CYTOCHROME ™ STAIN

(Modified - Leishman's)

BIO LAB DIAGNOSTICS
ISO 9001:2015
ISO 13485:2016
CE

PRINCIPLE

Combination of Xanthine and thiazine dyes explained by Romanowsky are known to be selectively staining acidophilic and basophilic cellular elements. BIOLAB'S Highly purified Polychromated Methylene Blue and Eosin stains demonstrate good differentiation of blood cells and hemoparasites.

REAGENT COMPOSITION

1. CYTOCHROM STAIN	**500 mL**
Methylene blue	4.3 mMol/L
Eosin	4.3 mMol/L

Contains polychromating materials, preservatives and stabilizers.

2. STOCK BUFFER 2X	**500 mL**
Phosphate	0.2 Mol/L

Contains preservatives and stabilizers.

Working Reagent Preparation

Dilute Stock Buffer 1 to 2 with distilled water.

Stock Buffer	1 mL
Distilled water	1 mL

Reagent 1 is ready to use.

STORAGE AND STABILITY

When stored at 25-35°C and protected from light, the reagents are stable until the expiry date stated on the label.

SAMPLES

Direct peripheral smear by finger prick is preferred. Whole blood sample may be collected in K_3 EDTA sample collection tube (Purple cap). (All samples should be handled as potential infective agents as no laboratory methods make conclusive finding for its safety. Therefore, adequate protective laboratory measures should be taken while handling such materials).

THIN SMEAR

A drop of blood can be spread with a fine edge spreader. Dry the smear at air and it is preferable to stain all smears as early as possible.

THICK SMEARS

In mass or conclusive screening of hemoparasites it is preferable to prepare thick smears by collecting 1-2 drop of blood on a clean glass slide and spread it to the shape of a coin. Dry at air.

LIMITATIONS

Over staining of Stain cause darker RBC resulting in poor staining of MP. Cytochrom stain Kit is only a microscopy stain that stains cellular elements and hemoparasites. Sample collected during relapsed cycle of plasmodium infection may not exhibit any parasite in the slide. For a conclusive finding it is necessary to screen sample collected from patients at different times.

Revised 7.4.2014 KI

PROCEDURE

A. FOR THIN SMEARS

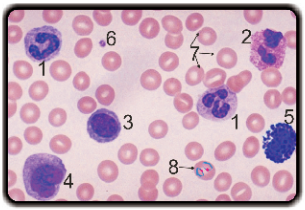
Arrange air dried smears in a slide rack keeping the smear side up and cover the smear with 0.5 mL (8-10 Drops) **CYTOCHROM™** stain. Wait for 60-80 seconds and add double the quantity (1 mL) of working buffer. Wash carefully after 6 minutes. Clean the bottom part of the slide with cotton wool. Air dry the smear and see under 100 x objective of a microscope.

B. FOR THICK SMEARS WITH DEHAEMOGLOBINIZING

1. Arrange air dried unfixed thick smears in a slide rack keeping the smear side up.
2. Dilute one volume of **CYTOCHROM™** stain with two volume of Buffer, mix well and cover the smear with diluted stain. Wait for 6 minutes and carefully remove stain by briskly flushing with plenty of water. Dry at air and see under oil immersion objective.

RESULTS

Fully comparable with Romanowsky Hematology Stain results. (100 x)



1. Neutrophils :(Segmented polymorph with purple granules). **2. Eosinophils :** (Polymorph with large orange granules). **3. Lymphocytes** : (Dark blue nuclei with clear blue cytoplasm). **4. Monocytes :** (Kidney shaped nucleus with smoky blue cytoplasm). **5. Basophils** : Polymorph with large blue granules. **6. Platelet** : (Small pale to dark bluish cells). **7. RBCs :** (Pinkish red). **8. Malaria Parasites** (P. Falciparum) : Pale bluish cytoplasm and Red Schuffner's Dot (Trophozoit).

B. THICK SMEAR

1. Label all smears with appropriate patient identification marks and arrange in a slide holder.
2. Dehaemoglobinize by dipping in a jar containing distilled water for 1 minute. Carefully remove from water.

Note : Unfixed thick smears may run out from the slides therefore, all steps of Dehemoglobinization and washing must be without much agitation.

3. Proceed to Bulk staining procedure of thin smear step No. 2.

BULK STAINING PROCEDURE

Using staining tanks number of slides can be stained with relatively less time.

A. THIN SMEAR

1. Label all smears with appropriate patient identification marks. Arrange in a slide holder. Fix thin smear by immersing in a tank filled with methanol or spraying with Easy Fix™ Fixative.

2. In a staining tank dilute one volume of **CYTOCHROM™** stain with two volume of Working Buffer, mix well. Place the slides in the stain for 6 minutes. Care to be taken to immerse the smear in stain completely. Carefully remove the slides and briskly flush the stain with plenty of tap water. Dry the smears at room temperature or in an oven thermostatically controlled to 50-60°C. See under oil immersion objective.

WARNING

Flammable solution. Keep away from heat and fire. This reagent system is for in vitro use Only. This reagent system is containing preservatives and components that have not established for safety if contacted on broken skin or eye or taken orally. In case of such incidents wash off with plenty of water, or consult a physician.

QUALITY CONTROL

The performance of stain must be periodically checked by known positive and known negative samples. The accuracy of reporting Malaria parasite or cells is subject to the professional experience of each person as well as the use of a good optical system that could make clear magnification of the smear.

BIBLIOGRAPHY

1. Mac. Neal, J.A.M.Med. Assoc. 78, 1112 (1922).
2. Biological Stain 9th Ed. The Williams & Wilkins Co. Balti more, MD 1977 P. 424.
3. J.Med Res. 7, 138 (1902).
4. "Histopathological technic and pract histochem. The Blackston Co., New York, N.Y. 1954.

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