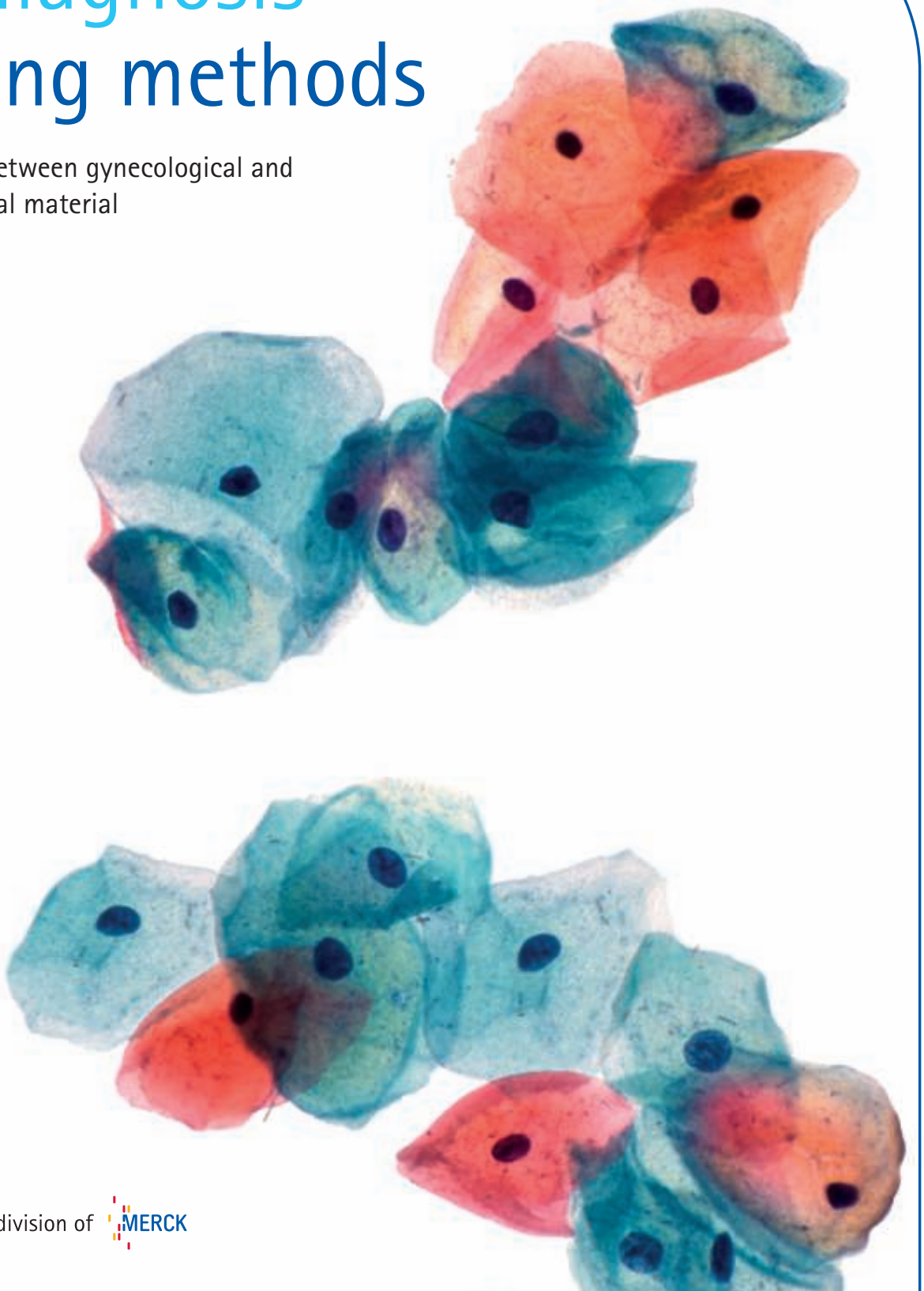
Cytodiagnosis staining methods

Differentiating between gynecological and non-gynecological material



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Content

Introduction

Cytodiagnosis is the diagnosis of disease through the microscopic examination of cells (of human, animal or plant origin) collected by various means.

