# CYTOCHROME STAIN WITH BUFFER

METHOD - MODIFIED LEISHMAN'S STAIN PRODUCT CODE - ST13

#### INSTRUCTIONS FOR USE



## INTENDED USE: Staining for Blood cells and Hemoparasites.

#### SUMMARY AND EXPLANATION

Leishman's stain, is used in microscopy for staining blood smears. It provides excellent stain quality. It is generally used to differentiate and identify leucocytes, malaria parasites, and trypanosomas. It is based on a methanolic mixture of "polychromed" methylene blue (i.e. demethylated into various azures) and eosin. The methanolic stock solution is stable and also serves the purpose of directly fixing the smear eliminating a prefixing step.

#### **PRINCIPLE**

Blood smears are prepared and fixed (using the Fixative). The cellular components when stained turn blue (basophilic) or orange granules (acidophilic). The color intensity can be varied by adjusting the staining time.

#### REAGENTS

Leishman's Stain Methylene blue.

Eosin

Buffer Phosphate Buffer

#### **PRECAUTIONS**

This product is for in Vitro diagnostics use and should be used by properly trained individuals. Precautions should be taken against the dangers of microbiological hazards by properly sterilizing specimens, containers and media after use. Directions should be read and followed carefully.

# **STORAGE**

Store product in its original container at room temperature until used. Keep container tightly closed during storage.

#### PRODUCT DETERIORATION

This product should not be used if:

- 1. The color has changed.
- 2. The expiration date has passed.
- 3. There are other signs of deterioration.

### MATERIALS REQUIRED BUT NOT SUPPLIED

- 1. Fixative (Methanol).
- 2. Microscope slides.
- 3. Microscope with oil immersion lens.
- 4. Immersion oil.
- 5. Fresh blood smears or other quality control slides.

### SPECIMEN COLLECTION AND PREPARTION

Blood Specimens: For optimal stain results, make smears from blood without anticoagulant, such as that obtained from a finger prick or ear lobe puncture. Blood obtained by vein puncture can be used. However, it is preferable to use the blood remaining in the needle because it is anticoagulant free. Fresh blood smears provide optimum results.

Thin Smears: The thin smear is prepared in the same manner as for a differential leukocyte count. Place one drop of blood near one end of a glass microscope slide. Hold a second spreader slide at a 40-45  $^{\rm o}$  angle, draw into the drop of blood, and allow it to spread to the width of the slide. Rapidly and smoothly push the spreader slide to the other end of the slide, pulling the blood behind it. A well-prepared smear is thick at one end and thin at the other.

#### **REAGENT PREPARATION: Working Reagent Preparation**

- 1. Buffer Reagent 1 ml + Distilled water 1 ml
- 2. Lesihman's stain ready-to-use.

#### **PROCEDURE**

- 1. Spray Fixative on Slides. Drain excess. Dry in Air.
- 2. Pour Leishman's stain on fixed slide. Keep it for 60 sec.
- 3. Add 1 ml of diluted buffer.
- 4. Wash with Distilled water after 5-6 minutes.
- 5. Allow the smear to air dry.

Examine under oil immersion microscope.

#### RESULTS AND INTERPRETATION

Refer to appropriate references for appearance of blood and tissue parasites and/or differential characteristics of Giemsa stained blood and tissue smears. After staining as specified under directions cells are observed under microscope.

#### RESULTS

Neutrophils Purple Granules Eosinophils Large Orange Granules Lymphocytes Dark Blue Nuclei Monocytes Kidney Shaped Smoky Blue Large Blue Granules Basophils

**Cellular Components** 

Pinksh Red RBC

Malaria Parasite Varying shades of Blue **Platelets** Pale or Dark Blue

## QUALITY CONTROL

All lot numbers of Leishman's Stain Kit have been tested and found to be acceptable. The patient smear can serve as quality control to verify the efficacy of the staining reagents. Parasites can be expected to stain correctly. In addition, a smear made from a patient blood specimen (previously identified as positive) with at least one parasite per oil immersion field may also be included to verify differential staining characteristics and compare with specimen stain results. If aberrant quality control results are noted, patient results should not be reported.

# **BIBLIOGRAPHY**

- Biological Stain 9th Edition The Williams & Wikins Co. Baltimore MD1977P.424.
- 2. Mac.Neal.J.A.M.Med.Assoc.78,1112(1922).

 $|\mathbf{i}|$ IVD SYMBOLS: Read Instruction for use In Vitro Diagnostic Use Only Storage Temperature Manufactured by Expiry Date ANAMOL LABORATORIES PVT. LTD.

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