



PRODUCT SPECIFICATION SHEET

LEISHMAN STAIN

Use

Leishman stain and Wrights stain are most commonly used stains in haematology laboratory, especially for visualizing malarial parasites. They can be also used for routine blood smear staining procedures.

Principle

The polychromic staining solutions (Wrights, Leishmans) contain methylene blue and eosin. These basic and acidic dyes induce multiple colours when applied to cells. Methanol acts as fixative and also as a solvent. The fixative does not allow any further change in the cells and makes them adhere to the glass slide. The basic component of white cells (i.e. cytoplasm) is stained by acidic dye and they are described as eosinophilic or acidophilic. The acidic components (e.g. nucleus with nucleic acid) take blue to purple shades by the basic dye and they are called basophilic. The neutral components of the cell are stained by both the dyes.

Storage And Stability

1. Store the bottle in dry, cool and dark place.
2. The shelf life of reagents is as per the expiry date mentioned on the reagent bottle labels.

Procedure

Fixing and staining procedure for blood smears

1. Stain provided contains methanol so it does not require separate fixing.
2. If staining is to be done later, smear can be fixed using methanol for 2-3 min.
3. Cover the slide with **Leishman Stain for 5 min.** This also allows fixation of the smear.
4. Add on the slide **Buffered Water (pH 7.0)** of about double the volume of the stain, allow staining to continue for **5-7 min.**, a metallic sheen should be formed on top of this mixture. Staining time may have to be adjusted according to the reaction of the stain. Reduce the time if overstained, increase the time if poorly stained.
5. Wash the stain off in the stream of buffered water until it has acquired a pinkish tinge. Do not tip off the stain, this will leave a deposit of stain on the blood film and will hamper microscopic examination.

Drying of blood film

1. Shake off the buffered water adhering to the slide and set the slide in an upright position in a drying rack. Keep the smeared surface of the slide facing down. This will avoid picking up dust.
2. After complete drying, observe the stained slide under oil immersion lens.





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Composition of buffered water:

| Ingredients | Formula / Litre |
|--|-----------------|
| Di sodium hydrogen phosphate. Di hydrate ($\text{Na}_2\text{HPO}_4 \cdot 2\text{H}_2\text{O}$) | 3.76 gms |
| Potassium dihydrogen phosphate. Anhydrous (KH_2PO_4) | 2.10 gms |
| D/W (q.s.) | 1000 ml |
| To achieve the required pH addition of Na_2HPO_4 can be done. | |

Result

Morphology and staining properties :

Granulocytes: These are cells with granulated cytoplasm which stain faint pink. These include Neutrophils, Eosinophils and Basophils.

Neutrophils: Pale pink cytoplasm with fine mauve coloured granules, include band and segmented forms (normally 3-4 lobed) of nucleus.

Eosinophils: Cytoplasm stains faint pink, contains large red orange granules and bilobed nucleus.

Basophils: Cytoplasmic granules appear large, dark blue black which fill the cell and obscure nucleus

Lymphocytes: Large size lymphocytes have clear blue cytoplasm on the margins of the nucleus. In smaller lymphocytes, dark violet coloured nucleus fills the entire cell and has a rim of clear cytoplasm

Packaging

Product Name : **LEISHMAN STAIN**

Product Code : **SI021**

Available Pack sizes : **500ml/125ml**

Further Information

For further information please contact your local MICROMASTER Representative.



MICROMASTER LABORATORIES PRIVATE LIMITED

SI021Pss, QAD/FR/024,Rev.00/01.01.2018.

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| Prepared By | Checked By | Approved By |
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| Microbiologist | Head Quality Control | Head Quality Assurance |

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