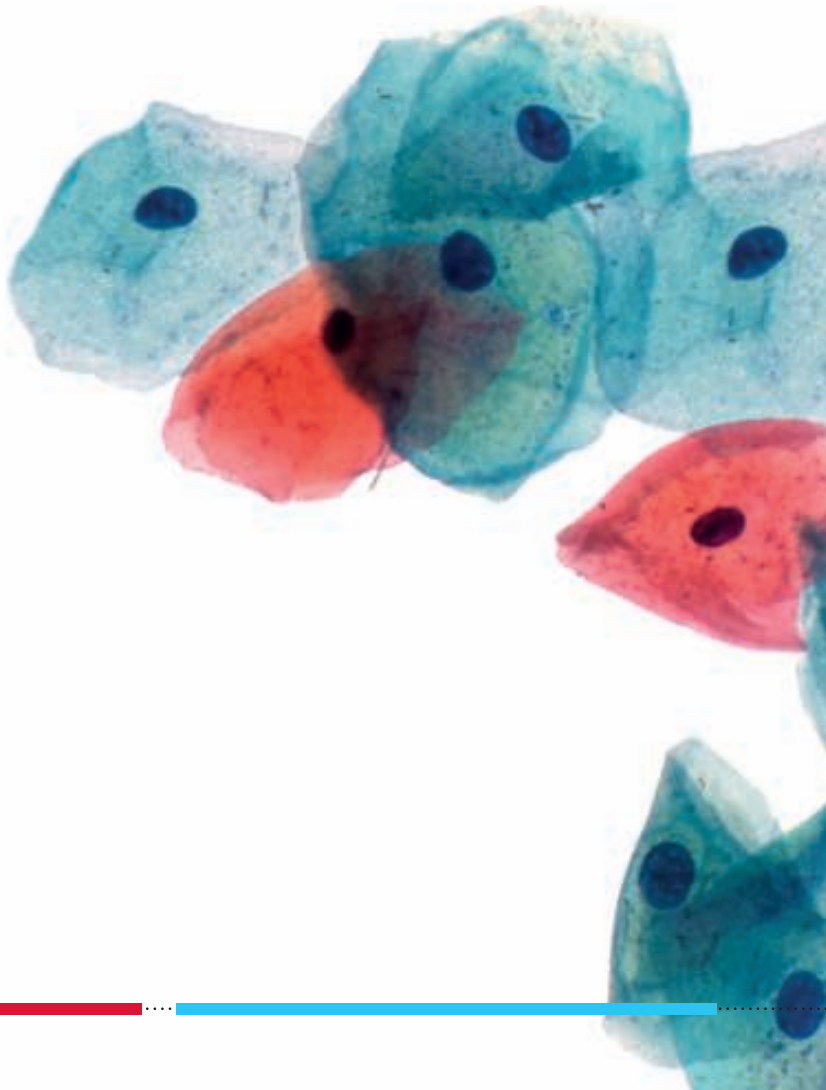
In the case of human cytodiagnosis, there are two areas of cytology – gynecological and non-gynecological – in which specimen material is examined for the presence of malignant and premalignant cells, which, through certain procedures, may be classified as benign, inflammatory, degenerative or malignant.

The name Papanicolaou is closely associated with human cytology, cervical screening and the staining of specimen material. George N. Papanicolaou began his study of cervical cytology in 1917. Among his many achievements was demonstrating the effects of the hormone cycle on the epithelium. In 1943, he published the results of his research into the diagnosis of cervical cancer, which involved fixing cervical cells while still moist, and staining them in 3 stages with competing dyes. The specimens were dehydrated, cleared with xylene (nowadays, a non-aromatic xylene substitute is preferred), and mounted under a coverslip. This staining technique, known as the Pap stain, revolutionized cytology and is still the gold standard today.

The Papanicolaou stain is also used for non-gynecological (clinical) material. For instance, specimens of sputum or urine, containing squamous epithelial or similar cells, demonstrate excellent results when stained according to the Papanicolaou technique. Stains normally used in hematology, such as Pappenheim's or Giemsa's stain, may also be applied. The choice of stain largely depends on the experience and preference of the investigator. Based on the number of slides to be prepared, the staining method selected can be applied either manually with the aid of cuvets, or mechanically in automated staining systems. For some years now, fully automated evaluation systems, originally limited to use in quality control, are now also permitted for screening. These systems process the Papanicolaou-stained material, mark abnormal cells or groups of cells, and save the images in a gallery for retrieval at any time.

