1.06888.0004

Microscopy

Papanicolaou’s solution 2a Orange G solution (OG6)

for cytology

For professional use only

IVD In Vitro Diagnostic Medical Device

CE

Intended purpose

This “Papanicolaou’s solution 2a Orange G solution (OG6) - for cytology” is used for human-medical cell diagnosis and serve the purpose of the cytological investigation of sample material of human origin. It is a ready-to-use staining solution that when used together with other in vitro diagnostic products from our portfolio makes cytological target structures evaluable for diagnostic purposes (by fixing, staining, counterstaining, mounting) in human gynecological and clinico-cytological specimen materials, for example cervical smears.
Unstained structures are relatively low in contrast and are extremely difficult to distinguish under the light microscope. The images created using the staining solutions help the authorized and qualified investigator to better define the form and structure in such cases. Further examinations may be necessary to reach a definitive diagnosis.

Principle

Most used staining procedure for cytological specimen is Papanicolaou’s technique and is intended for the the staining of exfoliative cells in cytological specimens.

In the first step, the cell nuclei are stained either progressively or regressively with a hematoxylin solution. Nuclei are stained blue to dark violet.

In the progressive hematoxylin staining method, staining is carried out to the endpoint, after which the slide is blued in tapwater.

With the regressive method the material is over-stained and the excess of staining solution is removed by acid rinsing steps, followed by the bluing step.
The structures of nuclei are more differentiated and better visible by the regressive method.

The second staining step is cytoplasmic staining by orange staining solution, especially for demonstration of mature and keratinized cells. The target structures are stained orange in different intensities.

In the third staining step is used the so-called polychrome solution, a mixture of eosin, light green SF and Bismarck brown. The polychrome solution is used for demonstration of differentiation of squamous cells.

Papanicolaou’s solution 2a Orange G solution (OG6) gives a pale, yellow-orange staining result with mature and keratinized squamous cells.

Sample material

Gynaecological and non-gynaecological specimen as sputum, urine, smears from fine needle aspiration biopsies (FNAB), effusions, rinses

Reagents

Cat No. 1.06888.0004
Papanicolaou’s solution 2a Orange G solution (OG6) for cytology — 20x 1 l

Also required:

for nucleus staining:

Cat. No.	105175	Hematoxylin solution modified acc. to Gill II for microscopy	500 ml, 2.5 l
or			
Cat. No.	109253	Papanicolaou’s solution 1a Harris hematoxylin solution for cytology	500 ml, 1 l, 2.5 l
or			
Cat. No.	109254	Papanicolaou’s solution 1b Hematoxylin solution S for cytology	500 ml, 2.5 l

for differentiation:

Cat. No.	109271	Papanicolaou’s solution 3a polychromatic solution EA 31 for cytology	500 ml, 2.5 l
or			
Cat. No.	109272	Papanicolaou’s solution 3b polychromatic solution EA 50 for cytology	500 ml, 1 l, 2.5 l

for regressive staining (see “Procedure”)**:**

Cat. No.	100316	Hydrochloric acid 25% for analysis EMSURE®	1 l, 2.5 l
Cat. No.	106329	Sodium hydrogen carbonate for analysis EMSURE® ACS,Reag.Ph Eur	500 g, 1 kg, 5 kg

Sample preparation

The sampling must be performed by qualified personnel.

All samples must be treated using state-of-the-art technology.
All samples must be clearly labeled.
Suitable instruments must be used for taking samples and their preparation. Follow the manufacturer’s instructions for application / use.

When using the corresponding auxiliary reagents, the corresponding instructions for use must be observed.

Fixation of smear samples

Wet fixation immediately with spray fixative M-FIX® for min. 10 min or wet fixation immediately in ethanol 96 % for min. 30 min.
When the smears are fixed with M-FIX®, rinsing steps 1 - 4 in the ascending ethanol sequence prior to staining can be omitted.

Reagent preparation

The Papanicolaou’s solution 2a Orange G solution (OG6) used for staining is ready-to-use, dilution of the solution is not necessary and merely produces a deterioration of the staining result and the stability.
It is recommended to filter the solution prior to its use.

Hydrochloric acid 0.1 %, aqueous

For preparation of approx. 100 ml solution mix:

Distilled water	100 ml
Hydrochloric acid 25 %	0.4 ml

Sodium hydrogen carbonate solution 1.5 %

For preparation of approx. 1000 ml of solution, add and dissolve:

Sodium hydrogen carbonate	15 g
Distilled water	1000 ml

Procedure

Progressive staining

Staining in the staining cell

The slides must be immersed and moved briefly in the solutions, simple immersion alone yields inadequate staining results.

The slides should be allowed to drip off well after the individual staining steps, as a measure to avoid any unnecessary cross-contamination of solutions.

The stated times should be adhered to guarantee an optimal staining result.

