

**Category:** Malaria OPD

**Title:** Standard Operating Procedures for Staining of Peripheral Smear for diagnosis of Malaria.

**SOP No.:** 03/04

**Effective Date:** 01<sup>st</sup> January 2025

**Review date** 31<sup>st</sup> December 2025

Department of Clinical Pharmacology, 1st Floor, New MS Building,  
Seth GS Medical College & KEM Hospital, Parel, Mumbai 400012

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## Table of Contents

No.	Contents	Page No.
1	Purpose	2
2	Scope	2
3	Responsibility	2
4	Detailed Instructions	3
5	Appendix	4
6	References	4

### 1. Purpose

To outline procedure for staining of thick and thin peripheral blood smear for diagnosis of malaria of patients referred from Medicine and General Out Patient Department (OPD).

### 2. Scope

This SOP is limited for staining of thick and thin peripheral blood smear for diagnosis of malaria.

### 3. Responsibility

Laboratory Technician

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#### **4. Detailed instructions**

- **Staining of thick peripheral smear for diagnosis of malaria.**
- Immerse slide in solution II of JaswantSingh and Bhattacharji stain(JSB) for 10 seconds.
- Wash for 2 seconds in a jar containing tap water.
- Stain with solution I of JSB for 1 second.
- Wash for 2 seconds in a jar containing tap water.
- Immerse in solution I again for 10 seconds.
- Wash for 2 seconds in a jar containing tap water till the smear gives a pink background.
- Dry under heat for 10 minutes and examine under microscope using oil immersion lens.
- **Staining of thin peripheral smear for diagnosis of malaria.**
- Fix the thin smear in methyl alcohol for a second or two.
- Immerse slide in solution I of JSB stain for 10 seconds.
- Wash for 2 seconds in a jar containing tap water
- Stain with solution II of JSB stain for 1 second.
- Wash for 2 seconds in a jar containing tap water.
- Immerse in solution I of JSB stain again for 10 seconds.
- Wash for 2 seconds in a jar containing tap water.
- Dry under heat for 10 minutes and examine under microscope using oil immersion lens.

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## **5. Appendix**

### **J. S. B. Stain:**

Jaswant Singh and Bhattacharji (1944) devised a rapid Romanowsky's method of staining malarial parasites by a water soluble stain, which consists of two solutions.

Solution I is made with the following ingredients: methylene blue 0.5 g, potassium dichromate 0.5 g, sulphuric acid (1% by volume) 3ml, potassium hydroxide (1%) 10 ml and water 500 ml.

Solution II is prepared by dissolving 1 g of eosin (water soluble) in 500 ml of tap water.

## **6. Reference:**

K.D. Chatterjee *Parasitology (Protozoology and Helminthology) in Relation to Clinical Medicine* 215.