VOLU-SOL
BUSINESS OF CHEMICALS

HEMATOLOGY BUFFER POWDER PH 7.1 (4.4 GM.)

Catalog Item: VHB-001 (1 packet – 4.4 gr)
VHB-010 (1 box of 10) VHB-024 (1 box of 24)
Order online at **www.volusol.com**

INTENDED USE: In hematological staining to clearly define individual cells, their nuclear detail, and cytoplasmic structure for microscopic examination.

SUMMARY AND PRINCIPLES: In 1901, 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. Many observers note that Giemsa's Stain yields great color intensity, sharpness of cellular detail, and is unsurpassed for the demonstration of blood parasites.

SPECIMEN PREPARATION: Either 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. **1)** Dissolve one vial of powder in 3.8 liters (one gallon of deionized water and mix thoroughly. **2)** The pH of the solution can be checked on a glass electrode pH meter. The pH of the buffer solution should be 7.15 ± 0.1.

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 Wright and Wright-Giemsa Stain are composed of eosinites of polychrome methylene-blue, in methanol, which yield a carefully monitored spectrum of eosinated homologs, which provides a richness of color. The forgoing situation is resolved if a buffer solution of correct pH is employed. The alcoholic solution delivers the required spectral variety of compound dye molecules to the cellular surfaces, while a buffer of the correct pH dissociates and hydrates the chromophores to increase the permeability of the cellular surfaces. Once differential staining has taken place, it becomes a function of the rinse to halt the process and remove any precipitate which may have accumulated during the buffering process.

PROCEDURE: Add 1 Packet to 1 gallon of deionized water. Refer to the Hemastainer Operator's Manual for procedure and suggested staining times.

EXPECTED RESULTS: Alcoholic solutions of compound dyes stain poorly, while aqueous solutions of dyes stain well. The reaction of cytoplasm to neutral 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 and buffering times for desired results. **LEUKOCYTE NUCLEI:** should appear bluish-purple. Acid stain yields pale blue and dark blue leukocyte nuclei. **EOSINOPHILIC GRANULES:** should appear red; acid stain yields brilliant and distinct red granules, whereas an alkaline stain yields dark, prominent, neutrophilic granules. **NEUTROPHILIC GRANULES:** should appear violet to pink; acid stain yields pale neutrophilic granules whereas an alkaline stain yields dark, prominent, neutrophilic granules. **LYMPHOCYTE CYTOPLASM:** should appear sky-blue; acid stain yields pale blue cytoplasm, whereas alkaline stain yields grey or lavender lymphocyte cytoplasm. **RBC'S:** pink-tan color with degrees of chromasia; **WBC'S:** nuclei with bright, bluish-purple chromatic, 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. ***NOTE:** RBC's should appear buff-pink; acid stain will render them bright red or reddish-orange, whereas alkaline stain will render them blue or green.*

STORAGE AND EXPIRATION: If smears cannot be stained within 4-6 hours of preparation, they should be fixed in absolute methanol.

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. R.D. Lillie, Biological Stains 8th Ed., The Williams & Williams Company, Baltimore, c. 1969.
2. R.D. Lillie, Histopathologic Technic and Practical Histochemistry, 3rd ed., McGraw-Hill, New York, c. 1965.
3. S.J. Singer and Garth T. Nicholson, "The Fluid Mosaic Model of the Structure of Cell Membranes," Science, Vol. 175, Feb. 1972.