

Histopathology Stains

500ml bottles

HS006: Harris' Hematoxylin

HS007: Orange G-6 solution

HS009: Polychromic solution EA-50



Papanicolaou's Stains

Intended use

The Papanicolaou's stains are intended for cellular staining in histocytology.

For *in vitro* diagnostic use only.

For professional use only.

Clinical significance

The Papanicolaou test is performed to diagnose cervical cancer and cancer in other organs, since it makes possible to detect changes in cells which may be cancer precursors.

Principle

After fixation, cells are treated with the Hematoxylin nuclear stain and counterstained with a mixture of cytoplasmic stains (Orange G-6, Eosin and Light green). The Hematoxylin nuclear stain is composed of oxidized hematoxylin and metallic ions (di- or trivalent) that bind anionic regions in the tissue such as DNA phosphate groups.

Cytoplasmic stains are acidic dyes that bind to basic cellular components such as the cytoplasm and other extracellular components, resulting in a differential staining of the various components. Eosin can penetrate tissue structures and react with basic protein groups. Orange G-6 is an acidic dye that penetrates dense structures even better than eosin, thereby staining highly keratinised cells orange to yellow. The polychromic EA stains combine the staining properties of Eosin with the ones of Light green, a less acidic dye, giving different shades to the final coloration of the cytoplasm.

Reagent composition

Harris' Hematoxylin (1 x 500 ml):

Hematoxylin, CI n°75 290	0.50%
Aluminium Sulphate	6%
Ethylene Glycol	15%

Orange G-6 solution (1 x 500 ml):

Orange G	0.25%
Ethanol	90%

Polychromic Solution EA-50 (1 x 500 ml):

Eosin	0.20%
Light green	0.05%
Ethanol	80%
Methanol	10%

Caution

Orange G-6 solution (HS007): Danger: H225 – Highly flammable liquid and vapour. Precautionary: P210 – Keep away from heat, hot surfaces, sparks, open flames and other ignition sources. No smoking. P233 – Keep container tightly closed. P280 – Wear protective gloves/protective clothing/eye protection/face protection. P303+P361+P353: IF ON SKIN (or hair): Take off immediately all contaminated clothing. Rinse skin with water/shower. P370+P378 – In case of fire: Use the means described in point 5 of the Safety Data Sheet. P403+P235 – Store in a well-ventilated place. Keep cool. P501 – Dispose of contents/container according to point 13 of the Safety Data Sheet.

Polychromic solution EA-50 (HS009): contains methanol. Danger: H225 – Highly flammable liquid and vapour. H302+H312+H332 – Harmful if swallowed, in contact with skin or if inhaled. H370 – Causes damage to organs. Precautionary: P210 – Keep away from heat, hot surfaces, sparks, open flames and other ignition sources. No smoking. P233 – Keep container tightly closed. P260 – Do not breathe dust/fumes/gas/mist/vapours/spray. P280 – Wear protective gloves/protective clothing/eye protection/face protection. P308+P311 – IF exposed or concerned: Call a POISON CENTER/doctor. P321 – Specific treatment. P370+P378 – In case of fire: Use the means described in point 5 of the Safety Data Sheet.

Preparation

All the reagents are ready for use.

Storage and stability

When stored at 15-30°C and protected from light, reagents will remain stable until the expiration date stated on the label. Containers must always be kept tightly closed.

A light precipitate may form for some reagents over time. This, nevertheless, does not affect their functionality.

Additional equipment

- Ethanol, different concentrations
- Xylene or Xylene substitute
- Mounting media
- Microscope

Samples

Cytological samples from different origin: gynaecological, sputum, liquid biopsies. Histological samples.

Handling of the samples must be carried out in accordance with the established protocols in each laboratory for the preparation of samples for staining with the Papanicolaou method.

Handle the samples carefully due their potentially infectious nature.

Test procedure

1. Smears that are fixed with a spray have to be submerged in water for 5 – 10 min. so that the carboxymethylcellulose (CMC) used on them gets well dissolved. Do not move the smears in order to avoid removal of the cell mass.
2. 1 min. immersion in Harris' Hematoxylin.
3. Rinse with water for 15 sec.
4. Rinse with Ethanol-HCl (0.25%) for 10 sec.
5. Rinse with water for 15 sec.
6. Rinse with water-ammonia (NH₃ 0.05%) for 5 sec.
7. Rinse with water for 15 sec.
8. Immersion for 10 sec. in Ethanol 96° (twice, using different trays).
9. Immersion for 3 min in Orange G-6 solution.
10. Rinse with Ethanol 96° for 15 sec (twice, using different trays).
11. Immersion for 3 min. in EA-50 solution.
12. Rinse with Ethanol 96° for 15 sec (twice, using different trays).
13. Immersion for 15 sec. in Absolute Ethanol (twice, using different trays).
14. Immersions for 15 sec. in Ethanol/Xylene (1:1).
15. Immersion for 15 sec. in Xylene.
16. Immersion for 2 min. in Xylene.
17. Mount immediately with mounting media.

Results

Nucleus:	blue-violet colour
Acidophilic cytoplasm:	reddish colour
Basophilic cytoplasm:	blue colour
Keratinized cytoplasm:	orange colour

Quality Control

The use of QC samples is recommended in order to assess the appropriate staining of the sample components. Each laboratory should establish its own QC scheme and corrective actions if the controls do not fulfil the established criteria.

We recommend to follow the QC practices defined by the CLSI.

Remarks

The above test procedure is stated as a guide. The Cypress Diagnostics stains can be adapted to the different variables of the methodologies used. The rinsing time with water after Hematoxylin immersion can vary with the staining time used: at longer staining times, longer rinsing times are required.

Clinical diagnosis should not be made on a single test result; it should integrate clinical and other laboratory data.

References

1. Marshall, P.N., Galbraith, W., Bacus, J.W., (1979). Anal. Quant. Cytology, 1, 169-178.
2. Baker, J.R., (1962), Quart. J. Micr. Sci., 103, 493-517.
3. Boon, M.E., Drijver, J.S., Routine Cytological staining techniques, 1st. ed (1986).
4. CLSI Guidelines and Standards, CLSI, Wayne, PA.

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