Instruction manual
Harris' hematoxylin solution
(Papanicolaous solution 1a)

"Harris' hematoxylin solution (Papanicolaous solution 1a)" is used for IVD cytological cancer and cycle diagnostics in human medicine cell diagnostics and serves for the cytological examination of human samples. It is a ready-to-use dye solution that makes cytological target structures in human-gynaecological and clinical-cytological specimen evaluable for diagnostics (by means of fixation, staining, counter-staining, mounting).

Papanicolaou staining is the most commonly used staining method for cytological material. It enables a comprehensive statement on dignity, hormone status and vaginal flora. Furthermore, it can be used for staining in cancer diagnostics.

Staining mechanism:
The first step of the H&E-staining mechanism is a coulomb interaction of the positively charged nuclear stain (hematoxylin) with the negatively charged phosphate groups of the nucleic acids in the cell nucleus. The nuclei appear in blue to dark violet. The second step is the cytoplasm staining with an orange dye solution which particularly visualizes mature and horny cells. The target structures are stained in different intensities of orange.

A differentiation is made between the progressive hematoxylin staining, which entails staining until the endpoint and subsequent blueing and fixation in tap water (which will be described below), and the regressive method. In the latter, overstaining with hematoxylin occurs and the excessive dye is removed in acidic differentiating steps. Here too, blueing and fixation of the staining takes place with tap water. With the regressive staining, the nucleus structures appear more differentiated and are more clearly visible.
In the third staining step, a so-called polychromatic solution is used, which is a mixture of eosin Y, light green SF and Bismarck brown. The polychromatic solution represents the differentiation of the squamous epithelium.

Tissue used:
Slices of formalin-fixed, paraffin-embedded tissue (3-4 µm thick paraffin slices) or frozen sections serve as the source material as well as clinical material from cytology, e.g. urine sediment, sputum, smears of fine-needle aspiration cytology (FNAC), flushing liquids, imprints, effusions, may be used.

Sample preparation:
Sample collection must be performed by qualified personnel.

All samples must be handled according to the state of the art. All samples must be labelled unambiguously. Suitable instruments must be used for sample collection and preparation; the manufacturer's instructions for their application/use must be followed.

Slices must be dewaxed and rehydrated in typical manner.

Reagent preparation:
The "Harris' hematoxylin solution (Papanicolaous solution 1a)" used for staining is ready-to-use; diluting of the solution is not necessary and would minimize the staining result and its shelf-life. It is recommended to filter the solution prior to use.

Performing the progressive staining
Staining in the staining cuvette

The slides must be immersed in the solutions and be shortly moved; simply putting them in will render insufficient staining results. The slides should be well drained after each staining step to avoid unnecessary carryover of solutions. To achieve an optimal staining result, adhere to the times indicated.

Slide with fixated smear	
Ethanol 96%	10 sec.
Ethanol 80%	10 sec.
Ethanol 70%	10 sec.
Ethanol 50%	10 sec.
Distilled water	20 sec.
"Harris' hematoxylin solution (Papanicolaous solution 1a)" art. no.: 6.00.05.0002.07	3 min.
Running tap water	3 min.
Ethanol 70%	30 sec.
Ethanol 80%	30 sec.
Ethanol 96%	30 sec.
Orange G-solution (Papanicolaous solution 2a)	3 min.
Ethanol 96%	30 sec.
Ethanol 96%	30 sec.
Polychromatic solution EA 31 (Papanicolaous solution 3a)	3 min.
Ethanol 96%	30 sec.
Ethanol 96%	30 sec.
Ethanol 100%	5 min.
Mixture of: Ethanol 100% + Xylene	2 min.
Xylene	5 min.
Xylene	5 min.
Mounting with EUKITT® (art. no.: 6.00.01.0001), EUKITT® neo (art. no.: 6.00.01.0003) or EUKITT® UV (art. no.: 6.00.01.0005). With EUKITT® UV or EUKITT® neo, the last three xylene steps may be omitted.	

After dehydration (increasing alcohol concentration), histological specimens may clarify with xylene and be mounted and stored with non-

aqueous mounting medium (e.g. EUKITT®, EUKITT® neo or EUKITT® UV) and coverslip.
For analysis of stained specimens with microscopic enlargement >40x, the use of immersion oil is recommended.

Performing the regressive staining
Staining in the staining cuvette
The slides must be immersed in the solutions and be shortly moved; simply putting them in will render insufficient staining results. The slides should be well drained after each staining step to avoid unnecessary carryover of solutions. To achieve an optimal staining result, adhere to the times indicated.