Slide with fixed smear	
Ethanol 96 %*	10 sec
Ethanol 80 %*	10 sec
Ethanol 70 %*	10 sec
Ethanol 50 %*	10 sec
Distilled water	20 sec
Hematoxylin solution modified acc. to Gill II or Papanicolaou's solution 1a Harris hematoxylin solution or Papanicolaou's solution 1b Hematoxylin solution S	3 min
Running tap water	3 min
Ethanol 70 %	30 sec
Ethanol 80 %	30 sec
Ethanol 96 %	30 sec
Papanicolaou's solution 2a Orange G solution or Papanicolaou's solution 2b Orange II solution	3 min
Ethanol 96 %	30 sec
Ethanol 96 %	30 sec
Papanicolaou's solution 3a polychromatic solution EA 31 or Papanicolaou's solution 3b polychromatic solution EA 50	3 min
Ethanol 96 %	30 sec
Ethanol 96 %	30 sec
Ethanol 100 %	5 min
Mixture consisting of: Ethanol 100 % + Neo-Clear® or xylene (1 + 1)	2 min
Clarify with Neo-Clear® or xylene.	5 min
Clarify with Neo-Clear® or xylene.	5 min
Mount the Neo-Clear®-wet slides with Neo-Mount® or the xylene-wet slides with e.g. Entellan® new and cover glass.	

* These steps can be omitted when smears are fixed with M-FIX®.

After dehydration (ascending alcohol series) and clarification with xylene or Neo-Clear®, cytological samples can be mounted with water-free mounting agents (e.g. Entellan® new, DPX new, or Neo-Mount®) and a cover glass and can then be stored.

The use of immersion oil is recommended for the analysis of stained slides with a microscopic magnification >40x.

Regressive staining

Staining in the staining cell

The slides must be immersed and moved briefly in the solutions, simple immersion alone yields inadequate staining results.

The slides should be allowed to drip off well after the individual staining steps, as a measure to avoid any unnecessary cross-contamination of solutions.

The stated times should be adhered to guarantee an optimal staining result.

Slide with fixed smear	
Ethanol 96 %*	10 sec
Ethanol 80 %*	10 sec
Ethanol 70 %*	10 sec
Ethanol 50 %*	10 sec
Distilled water	10 sec
Hematoxylin solution modified acc. to Gill II or Papanicolaou's solution 1a Harris hematoxylin solution or Papanicolaou's solution 1b Hematoxylin solution S	5 min 6 min 5 min
Distilled water	10 sec
Hydrochloric acid 0.1%, aqueous	10 sec
Distilled water	10 sec
Sodium hydrogen carbonate solution 1.5 %	1 min
Running tap water	3 min
Ethanol 70 %	30 sec
Ethanol 80 %	30 sec
Ethanol 96 %	30 sec
Papanicolaou's solution 2a Orange G solution or Papanicolaou's solution 2b Orange II solution	3 min
Ethanol 96 %	30 sec
Ethanol 96 %	30 sec
Papanicolaou's solution 3a polychromatic solution EA 31 or Papanicolaou's solution 3b polychromatic solution EA 50	3 min
Ethanol 96 %	30 sec
Ethanol 96 %	30 sec
Ethanol 100 %	5 min
Mixture consisting of: Ethanol 100 % + Neo-Clear® or xylene (1 + 1)	2 min
Clarify with Neo-Clear® or xylene.	5 min
Clarify with Neo-Clear® or xylene.	5 min
Mount the Neo-Clear®-wet slides with Neo-Mount® or the xylene-wet slides with e.g. Entellan® new and cover glass.	

* These steps can be omitted when smears are fixed with M-FIX®.

After dehydration (ascending alcohol series) and clarification with xylene or Neo-Clear®, cytological samples can be mounted with water-free mounting agents (e.g. Entellan® new, DPX new, or Neo-Mount®) and a cover glass and can then be stored.

The use of immersion oil is recommended for the analysis of stained slides with a microscopic magnification >40x.

Result

Staining with	3a / EA 31	3b / EA 50
Cytoplasm cyanophilic (basophilic) eosinophilic (acidophilic) keratinized	blue-green to green pink pink-orange	blue-green pink pink-orange
Erythrocytes	red	
Nuclei	blue to dark violet	
Microorganisms	grey-blue, grey-green	

Technical notes

The microscope used should meet the requirements of a medical diagnostic laboratory.
When using automatic staining systems, please follow the instructions for use supplied by the supplier of the system and software.
Remove surplus immersion oil before filing.

Diagnostics

Diagnoses are to be made only by authorized and qualified personnel.
Valid nomenclatures must be used.
This method can be supplementarily used in human diagnostics.
Further tests must be selected and implemented according to recognized methods.
Suitable controls should be conducted with each application in order to avoid an incorrect result.

Storage

Store the Papanicolaou's solution 2a Orange G solution (OG6) - for cytology at +15 °C to +25 °C.

Shelf-life

The Papanicolaou's solution 2a Orange G solution (OG6) - for cytology can be used until the stated expiry date.

After first opening of the bottle, the contents can be used up to the stated expiry date when stored at +15 °C to +25 °C.

The bottles must be kept tightly closed at all times.

Avoid exposure to heat.

In the Papanicolaou's solution 2a Orange G solution (OG6) - for cytology there may be occasional precipitations. This has no effect on the quality of the solution and do not affect the diagnosis.

