VOLU-SOL
BUSINESS OF CHEMICALS

ACID FAST STAIN KIT & REAGENTS

AFB KIT BRILLIANT GREEN (VAF-300) **AFB KIT METHYLENE BLUE** (VAF-900)

COMPONENTS:

ACID ALCOHOL DEC.	ACID FAST STAIN	BRILLIANT GREEN	METHYLENE BLUE
VAA-008 (8 oz.)	VAF-008 (8 oz.)	VBG-008 (8 oz.)	VBC-008 (8 oz.)
VAA-016 (16 oz.)	VAF-016 (16 oz.)	VBG-016 (16 oz.)	VBC-016 (16 oz.)
VAA-032 (32 oz.)	VAF-032 (32 oz.)	VBG-032 (32 oz.)	VBC-032 (32 oz.)
VAA-128 (128 oz.)	VAF-128 (128 oz.)	VBG-128 (128 oz.)	VBC-128 (128 oz.)

CARBOL FUCHSIN /ZIEHL NEELSEN VZN-008 (8 oz.) VZN-032 (32 oz.)

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

SUMMARY AND PRINCIPLES: Volu-Sol's Acid Fast Stain is based on the popular Kinyoun Modified Stain Procedure which requires no heating of the stain. Acid fast stain's function is to brilliantly stain acid fast organisms and resist decolorizing after acid alcohol treatment. This staining allows the rapid differentiation of acid fast organisms from non-acid fast organisms. Acid fastness can be determined by the selective permeability of the cytoplasmic membrane. The brilliance of red coloration is due to the retention of the dye (carbol fuchsin) within the cell membrane. If the cell is mechanically disrupted, the acid-fast property will be lost. Although the carbol fuchsin stain penetrates both acid fast and non-acid fast cells, only acid fast organisms retain the stain after being treated with acid alcohol solution. Following treatment with the respective counterstain, acid fast organisms will appear bright red, while non-acid fast organisms will appear light green (brilliant green counterstain) or light blue (methylene blue counterstain).

SPECIMEN PREPARATION: **SPUTUM**: A loop full of the specimen (preferably a portion of purulent, blood, or caseous material) is spread over a small area of a clean, dry glass slide to make a thin smear. Allow to dry, gently heat fix over an open flame and proceed with staining. **GASTRIC AND URINE SAMPLES**: Centrifuge 10-15 ml. of specimen to concentrate organisms. Proceed to prepare smear. **HISTOLOGY SECTIONS**: Prepare according to published techniques.

PROCEDURE: **SMEARS:** Flood slide with Volu-Sol's Acid Fast Stain for 2 minutes after fixing the smear (heating unnecessary). Rinse slide with tap water. Decolorize smear with gentle stream of Volu-Sol's Acid Alcohol Decolorizer until stain no longer runs off slide. Rinse slide with tap water. Counterstain slide for 2 minutes with either Volu-Sol's Brilliant Green or Methylene Blue Counterstain (based on preference). Rinse slide with tap water, drain, and either air dry or blot gently with absorbent paper. Examine dried, stained slide under oil immersion objective by careful observation. Positive smears should be reported according to established recommendations. **HISTOLOGY SECTIONS:** Section paraffin tissue blocks in the normal manner. Process sections through deparaffinization and rehydrate with water. Stain slides for 2 minutes in Volu-Sol's Acid Fast Stain with gentle agitation. Rinse slides in gently running tap water. Decolorize sections individually in gentle stream of Volu-Sol's Acid Alcohol Decolorizer until stain no longer runs off of slide. Rinse slide in gently running tap water. Counterstain sections in either Volu-Sol's Brilliant Green or Methylene Blue Counterstain for two minutes with occasional gentle agitation. Rinse slides in gently running tap water. Finish processing for permanent mounting with acetone for 10 seconds, acetone: xylene (1:1) for one minute, and xylene for two minutes.

EXPECTED RESULTS: Acid fast organisms will appear as brightly stained red bodies. Typical acid fast bacilli will be red, slender, slightly curved, long or short rods, and sometimes beaded or granular. Atypical forms are usually thick or diptherial, very long, or sometimes coccoidal. Background material and other organisms will stain light green with the brilliant green counterstain or light blue with the methylene blue counterstain.

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. After prolonged storage, some separation of the phenol from the carbol fuchsin may occur. Should this occur, shake the tightly capped bottle vigorously prior to use.

REFERENCES:

1. Clark, G., ed., Staining Procedures, 3rd ed., 1973, the Williams and Wilkins, Co., Baltimore.
2. Lillie, R.D., Biological Stains, 8th ed., 1973, the Williams and Wilkins, Co., Baltimore.
3. Lillie, R.D., Histopathologic Technic and Practical Histochemistry, 3rd ed., 1965, McGraw Hill, New York.
4. Diagnostic Standards and Classifications of Tuberculosis, National Tuberculosis and Respiratory Disease Assoc., 1969, New York.
5. Bailey, R.V., and Scott, E.G., Diagnostic Microbiology, 4th ed., 1974, C.V. Mosby Co., St. Louis.