Slide with fixated smear	
Ethanol 96%	10 sec.
Ethanol 80%	10 sec.
Ethanol 70%	10 sec.
Ethanol 50%	10 sec.
Distilled water	10 sec.
"Harris' hematoxylin solution (Papanicolaous solution 1a)" art. no.: 6.00.05.0002.07	6 min.
Distilled water	10 sec.
Hydrochloric acid 0.1% aqueous	10 sec.
Distilled water	10 sec.
Sodium hydrogen carbonate solution 1.5% aqueous	1 min.
Running tap water	3 min.
Ethanol 70%	30 sec.
Ethanol 80%	30 sec.
Ethanol 96%	30 sec.
Orange G-solution (Papanicolaous solution 2a)	3 min.
Ethanol 96%	30 sec.
Ethanol 96%	30 sec.
Polychromatic solution EA 31 (Papanicolaous solution 3a)	3 min.
Ethanol 96%	30 sec.
Ethanol 96%	30 sec.
Ethanol 100%	5 min.
Mixture of: Ethanol 100% + Xylene	2 min.
Xylene	5 min.
Xylene	5 min.
Mounting with EUKITT® (art. no.: 6.00.01.0001), EUKITT® neo (art. no.: 6.00.01.0003) or EUKITT® UV (art. no.: 6.00.01.0005). With EUKITT® UV or EUKITT® neo, the last three xylene steps may be omitted.	

After dehydration (increasing alcohol concentration), histological specimens may clarify with xylene and be mounted and stored with non-aqueous mounting medium (e.g. EUKITT®, EUKITT® neo or EUKITT® UV) and coverslip.
For analysis of stained specimens with microscopic enlargement >40x, the use of immersion oil is recommended.

Evaluation:

Staining with	3a / EA 31
Cytoplasm -cyanophilic (basophilic) -eosinophilic (acidophilic) -horny	 blue-green to green pink pink-orange
Erythrocytes	red
Nuclei	blue to dark violet
microorganisms	grey-blue
trichomonads	grey-green

Technical information:
The microscope used should comply with the requirements of a medical-diagnostic lab. If histoprocessors or stainers are used, the hardware and software manufacturers' instructions are to be followed. Remove excessive immersion oil prior to archiving.

Diagnostics:
Only authorized and trained personnel may give diagnoses. Valid nomenclature is to be used. Follow-up tests are to be chosen and performed according to recognized methods.

Storage:
"Harris' hematoxylin solution (Papanicolaous solution 1a)" must be stored at +15 °C to +25 °C (+59 °F to +77 °F). Storage temperatures below +15 °C (+59 °F) may cause dye precipitation. In that case, the dye solutions should be put into a water bath at approximately 60 °C (140 °F) for 2-3 hours and be filtrated prior to use.


Warning:
Please read all information carefully before use.


Biohazard warning:
Use appropriate personal protective equipment when handling potentially infectious sample material.


Do not use if packaging is damaged:
If the packaging is damaged, this may lead to leakage of "Harris' hematoxylin solution (Papanicolaous solution 1a)". In general, be aware of the dangers of wetting and take appropriate safety measures to prevent this (e.g. wearing gloves).


Use by:
"Harris' hematoxylin solution (Papanicolaous solution 1a)" may be used until the stated expiry date.

After first opening, store bottle at +15 °C to +25 °C (+59 °F to +77 °F) and use until expiry date.

Always keep bottles properly closed.

Directions for Use:
"Harris' hematoxylin solution (Papanicolaous solution 1a)" is ready-to-use and may be applied without further preparation steps.

Follow the usage instructions:
"Harris' hematoxylin solution (Papanicolaous solution 1a)" should be used in accordance with the usage instructions provided by relevant system and/or reagent supplier, or according to your own tried-and-tested methods.

National guidelines for occupational safety and quality assurance are to be observed. Microscopes equipped according to the standard are to be used.

Warning:
For professional use only!

To avoid errors, application must be performed by trained personnel.

Disposal instructions:
Dispose of packaging according to existing disposal regulations. Used solutions and solutions with expired shelf-life must be disposed of as hazardous waste, the local disposal regulations are to be observed. REGULATION (EC) No 1272/2008 on classification, labelling and packaging of substances and mixtures, amending and repealing Directives 67/548/EEC and 1999/45/EC, and amending Regulation (EC) No 1907/2006 applies within the EU.

"Harris' hematoxylin solution (Papanicolaous solution 1a)":
Art. no.: 6.00.05.0001.07.04.01 1000 ml

Manufacturer:

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Germany

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http://www.eukitt.de

“Harris’ hematoxylin solution (Papanicolaous solution 1a)”
Art. no.: 6.00.05.0001.07.04.01

Hazard and precautionary statements:

Causes serious eye damage (H318). Wear protective gloves/protective clothing/eye protection/face protection (P280). IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing (P305 + P351 + P338). Immediately call a POISON CENTER or doctor/physician (P310).