R03087-76

Microscopy

Leishman Stain

IVD

In Vitro Diagnostic Medical Device

Intended Use

Leishman Stain is used for staining blood smears to differentiate blood corpuscles, malarial parasites, trypanosomers, etc. It can be used as an alternative to Giemsa and for use in staining bone marrow sections. It serves the purpose of investigating sample material of human origin.

Principle

Leishman stain is a Romanowsky neutral dye stain based on the combination of methylene blue azure as the basic dye component and eosin as the acidic dye component. Romanowsky modified Erlich's earlier finding of a neutral dye which offered the ability to identify acidophilic, basophilic and neutrophilic granules of leukocytes. The staining of the nuclei is due to the molecular interaction of the Eosin Y dye and a complex of Azur B with DNA. Both dyes assemble to form an Eosin Y-Azur B-DNA complex and the intensity of the resulting stain depends on the content of the Azur B and the ratio of the Azur B to Eosin Y. Furthermore , the resulting stain can vary depending on the influence of fixation, staining times, pH value of the solutions or buffer solutions.

Sample material

Fresh native whole blood or bone marrow smears, clinical cytological material such as urine sediment, sputum, smears from fine needle aspirate biopsies (FNAB), rinses and imprints.

Reagent

Cat. No. R03087-76 Leishman Stain
1 L

Also required:

Cat. No. 65044A -85 Hemacolor® Solution I 4L

Cat. No 1217 Buffer, Phosphate pH 6.4 1 L, 4L
Or
Cat. No. 1218 Buffer, Phosphate pH 6.8 1 L, 4L
Or
Cat. No. 1219 Buffer, Phosphate pH 7.0 1 L, 4L
Cat. No. 6442 Water, Deinonized Distilled, ASTM Type II 4 L, 20 L

Sample preparation

The sampling must be performed by qualified personnel. All samples must be clearly labeled. Suitable instruments must be used for collecting and preparing samples. Follow the manufacturer's instructions for application/use.

Reagent Preparation

Please note Leishman Stain is provided as a concentrate.

- Buffer Diluent: Place 30 ml of phosphate buffer pH 6.4, 6.8 or 7.0 into a vessel. Add 100 ml of Deionized Distilled Water, ASTM Type II.
- Stain-Buffer mixture: Combine 20 mL of Leishman Stain concentrate with 100 mL of Buffer Diluent (directions for preparation above). Mixture is stable for 3 hours from time of preparation.

Staining Procedure

Procedure: Flood Slide Method

Equipment Needed: Staining rack, forceps, Pasteur pipets.

1. Place slide on staining rack and flood with methanol (fixative) volume 1-2 mL for 1 minute. Drain excess methanol.
2. Flood slides with 20 drops Leishman Stain Solution and allow to stand for 1 minute. Do not rinse.
3. Apply 30 drops of Buffer Diluent. Mix gently by rocking the slide. Allow to stand for 3 minutes. A greenish metallic sheen should appear on the surface of the mixture.
4. Drain stain-buffer mixture and rinse slide with 5-10 ml deionized water for 10-15 seconds.
5. Air dry prior to examination.
6. Examine microscopically (see Results).

Procedure: Dip Slide Method

Equipment Needed: Three Coplin jars, forceps

1. Place slides in methanol (fixative) for 30 seconds.
2. Place slides in Leishman Stain for 3 minutes
3. Place slides in Stain-Buffer mixture for 6 minutes. See Reagent preparation section – "Stain Buffer Mixture".
4. Remove slide from Stain-Buffer mixture and rinse with 10-15 ml of deionized water.
5. Allow slides to dry.
6. Examine microscopically (see Results).
   Note: For best results, Coplin jars should be covered when not in use.

Procedure: Midas®III- Plus Automated Slide Stainer

Stain/Buffer preparation

- Place 50 ml of Leishman Stain into vessel.
- Add 75 ml of Phosphate Buffer pH 6.8 (or pH 6.4 or pH 7.0)
- Add 175 ml Deionized Distilled Water, ASTM Type II.
- Mix and let stand 10 minutes before use.

All stations default to dipping activated. This is the suggested staining protocol for the use of Harleco® Stains.

