Hematoxylin (Harris)

S034

Intended use

Haematoxylin (Harris) is Recommended as nuclear stain for Immunohistochemical and cytochemical staining.

Composition**

Ingredients

Haematoxylin	5.0 gm
Ammonium or potassium alum	100.0 gm
Mercuric oxide	2.50 gm
Alcohol 95%	50.0 ml
Distilled water	1,000.0 ml

**Formula adjusted, standardized to suit performance parameters

Directions

1. Flame the slide and place in xylene for 3-4 minutes. Repeat xylene treatment with agitation.
2. Dip in 100% absolute alcohol for 30-60 seconds. Next, dip in 90%, 80% and in 70% absolute alcohol. Wash in tap water and rinse in distilled water.
3. Stain in Haematoxylin (S034) for 3-5 minutes. Wash in tap water.
4. Dip in 0.5% (v/v) hydrochloric acid.
5. Rinse in tap water for 30-60 seconds.
6. Dip in dilute ammonia water till section appears blue.
7. Wash in tap water and then rinse in 95% alcohol.
8. Agitate in eosin solution for 10-60 seconds. Drain stain solution.
9. Dip slide in 70% alcohol for 30-60 seconds.
10. Place in 95% alcohol for 30-60 seconds.
11. Place in absolute alcohol (2 changes, 30-60 seconds each).
12. Place the slide twice in xylene for 30-60 seconds.
13. Drain excess xylene and mount on DPX or Canada balsam with a cover slip.

The first 2 steps of the procedure are collectively referred to in all staining procedures as “deparaffinize.” Steps 3-8 are referred to as “staining”. The last 5 steps are referred to in all staining methods as “dehydrate, clear, and mount”.

Principle And Interpretation

Hematoxylin is extracted from logwood with hot water, and then precipitated out from the aqueous solution using urea. Hematoxylin itself is not a stain. Its major oxidation product is Hematein that is a natural dye responsible for colour properties. Hematein can be produced by natural oxidation on exposure to light and air. Ehrlichs and Delafields hematoxylin solutions are examples of naturally ripened hematoxylins. Chemical oxidation uses sodium iodate (e.g., Mayer's hematoxylin) or mercuric oxide (e.g., Harris hematoxylin). Hematein is anionic, having poor affinity for tissue, and is inadequate as a nuclear stain without the presence of a mordant.

The mordants used are salts of aluminum, iron, tungsten. Harris hematoxylin is chemically ripened with mercuric oxide alum hematoxylin. As mercuric oxide is highly toxic and have detrimental effect on automated staining machines. It is general purpose hematoxylin and gives particularly clear nuclear staining and used as a progressive stain in diagnostic exfoliative cytology. Haematoxylin and eosin are the principle stains used for the demonstration of nucleus and the cytoplasmic inclusions. Here, acid reacting components of the cell combine with alkaline dyes and the alkaline area react with acid dyes.

Type of specimen

Clinical samples: Blood sample

Specimen Collection and Handling

For clinical samples follow appropriate techniques for handling specimens as per established guidelines.
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 medium is expected when used as per the direction on the label within the expiry period when stored at recommended temperature

Quality Control

- **Appearance :** Red to purple coloured solution.
- **Clarity :** Clear without any particles.
- **Microscopic Examination :** Staining is carried out staining characteristics is observed under micro scope by using oil immersion lense.
- **Results :** Nuclei: Blue
  Cytoplasm: Pink

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

Reference

1. Wehr H. M. and Frank J. H., 2004, Standard Methods for the Microbiological Examination of Dairy Products, 17th Ed., APHA Inc., Washington, D.C.

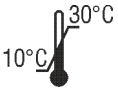
2. Isenberg, H.D. Clinical Microbiology Procedures Handbook. 2nd Edition.

3. Jorgensen, J.H., Pfaller, M.A., Carroll, K.C., Funke, G., Landry, M.L., Richter, S.S and Warnock., D.W. (2015) Manual of Clinical Microbiology, 11th Edition. Vol. 1.

4. Shanhooltzer, C.J., P. Schaper, and L.R. Peterson. 1982. Concentrated Gram stain smear prepared with a cytospin centrifuge. J. clin. Microbiol.16:1052-1056

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

6. Newell J.E. and Dukee., 1961, Workshop on urine analysis and renal function studies, the routine examination of urine in laboratory, Chicago, American Society of Clinical Pathologist.



Storage temperature

Do not use if package is damaged

In vitro diagnostic medical device

CE Marking

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