm. **EOSINOPHILS:** Bright red or reddish-orange granules in cytoplasm. **BASOPHILS:** Deep purple and violet-black granules in cytoplasm. **PLATELETS:** Clearly demarcated red-purple granules in light blue cytoplasm.

STORAGE AND EXPIRATION: If smears cannot be stained within 4-6 hours of preparation, they should be fixed with dip stain I. Store stains at room temperature (70-77.9 °F/ 20-25.5 °C). Maximum intended shelf life is printed on the label.

WARNING: Danger! Flammable. Vapor Harmful. For in vitro diagnostic use only. May be fatal or cause blindness if ingested. Cannot be made nonpoisonous. Keep away from heat and open flame. Avoid repeated or prolonged breathing of vapor. Use only with adequate ventilation.

REFERENCES:

1. Wright, J.H., Med. Res., 7 138 (1902)
2. Clinical Diagnosis by Laboratory Methods, 15th ed., Davidsohn, I., and Henry, J.B., Saunders, 1974.
3. Wintrobe, M.M., 7th ed., Lea & Febiger, Philadelphia: Clinic Hematology.