Products for cytology that are intended for the examination of human specimens are now classed as in vitro diagnostic medical devices (IVDs). Since 7 December 2003, such devices require CE certification if they are to be used in European Union countries. In other words, every product should include a dossier containing data on product development (in the case of new products), production, quality control and risk management. All labels, package inserts and promotional material must bear the CE mark, and IVDs may now only be used if they carry this mark. If these or similar products were not designed as IVDs, they must clearly state that they are not allowed to be used as such. The basis for CE certification is "Directive 98/79/EC of the European Parliament and of the Council" of 27 October 1998 on in vitro diagnostic medical devices.

Gynecological cytology



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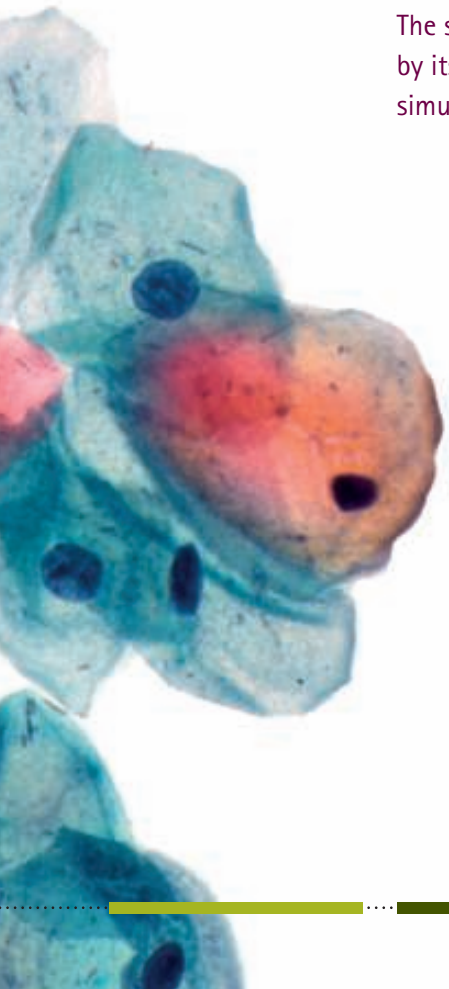
Cervical screening is a well-established method in industrialized countries. Thanks to its simplicity and outstanding precision, it has contributed significantly to the decrease in cervical carcinomas. The resounding success of cervical screening is also partly due to the fact that changes in cells from all layers of the squamous epithelium can be found within the basal membrane and assigned to defined groups; even changes beyond the basal membrane that behave invasively can be diagnosed and classified based on precisely defined criteria. Progression from mild changes in the epithelium to the development of cervical carcinoma takes years. Thus, cervical carcinomas can be largely prevented through careful screening.

The notable achievements of cervical screening are:

- Decline in cervical carcinoma mortality
- Decrease in number of newly diagnosed invasive carcinomas or stage II-IV cervical carcinomas
- Increase in number of detected precancerous states in the total number of screenings

The success and efficiency of cervical screening is measured by its ability to detect precancerous changes (sensitivity) while simultaneously preventing false-positive diagnoses (specificity).



Material

In cytodiagnosis cells are loosened from the tissue mass and transferred to a cytological slide, examined and evaluated. The architecture of the tissue is not recognizable and cannot play a part in the evaluation. Cytological testing presupposes a high degree of expertise. Cytology has the advantage that collection of material can be undertaken without serious inconvenience to the patient; time and equipment requirements are modest and, where necessary, repeat testing is nearly always possible.

Microscopic examination of the material can take place almost immediately, depending on the fixation, preparation and staining techniques employed. These features make cytodiagnosis suitable for screening larger groups, e.g. for cervical screening. One critical point in cytological diagnosis is that the results of cytological investigations relate directly to the location from which the specimen material was collected. For the sake of their efficiency and reputation, sampling and preparation techniques must be thoroughly mastered and controlled.

Gynecological material

In gynecology cells are collected from 2 points – from the surface of the portio and within the cervical canal – and smeared onto a glass microscope slide in the traditional way. Various collecting systems such as cotton swabs, wooden spatulae, various plastic spatulae, and cervical brushes, so-called cytobrushes, are employed. Cotton swabs, the traditional collection system, have the disadvantage that the soft, absorbent surface does not always enable a representative sample to be collected and that some material remains in on the cotton during spreading onto the slide. In brush biopsies more endocervical cells and blood may be present in the smears, and this may lead to evaluation difficulties, especially at the start.