For Peripheral Blood Smears

Solution	Station	Time
Fixative	2	30 seconds
Leishman Stain	3	3 minutes
Stain-Buffer Mixture	4	6 minutes
Rinse	5	1.5 minutes
Dry	6	3 minutes

For Bone Marrow Aspirates

Solution	Station	Time
Fixative	2	30 seconds
Leishman Stain	3	10 minutes
Stain-Buffer Mixture	4	20 minutes
Rinse	5	1.5 minutes
Dry	6	3 minutes

Results

Cell Type	Nuclei	Granules	Cytoplasm
Erythrocytes		Yellowish Red	Yellowish Red
Polymorpho-nuclear Neutrophilic Leucocytes	Dark Blue to Purple	Reddish Lilac	Pale Pink
Basophilic Leucocytes	Purple or Dark Blue	Dark Purple to Black	
Eosinophilic Leucocytes	Blue to Purple	Red to Orange Red	Blue
Lymphocytes	Dark Purple		Sky Blue
Platelets		Violet to Purple	

Application Notes:

The microscope used should meet the requirements of a medical diagnostic laboratory.

The freshly prepared staining solutions should be filtered before use.

For more basophilic staining use Phosphate Buffer pH 6.8 or pH 7.0.

Stain and or stain/buffer times may need to be adjusted if a different pH is used.

Midas®III Plus Automated Slide Stainer: Maximum rinse water flow rate should not exceed 2,000 ml/minute on the Midas® III – Plus Automated Slide Stainer.

For best staining results use Deionized or Distilled Water.

Technical Notes for Manual Staining Procedures

1. Experimentation and adjustment to staining times may be required to obtain optimal results and cell differentiation.
2. Best results are obtained when the following are observed:
   a. Slides are clean and free of grease and debris.
   b. Methanol fixative is acetone free.
   c. Blood smears are freshly prepared.
   d. Blood smears are prepared as a very thin layer on slide.
3. Staining intensity can be increased by extending the timing in steps 2 and 3. However, this will only have moderate effects on the intensity.
4. pH 6.4 buffer will produce acidophilic results. RBC's will be pink in color (step 3).
5. pH 6.8 buffer will produce neutrophilic results. RBC's will appear yellowish-pink to tan (step 3).
6. Distilled water will product basophilic results. RBC's will appear gray to blue-gray (step 3).
7. For best results read the "feathered" end of the stained slide.

Diagnostics

Diagnoses are to be made only by authorized and trained personnel. Valid nomenclature must be used.
Further tests must be selected and implemented according to recognized methods. Suitable controls should be conducted with each application to avoid an incorrect result.

Storage

15 deg C to 25 deg C

Shelf-life

After first opening of the bottle of Leishman stain, the contents can be used up to the stated expiry date when stored at +15 deg C to +25 deg C. The bottle must be tightly closed at all times.

Additional instructions

For professional use only.
The application must be carried out by qualified personnel only.

National guidelines for work safety and quality assurance must be followed.

If necessary use a standard centrifuge suitable for medical diagnostic laboratory.

Protection against infection

Effective measures must be taken to protect against infection in line with laboratory guidelines.

Instructions for disposal

The package must be disposed of in accordance with the current disposal guidelines. Used solutions and solutions that are past their shelf-life must be disposed of as special waste in accordance with local guidelines.

Auxiliary reagents

Cat. No. 64969	Harleco® Krystalon™ Mounting Medium	50 mL, 500 mL
Cat. No 1217	Buffer, Phosphate pH 6.4	1 L, 4L
Cat. No. 1218	Buffer, Phosphate pH 6.8	1 L, 4L
Cat. No. 1219	Buffer, Phosphate pH 7.0	1 L, 4L
Cat. No. 6442	Deinonized Distilled, ASTM Type II	4 L, 20 L
Cat. No. 65044A -85	Hemacolor® Solution I	4 L

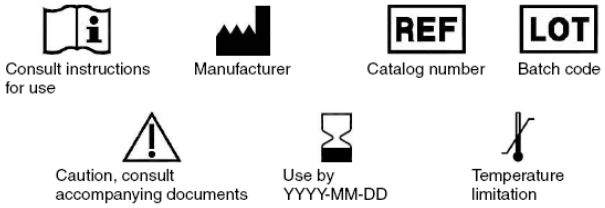
Hazard classification

Please observe the hazard classification printed on the label and the information given in the safety data sheet. The safety data sheet is available on the website and on request.

Literature

1. Loffler, H., Rasteter J., Haferlach, T., Atlas der Kilinischen Hamatologie, 2004, Springer-Vertag Berlin Heidelberg
2. Routine Cytological Staining techniques: Theoretical Background and Practice, Mathilde E. Boon, Johanna S. Drijver, 1986, Elsevier Science Publishing Company

3. Conn's Biological Stains: A Handbook of Dyes, Stains and Fluorochromes for use in Biology and Medicine, 10th Edition, (ed. Horobin, R.W. and Kiernan, J. A.). Blos, 2002



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