Wright/Wright-Giemsa Hematology Stain Kits and Reagents

Wright's Stain Kit VWS-300 (8 oz.) **Wright-Giemsa Stain Kit** VWG-300 (8 oz.)

Wright Stain:	Wright-Giemsa:	Buffer:	Rinse:
VWS-016 (16 oz.)	VWG-016 (16 oz.)	VWB-032 (32 oz.)	VHR-032 (32 oz.)
VWS-032 (32 oz.)	VWG-032 (32 oz.)	VWB-128 (128 oz.)	VHR-128 (128 oz.)
VWS-128 (128 oz.)	VWG-128 (128 oz.)	VWB-640 (640 oz.)	VHR-640 (640 oz.)

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Intended Use: A hematology stain is intended for use in differential staining procedures for blood, bone marrow, and the demonstration of 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. Many observers note that Giemsa's Stain yields great color intensity, sharpness of cellular detail, and is unsurpassed for the demonstration of blood parasites. This formulation results in rapid penetration of the cellular elements, producing an excellent, intense stain.

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, EDTA is recommended. If smears cannot be stained within 4-6 hours of preparation, they should be fixed in absolute methanol. Prefixing slides with methanol prior to staining is an excellent technique to enhance the quality of stain.

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 reactions. Wright and Wright-Giemsa Stains are composed of eosin and methylene blue. Alcoholic solutions of compound dyes stain poorly, while aqueous solutions of dyes stain well. 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: Three containers with tightly fitting covers or screw caps are filled with **1)** Volu-Sol's Wright or Wright-Giemsa Stain, **2)** Volu-Sol's Hematology Buffer, and **3)** Volu-Sol's Hematology Rinse. The recommended staining and buffering times which follow are applicable to peripheral blood smears or initial trials of the stain; bone marrow may require 2-3 times the exposure to the stain and buffer. Staining and buffering times should be adjusted to suit individual preferences. Fresh, unused buffer should be primed by adding a small amount of stain (approximately 0.5 ml. of stain for each 50 ml. of buffer) before processing the first batch of slides. Change buffer when an iridescent scum of stain forms on its surface or when the buffer becomes discolored to a dark blue. Change rinse when it becomes discolored to a medium blue (rinse change required more often than the buffer change). Replace the stain when the volume becomes insufficient. Keep stain covered when not in use to prevent evaporation. Dip slides in the stain for preferred staining time (approximately 60 seconds) and do not agitate. Drain or blot edge of slide (or slide holder) to remove excess stain. Dip slides in buffer for approximately 60 seconds. Increasing or decreasing the staining or buffering time will alter the color of the finished slide. Drain or blot edge of slide (or slide holder) to remove excess buffer. Dip slides in rinse for 2-10 seconds. Dry slides in vertical position, on an absorbent surface (e.g., paper towel). Do not blot smear. Apply oil and examine the slides microscopically. **Rack Procedure:** Place slides on staining rack. Apply sufficient stain to cover the smear. Leave stain on slides for approximately 1 minute. Add buffer to slides. Mix layers of stain and buffer by applying a current of air or by blowing on the slides. Allow the stain-buffer mixture to remain for 2 minutes (or as preferred). Wash stain-buffer mixture by flooding with rinse until the slides run clear. Remove slides from rack, wipe the back of the slides, and dry in vertical positions. ***Note:** Do not replenish by adding new stain to the old stain. *

Expected Results: The reaction of the cytoplasm to neutral staining is subject to many variables. The variable of the 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. **RBC's:** Pink-tan color. **WBC's:** Nuclei with bright, bluish-purple chromatin light blue nucleoli. **Lymphocytes:** Clear blue cytoplasm, red-purple granules may be present. Acidic stain yields pale blue cytoplasm, whereas alkaline stain yields gray or lavender lymphocyte cytoplasm. **Monocytes:** Bluish grey cytoplasm, azure granules usually present. **Neutrophils:** Light purplish-pinkish or lavender granules in cytoplasm. Acidic stain yields pale neutrophilic granules, whereas a basic stain yields dark, prominent neutrophilic granules. **Eosinophils:** Bright red or reddish-orange granules in cytoplasm. Acidic stain yields brilliant and distinct red granules, whereas basic stain yields deep gray or blue eosinophilic granules. **Basophils:** Deep purple and

violet-black granules in cytoplasm. **PLATELETS:** Red-purple granules in light blue cytoplasm.

STORAGE AND EXPIRATION: Store reagents at room temperature (70-77.9 °F/ 20-25.5 °C). Protect the stain from exposure to water vapor, and direct sunlight. Maximum intended shelf life is printed on the label. If stain is kept in staining solution for an extended period (e.g., several weeks), filter before use.

WARNING: For in vitro diagnostic use only. Toxic and Flammable. Vapor harmful. May be fatal or cause blindness if ingested. Cannot be made non-poisonous. Avoid prolonged breathing of vapor. Use with adequate ventilation.

REFERENCES:

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