As a second method was established the liquid based cytology In that method, specimen material is collected with a brush. The brush with the material is placed in a transport container filled with a preserving medium, and specially filtered, enabling inflammatory cells and blood to be largely separated out. A so-called monolayer smear is prepared. The microscopic image is very clean, the fields that are to be evaluated are smaller and exhibit tidy distribution of cells. The two methods are of equal merit because, on the one hand, the inclusion of blood and inflammatory cells provides important information and, on the other, because these inclusions can make microscopic examination more difficult.

Fixation

A precondition for exact cytological diagnosis is perfect fixation of sample material. In order to prevent the cells from drying out and shrinking, to maintain the specimen's structural features and to permit clear staining and differentiation, specimens must be fixed immediately after being taken and while still moist. If specimens are fixed too late, so-called artefacts can be found in Papanicolaou-stained smears on single cells or cell clusters. Artefacts have a brownish granular appearance and may impact negatively on diagnosis. The classic method of fixing is to immerse the microscope slides in 96% ethanol for 30 min. A more efficient and quicker way is to fix them with a spray fixative. Spray fixatives are aqueous-alcoholic solutions containing polyethylene glycol (PEG, Carbowax). They are suitable for all types of cytological material due to be stained by the Papanicolaou method.

Ethanol (ethyl alcohol) with a content of 96% or 100% (absolute) is another frequently used fixative. It works by extracting water from specimen materials without affecting or altering their structures or chemical constituents. The denaturant used to denature the alcohol is methyl ethyl ketone which behaves neutrally in the applications for which it is used. The sample material is used to be fixed for 30 min. in ethanol to preserve the fine structures of the cell material.

Product	Package size	Cat. No.
Ethanol absolute, for analysis EMSURE® ACS, ISO, Reag Ph Eur	1 L, 2.5 L	100983
Ethanol denatured with about 1% methyl ethyl ketone for analysis EMSURE®	1 L, 5 L	100974

M-FIX™ is a spray fixative consisting of alcohol and polyethylene glycol (PEG) in aqueous solution. It can be sprayed quickly and easily onto moist slides to build up a thin even layer which fixes the smear and stops the cells drying out. The still moist smear is sprayed immediately 3 times with M-FIX™ spray fixative so that the specimen is evenly wetted, but not drenched, with 0.3 to 0.6 ml of M-FIX™. Care should be taken that the spray is approximately 20 cm away from the object during spraying in order to avoid any potential loss of cells.

The alcohol contained in M-FIX™ spray fixative evaporates, leaving a protective film of polyglycol on the specimen. After approx. 10 min. the specimen is dry and can now be stained, stored or dispatched for further processing. Specimens prepared in this manner remain stable for a period of several weeks. During staining, care should be taken that the specimen is first immersed in distilled water or 50% ethanol for approx. 10 sec. in order to remove the polyglycol film. The descending alcohol series before the hematoxylin step in the Papanicolaou method can be omitted.

Advantages of M-FIX™ spray fixative

- Optimum protection against drying out
- Retention of staining properties
- Uniform covering of cell smears
- Simple procedure
- Specimens can be transported, mailed or stained after approx. 10 min
- Simple removal of fixative film in distilled water

Technical information

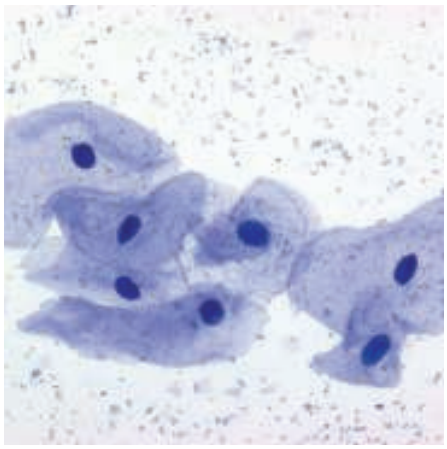
Any crystallization that may occur during transport or due to storage at varying temperatures can be easily resolved by shaking the bottle vigorously.

