Leishman's Stain Solution

Intended Use:
Leishman's Stain is recommended for use in microscopy for blood staining.

Composition**

<table>
<thead>
<tr>
<th>Ingredients</th>
<th></th>
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</thead>
<tbody>
<tr>
<td>Leishman stain</td>
<td>0.150 gm</td>
</tr>
<tr>
<td>Methanol, absolute</td>
<td>100.000 ml</td>
</tr>
</tbody>
</table>

**Formula adjusted, standardized to suit performance parameters

Directions
1) Freshly prepare and rapidly air dry blood film.
2) Cover the film with Leishman's Stain (S018S) and allow to act for 1 minute. Methanol in the stain fixes the preparation.
3) Add double the volume of distilled water to the slide and mix.
4) Allow the diluted stain to act for 10-12 minutes.
5) Wash the film with distilled water or phosphate buffer of pH 7.0, drain and dry in air and examine.

Principle And Interpretation
Leishman 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.

Type of specimen
Clinical samples: Blood sample

Specimen Collection and Handling
For clinical samples follow appropriate techniques for handling specimens as per established guidelines(1,2).
After use, contaminated materials must be sterilized by autoclaving before discarding.

Warning and Precautions
In Vitro diagnostic use only. Read the label before opening the container. Wear protective gloves/protective clothing/eye protection/face protection. Follow good microbiological lab practices while handling specimens and culture. Standard precautions as per established guidelines should be followed while handling clinical specimens. Safety guidelines may be referred in individual safety data sheets.

Limitations
1) To preserve morphology of cells, films must be fixed without delay and the films should never be left unfixed for more than a few hours.
2) Methanol used as fixative should be completely water free. As little as 1% water may affect the appearance of the films and a higher water content causes gross changes.
3) The red cells will also be affected by traces of detergent on inadequately washed slides.
4) Sometimes when thick films are stained they become overlaid by a residue of stain or spoil by the envelopes of the lysed red cells.

Performance and Evaluation
Performance of the stain is expected when used as per the direction on the label within the expiry period when stored at recommended temperature.

Quality Control
Appearance
Blue coloured solution.
Clarity
Clear without any particles
Microscopic Examination
Blood staining is carried out and staining characteristic is observed under microscope by using oil immersion lens.

Results

<table>
<thead>
<tr>
<th>Component</th>
<th>Stain Color</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nuclear &amp; Basophilic cytoplasmic components</td>
<td>Blue</td>
</tr>
<tr>
<td>Neutrophilic Granules</td>
<td>Lilac</td>
</tr>
<tr>
<td>Eosinophilic Granules</td>
<td>Orange</td>
</tr>
<tr>
<td>Nucleoli</td>
<td>Blue-violet</td>
</tr>
<tr>
<td>Red cells</td>
<td>Pink</td>
</tr>
<tr>
<td>Mature Monocytes</td>
<td>Grey blue kidney shaped</td>
</tr>
<tr>
<td>Lymphocytes</td>
<td>Blue with purple nucleus</td>
</tr>
</tbody>
</table>

Storage and Shelf Life
Store between 10 - 30°C in tightly closed container and away from bright light. Use before expiry date on label. On opening, product should be properly stored in dry ventilated area protected from extremes of temperature and sources of ignition. Seal the container tightly after use.

Disposal
User must ensure safe disposal by autoclaving and/or incineration of used or unusable preparations of this product. Follow established laboratory procedures in disposing of infectious materials and material that comes into contact with clinical sample must be decontaminated and disposed of in accordance with current laboratory techniques (3,4).

Reference
5. Staining Procedures;Fourth Edition ;Williams& Wilkins;Baltimore

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