VOLU-SOL
BUSINESS OF CHEMICALS

ALCOHOLIC GIEMSA STAIN (8/16 OZ.)

Catalog Item: VAG-008 (8 oz.) / VAG-016 (16 oz.)

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

INTENDED USE: A stain specifically formulated for **1)** the study of the morphology of blood; **2)** tissue cells; **3)** the demonstration of protozoan parasites; **4)** and the morphological analysis of chromosomes in cytogenetic applications.

SUMMARY AND PRINCIPLES: Volu-Sol's Alcoholic Giemsa is a unique and patented formulation, compounded for blood films, bone marrow, and cytogenetic procedures. It is the function of a stain to clearly define individual cells, their nuclear detail, and cytoplasmic structure for microscopic examination. This formulation results in a rapid penetration of cellular elements, producing a clearly defined cytoplasmic structure and distinct nuclear detail.

SPECIMEN PREPARATION: HEMATOLOGICAL USE: Prepare and fix blood smears using any preferred method. Davidson and Nelson specimen procurement, and patient preparation are recommended. **CYTOGENETIC USE: 1)** Buccal smear: Use clean slides, stored dry (label before use). Scrape buccal mucosa, wipe spatula clean, scrape again, and spread on one slide. Cover with second slide and press together. Slide them apart and place immediately into stain. **2)** Macro-Culture for Leukocytes: Using aseptic technique, draw 10 mL of venous blood into a sterile syringe containing 0.1 mL of heparin solution (injectable 1,000 USP heparin units/mL). Gently mix by inverting the syringe. Remove the needle, transfer the blood into a sterile tube, and let stand at room temperature for approximately 45 minutes, or until red cells are separated from leukocytes (a sterile centrifuge tube may be used and spun at 800-900 rpm for 5 min.). Remove leukocyte layer (buffy coat) with about 1 mL of plasma and transfer into sterile screw cap tube containing 8 mL of media of 10% fetal bovine serum and 1% phytohemagglutinin. Flame lip of tube and tighten cap firmly. Incubate in a horizontal position for 48-72 hours at 37° C. Add 0.9 mL colchicine solution to tube at the end of 48 hours and continue incubating for 2 more hours (colchicine concentration: 1 mg. colchicine per 100 mL H_20). Remove the tubes from the incubator after 2 hours and centrifuge at 800-900 rpm for 5 min. **HYPOTONIC TREATMENT:** Pour off the supernatant and add 5 mL of warm KCL (0.075 M) and gently mix with pipette. Incubate for 7 min. at 37°C. Centrifuge at 800-900 rpm for 5 minutes and pour off supernatant. **FIXATION:** Slowly add 5 ml of cold freshly made methanol-acetic acid fixative (3:1). Leave at room temperature for 30 minutes, then mix very gently with pipette and spin at 500-700 rpm for 5 minutes. Pour out old fixative and add just enough new fixative (0.25-0.5 mL) to make cell suspension adequate for making slides. ***NOTE:** At this time, if you leave the cells overnight in the refrigerator you can get a much better spreading of cells.* **SLIDE PREPARATION:** Dip a thoroughly cleaned slide into distilled water. Observe for cleanliness of slide by adherence of the water film. Place one to two drops of the cell suspension onto the center of the wet slide, and immediately pass through a small flame, igniting the fixative. Allow to dry thoroughly before standing. **HEMATOLOGICAL**: Delay in smearing will result in immediate and undesirable coagulation of the specimen. Use of an anticoagulant is recommended, EDTA is suggested. Cytogenetic buccal smears: The slides may remain in the ethyl alcohol 95% up to 24 hours without any harm. **LEUKOCYTE CULTURES:** Heparin is recommended for anticoagulation of blood collected for cultures. Although there are many commercial heparin preparations, some use phenol, which is toxic to leukocyte cultures as a preservative. Commercial diluting fluids may also contain toxic preservatives (e.g. benzyl alcohol).

