Volu-Sol, Inc.

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Wright and Wright-Giemsa Hematology Stain Kits and Reagents

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 EXPLANATION OF TEST: It is the function of a hematology stain to clearly define individual cells, their nuclear detail and their cytoplasmic structure for microscopic examination. In 1910, Paul Ehrlich synthesized a dye for use as a differential blood stain. His original mixture was preceded by the work of Romanovsky (1891). Between the years 1889 and 1901, other workers modified the Romanovsky stain. These efforts were eventually culminated by Wright's (1902) modification of Leishman's stain. Giemsa, in 1902 modified the stain of Leishman in an attempt to synthesize a neural stain with greater chemical purity. Many observers note that Giemsa's stain yields great intensity of color and sharpness of cellular detail and is unsurpassed for the demonstration of blood parasites. Volu-Sol's Wright and Wright-Giemsa Stains are a modification of Leishman's Stain. This formulation results in rapid penetration of the cellular elements, producing an excellent, intense stain.

PRINCIPAL OF THE PROCEDURE: A neutral stain is fundamentally a compound dye molecule which consists of acidic and basic chromophore groups, ionically-bound in alcoholic solution. The structures of cytoplasm exhibit differential affinities for the chromophore groups, based upon their charged-group interactions. Wright's and Wright-Giemsa's stains are composed of Eosin and Methylene blue. Alcoholic solutions of compound dyes stain poorly, while aqueous solutions of dyes stain quite well. The forgoing situation is resolved if a buffer solution of correct pH is employed.
Since an alcoholic solution can deliver the required spectral variety of compound dye molecules to the cellular surfaces, a buffer of the correct pH dissociated and hydrated 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 accumulated during the buffering process.

STORAGE INSTRUCTION:

1. Volu-Sol, Inc. products should be stored at room temperature, less than 86°F (30°C).
2. Protect the stain from exposure to water vapor, and direct sunlight.
3. Maximum intended shelf life of Volu-Sol, Inc. products is the date printed on the label.

SPECIMEN: Either capillary or venous blood is acceptable for making blood films. If no anticoagulant is employed, the blood film must be made immediately. Should an anticoagulant be required, EDTA is recommended. If smears can't 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.

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PROCEDURE FOR BLOOD SMEARS AND BONE MARROW
A: NOTES ON DIP PROCEDURE:

1. Three containers with tightly fitting covers or screw caps are filled with #1 Volu-Sol Wright or Wright-Giemsa Stain, #2 Volu-Sol Hematology Buffer, #3 Volu-Sol 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 as blood smears; staining and buffering times should be adjusted to suit individual preferences.

2. Fresh, unused buffer should be primed by adding a small amount of stain (approximately 0.5 ml of stain for each 50ml of buffer) before processing the first batch of slides.
3. Change buffer when an iridescent scum of stain forms on its surface or when the buffer becomes discolored a dark blue.
4. Change rinse when it becomes discolored a medium blue. This will be required more often than the buffer change.
5. When stain volume becomes insufficient, replace the stain. Do not replenish by adding new stain to the old stain.
6. To prevent evaporation, keep stain covered when not in use.
7. If stain is kept in the staining container for an extended period (e.g., several weeks), filter before use.

B: DIP PROCEDURE:

1. Dip slides in the Volu-Sol Stain for preferred staining time, approximately 60 seconds. Do not agitate.
2. Drain or blot edge of slide (or slide holder) to remove excess stain.
3. Dip slides in the Volu-Sol Buffer for approximately the same amount of time as in stain, 60 seconds. Increasing or decreasing staining or buffering time will alter the color of the finished slide.
4. Drain or blot edge of slide (or slide holder) to remove excess buffer.
5. Dip slides into the Volu-Sol Hematology Rinse for 2 to 10 seconds.
6. Wipe back of slide.
7. Dry slides in vertical position, on an absorbent surface (e.g., paper towel). Do not blot smear.
8. Apply oil and examine the slides microscopically.