Product	Package size	Ord. No.
M-FIX™ spray fixative	100 ml	1.03981.0102
M-FIX™ spray fixative (refill)	1 L	1.03981.1000

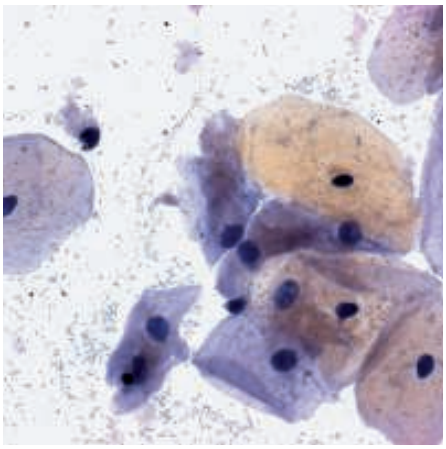
Staining – Papanicolaou stain

The important elements of this method are nuclear staining followed by orange and polychromic cytoplasmic staining.

Hematoxylin stain > Orange stain > Polychromic stain



1. Result

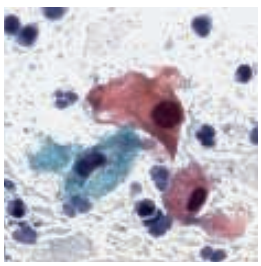


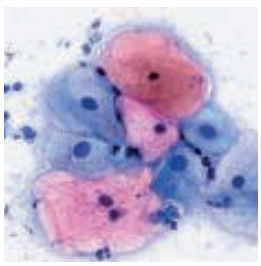
2. Result

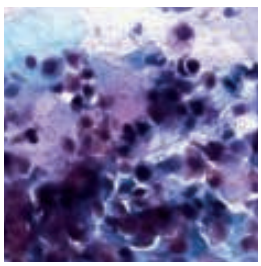
3. Result

Hematoxylin stain + Orange stain + Polychromic stain = Papanicolaou stain

Following Papanicolaou staining the cells are highly transparent, a feature which means diagnosis is possible even in areas of overlapping cells and when mucus and inflammatory cells are present.





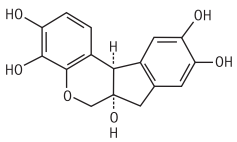


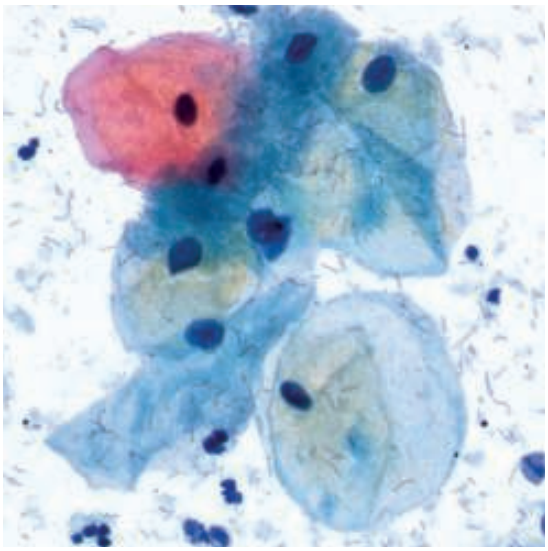
Classic Papanicolaou staining, Pap smear

Nuclear staining

Nuclear staining is accomplished using the natural dye, hematoxylin. Hematoxylin dye is mixed with a trivalent metal salt to produce a so-called hematoxylin lake, and it is this that is actually used to selectively stain the cell nuclei (DNA). The oxidized form, hematein, chelates with the trivalent metal ions (Al^{3+}, Fe^{3+}, Cr^{3+}) of certain alums. The chelated compound is used in an acid medium and, when rinsed with tap water, produces the characteristic blue color. This step also fixes the dye on the target structures. What makes the hematoxylin solutions special is the fact that, apart from the dye, all of the other components (an oxidizing agent, now iodate for safety reasons, and the metal ions), are present as salts. The constituents must be carefully matched so that oxidation occurs in a way that ensures there is always sufficient hematoxylin, i.e. hematein, present and that the oxidized dye is available in sufficient quantity throughout its entire declared useful life.

Hematoxylin
$C_{18}H_{14}O_6$ | C.I. 75290





Papanicolaou stain with Harris hematoxylin, Pap smear

The most commonly used staining solutions are listed below.

Harris' hematoxylin solution is the classic staining solution that has been sold without mercuric chloride for many years now and, with iodate as oxidizer, yields identical staining results and useful lives. Oxidation is such that using iodate to oxidize the hematoxylin is just the same as using mercury. With a dye content of approx. 5 g/l Harris' hematoxylin solution produces strong stains. The staining solution must be filtered before use.