PRINCIPLES OF THE PROCEDURE: A neutral stain is a compound dye molecule which consists of both acidic and basic groups ionically bound in an alcoholic solution. The structures of cellular protoplasm, based upon their charged group interactions, exhibit differential affinities for the chromophore groups. Volu-Sol's Alcoholic Giemsa Stain 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 Hematology Buffer) 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: Always do both right and left sides of cheek. Always run a known positive (female) control with the sample. **BLOOD SMEARS: 1)** Prepare 3 containers with tightly-fitting covers or screw caps (e.g., coplin jars) filled with *(1)* Volu-Sol's Alcoholic Giemsa Stain, *(2)* Hematology Buffer, and *(3)* Hematology Rinse. Fresh unused buffer should be primed by adding a small amount of stain (approximately 0.5 ml of stain for 50 ml of buffer) before processing the first batch of slides. Change buffer when an iridescent layer of stain forms on its surface or when the buffer becomes a discolored dark blue. Change rinse when it becomes discolored to medium blue. Changing rinse will be required more often than buffer change. When stain volume becomes insufficient, replace the stain. Do not replenish by adding new to the old stain. To prevent evaporation, keep stain covered when not in use. Filter before use if stain will be kept in staining container for an extended period (e.g., several weeks). **2)** Dip slides in diluted, alcoholic giemsa stain for 2 minutes (do not agitate). Drain or blot edge of slide to remove excess stain. Dip slides in buffer for approximately 2 minutes. Increasing or decreasing staining or buffering time will alter the color of the finished slide. Drain or blot edge of slide to remove excess buffer. Dip slides into the rinse for 2-10 seconds using quick dips, then wipe back of slide. Dry slides in vertical position,

on an absorbent surface (e.g. paper toweling - do not blot smear). Cover slip if desired, apply oil and examine microscopically. **3)** Rack Method Staining: Dropper bottles, wash bottles, or pipettes may be used to apply stain, buffer, and rinse to slides. Solutions are the same with the dip method. **4)** Rack Procedures: Place slides on staining rack. Cover smear with alcoholic giemsa. Leave stain on slides for approximately 6 minutes, then add buffer to slides. Mix layers of stain and buffer by applying a current of air or by blowing on the slides. An iridescent layer should form on the surface in 5-10 seconds. Allow the stain-buffer to mix by flooding with rinse until slide runs clear, then remove slides from rack. Wipe back of slides and proceed to dry in vertical position as described in dip method. **BUCCAL SMEARS:** After preparation of smear, continue as follows: *(1)* Alcoholic giemsa for 2 minutes. *(2)* Leukocyte culture for 10 minutes. *(3)* Buffer for 1 minute. *(4)* Clear in acetone for 10 seconds. *(5)* Clear in acetone: xylene (1:1) solution for 10 seconds. *(6)* Clear in xylene for 1 minute. *(7)* Remove slides from xylene and mount cover slip with Permount Mounting Medium. *(8)* Blot with paper towel.

EXPECTED RESULTS: 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 should appear buff-pink color with degrees of chromasia. WBC's should have nuclei with bright, bluish-purple chromatin, and 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.

STORAGE AND EXPIRATION: Store reagents at room temperature (70-77.9 °F/20-25.5 °C). Maximum intended shelf life is printed on the label.

WARNING: Danger! For in vitro diagnostic use only. Flammable. Vapor Harmful. 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 Histochemisty, 3rd ed., McGraw Hill, New York.
3. S.J. Singer and G.T. Nicholson, the Fluid Mosaic Model of the Structure of Cell Membranes, Science, Vol. 175, Feb 1972.
4. Davidson and Nelson, Clinical Diagnosis, 15th ed., W.B. Saunders Co. Philadelphia 1974.
5. S.A. Levinson and R.P. McPhate, Clinical Hematology, 6th ed., Lea & Febiger, Philadelphia. 1969.