C: RACK PROCEDURE:

1. Place slides on staining rack.

2. Apply sufficient Volu-Sol Stain to cover the smear. Leave stain on slides for approximately 1 minute.
3. Add approximately the same volume of Volu-Sol buffer as stain to slides.
4. Mix layers of stain and buffer by applying a current of air or by blowing on the slides.
5. Allow the stain-buffer mixture to remain for 2 minutes (or as preferred).
6. Wash stain-buffer mixture by flooding with Volu-Sol Hematology Rinse until the slides run clear.
7. Remove slides from rack. Wipe back of slides and proceed to dry in vertical positions described in dip method.

EXPECTED RESULTS:

The reaction of cytoplasm to neutral staining is subject to a great many variables as described earlier. 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. Specifically, RBC's should appear buff-pink; acidic stain will render them bright red or reddish-orange, whereas alkaline stain will render them blue or green.

1. **RBCs**: Pink-tan color.
2. **WBCs**: Nuclei with bright, bluish-purple chromatin light blue nucleoli.
3. **Lymphocytes**: Clear blue cytoplasm, red-purple granules may be present
4. **Monocytes**: Bluish grey cytoplasm, azure granules usually present.
5. **Neutrophils**: Light purplish-pinkish or lavender granules in cytoplasm.
6. **Eosinophils**: Bright red or reddish-orange granules in cytoplasm.
7. **Basophils**: Deep purple and violet-black granules in cytoplasm.
8. **Platelets**: Red-purple granules in light blue cytoplasm.

LIMITATION OF THE PROCEDURE: A truly representative blood smear is a diagnostic tool of inestimable value to the clinician. The course of a disease is open monitored by the routine differential, therefore, it is to be stressed that the information gathered from the blood smears is only as accurate as the preparation of the film, from specimen collection and spreading, to drying and final staining of the resultant smear.

1. All leukocyte nuclei should appear bluish-purple. Acidic stain yields pale blue and alkaline stain yields dark blue leukocytes nuclei.
2. Eosinophilic granules should appear red. Acidic stain yields brilliant and distinct red granules, whereas basic stain yields deep gray or blue eosinophilic granules.
3. Neutrophilic granules should appear violet to pink. Acidic stain yields pale neutrophilic granules, whereas a basic stain yields dark, prominent neutrophilic granules.
4. Lymphocyte cytoplasm should appear sky-blue. Acidic stain yields pale blue cytoplasm, whereas alkaline stain yields gray or lavender lymphocyte cytoplasm.

☠ TOXIC AND FLAMMABLE.
Vapor harmful. For in vitro diagnostic use only!. May be fatal or cause blindness if ingested. Cannot be made non-poisonous. Avoid prolonged breathing of vapor. Use with adequate ventilation

REFERENCES:

1. RD. Lillie, Biological stains, 8th ed., The Williams & Wilkins Co. Baltimore, c. 1969.
2. RD. Lillie, Histopathologic Technique and Practical Histochemistry. 3rd, McGraw-Hill. New York, c. 1965.
3. S.J. Singer and Garth T. Nicholson, "The Fluid Mosaic model of the structure of Cell Membranes", Sciences Vol. 175, Feb. 1972.
4. Israel Davidsohn and Douglas A Nelson, "'The Blood", Clinical Diagnosis, Israel Davidsohn and John Bernard Henry. Et al., 15 ed., W.B. Saunders Co. Philadelphia, c.1969.
5. Samuel a. Levinson and Robert P. MacFate, Clinical Laboratory. Diagnosis, 7th ed., Lea & Febiger. Philadelphia, c.1969

PACKAGING	Size	Catalog No.
Wright Stain	16 oz.	VWS-016
	32 oz.	VWS-032
	128 oz.	VWS-128
Wright Stain Kit	8 oz. Each	VWS-300
Wright-Giemsa Stain	16 oz.	VWG-016
	32 oz.	VWG-032
	128 oz.	VWG-128
Wright-Giemsa Stain Kit	8 oz. Each	VWG-300
Hematology Buffer	32 oz.	VWB-032
	1 gal.	VHB-0128
	5 gal.	VWB-640
Hematology Rinse	32 oz.	VHR-032
	1 gal.	VHR-128
	5 gal.	VHR-640

Larger sizes available upon request

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