Capacity

109253 Papanicolaou's solution 1a Harris hematoxylin solution
1500 - 2500 stainings / 500 ml

109254 Papanicolaou's solution 1b Hematoxylin solution S
1500 - 2500 stainings / 500 ml

105175 Hematoxylin solution modified acc. to Gill II
1000 - 1500 stainings / 500 ml

106888 Papanicolaou's solution 2a Orange G solution)
1500 - 2000 stainings / 500 ml

106887 Papanicolaou's solution 2b Orange II solution
1500 - 2000 stainings / 500 ml

109271 Papanicolaou's solution 3a polychromatic solution EA 31
1500 - 2000 stainings / 500 ml

109272 Papanicolaou's solution 3b polychromatic solution EA 50
1500 - 2000 stainings / 500 ml

Additional instructions

For professional use only.
In order to avoid errors, the application must be carried out by qualified personnel only.
National guidelines for work safety and quality assurance must be followed.
Microscopes equipped according to the standard must be used.
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. Information on disposal can be obtained under the Quick Link "Hints for Disposal of Microscopy Products" at www.microscopy-products.com. Within the EU the currently applicable 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.

Auxiliary reagents

Cat. No. 100316	Hydrochloric acid 25% for analysis EMSURE®	1 l, 2.5 l
Cat. No. 100579	DPX new non-aqueous mounting medium for microscopy	500 ml
Cat. No. 100974	Ethanol denatured with about 1 % methyl ethyl ketone for analysis EMSURE®	1 l, 2.5 l
Cat. No. 103699	Immersion oil Type N acc. to ISO 8036 for microscopy	100-ml dropping bottle
Cat. No. 103981	M-FIX® spray fixative for cytodiagnosis	100 ml, 1 l
Cat. No. 104699	Immersion oil for microscopy	100-ml dropping bottle, 100 ml, 500 ml
Cat. No. 105175	Hematoxylin solution modified acc. to Gill II for microscopy	500 ml, 2.5 l
Cat. No. 106329	Sodium hydrogen carbonate for analysis EMSURE® ACS, Reag. Ph Eur	500 g, 1 kg, 5 kg
Cat. No. 106887	Papanicolaou's solution 2b Orange II solution for cytology	500 ml, 2.5 l, 25 l
Cat. No. 107961	Entellan® new rapid mounting medium for microscopy	100 ml, 500 ml, 1 l
Cat. No. 108298	Xylene (isomeric mixture) for histology	4 l
Cat. No. 109016	Neo-Mount® anhydrous mounting medium for microscopy	100-ml dropping bottle, 500 ml
Cat. No. 109253	Papanicolaou's solution 1a Harris hematoxylin solution for cytology	500 ml, 1 l, 2.5 l
Cat. No. 109254	Papanicolaou's solution 1b Hematoxylin solution S for cytology	500 ml, 2.5 l
Cat. No. 109271	Papanicolaou's solution 3a polychromatic solution EA 31 for cytology	500 ml, 2.5 l
Cat. No. 109272	Papanicolaou's solution 3b polychromatic solution EA 50 for cytology	500 ml, 1 l, 2.5 l
Cat. No. 109843	Neo-Clear® (xylene substitute) for microscopy	5 l

Hazard classification

Cat. No. 1.06888.0004

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.

Main components of the product

Cat. No. 1.06888.0004

C.I. 16230	1.9 g/l
$H_3(Mo_{12}O_{40})P \times H_2O$	0.1 g/l

Other IVD products

Cat. No.	109269	Papanicolaou's solution 3d polychromatic solution EA 65 for cytology	100 ml, 2.5 l
Cat. No.	109270	Papanicolaou's solution 3c polychromatic solution EA 65 for cytology	100 ml
Cat. No.	109275	Shorr staining solution for hormonal cytodiagnosis	500 ml
Cat. No.	115355	CYTOCOLOR® Cytological standard stain acc. to Szczepanik for microscopy	6x 500 ml

General remark

If during the use of this device or as a result of its use, a serious incident has occurred, please report it to the manufacturer and/or its authorised representative and to your national authority.

Literature

1. Routine Cytological Staining Techniques: Theoretical Background and Practice, Mathilde E. Boon, Johanna S. Drijver, 1986, Elsevier Science Publishing Company
2. 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). Bios, 2002
3. Romeis - Mikroskopische Technik, Editors: Maria Mulisch, Ulrich Welsch, 2015, Springer Spektrum, 19. Auflage
4. Theory and Practice of Histological Techniques, John D Bancroft and Marilyn Gamble, 6th Edition
5. Gynäkologische Zytodiagnostik Lehrbuch und Atlas, Hans-Jürgen Soost, Siegfried Baur, Georg Thieme Verlag Stuttgart, Auflage, 1990

Consult instructions for use

Manufacturer

Catalog number

Batch code

Caution, consult accompanying documents

Use by YYYY-MM-DD

Temperature limitation

Status: 2020-Jul-30

Merck KGaA, 64271 Darmstadt, Germany,
Tel. +49(0)6151 72-2440
www.microscopy-products.com