Hematoxylin S is a strong hematoxylin solution with a dye content of 6 g/l. Its applications are in cytology and histology using progressive and regressive methods. The staining solution does not have to be filtered before use.

Gill's hematoxylin solutions contain various amounts of hematoxylin and use iodate as oxidizer, so they are more environment-friendly.
Hematoxylin solution acc. to Gill II is suitable for use in cytology. It delicately stains cell nuclei with its dye content of 2 g/l. The staining solution should only be used for the progressive method.
Hematoxylin solution acc. to Gill III stains more intensely and has a dye content of approx. 4 g/l. The solution is better suited for use in histology. Hematoxylin solution acc. to Gill III can be used for regressive as well as progressive staining. Gill's solutions do not have to be filtered before use.

Product	Package size	Cat. No.
Papanicolaou's solution 1a, Harris' hematoxylin solution	500 ml, 1 L, 2.5 L	109253
Papanicolaou's solution 1b, hematoxylin solution S	500 ml, 2.5 L	109254
Hematoxylin solution acc. to Gill II	500 ml, 2.5 L	105175
Hematoxylin solution acc. to Gill III	500 ml, 1 L, 2.5 L	105174

Cytoplasmic staining with orange staining solution

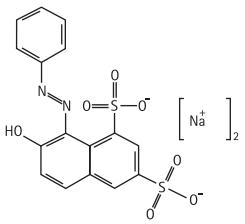
Papanicolaou stain with Orange G, Pap smear

The effect of the orange staining solution is particularly pronounced in smears with keratinized cells under acidic pH conditions, when the obviously orangeophilic cytoplasmic stain is recognizable and may point to the presence of hyperkeratosis, HPV infections or carcinoma cells. It is supposed that orange dyes have a ripening effect on the subsequent polychromic stain.

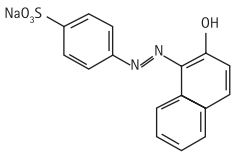
The products used are Orange G, which stains the target structures yellowish-orange, and Orange II, which colors the target structures reddish-orange.

Product	Package size	Cat. No.
Papanicolaou's solution 2a, Orange G	500 ml, 1 L, 2.5 L	106888
Papanicolaou's solution 2b, Orange II	500 ml, 2.5 L	106887

Orange G $C_{16}H_{10}N_2Na_2O_7S_2$ | C.I. 16230



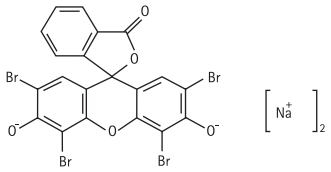
Orange II $C_{16}H_{11}N_2NaO_4S$ | C.I. 15510



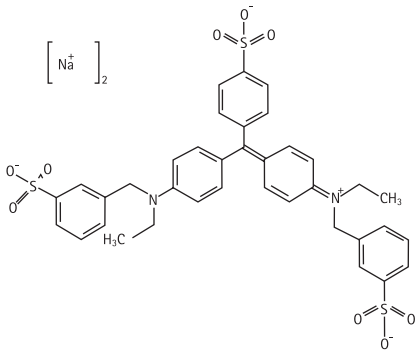
Cytoplasmic staining with polychromic staining solutions

The second staining step for cytoplasm is staining with a polychromatic mixture of Eosin G, Light Green SF and Bismarck Brown (vesuvine). Various EA modifications (EA stands for eosin azure) are known. They differ simply through the various concentrations of the individual dyes. Staining solutions commonly used in cytology are EA 31 and EA 50, while EA 65 is preferred for mucous material such as sputum, bronchial secretions and other non-gynecological material. Bismarck Brown reportedly does not have a staining effect but rather contributes to stabilizing the staining solution. As a result of their different molecular weights and the various pore diameters of the cell membrane, the two dyes Eosin G and Light Green SF compete for the same target structures and cause the cells to be differently stained at various cyclic stages. Mature squamous epithelial cells, nucleoli and ciliae, for instance, have a stronger affinity for Eosin G, while parabasal and intermediate cells appear green, blue-green or blue after being stained with Light Green SF.

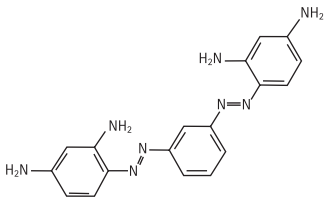
Eosin G $C_{20}H_6Br_4Na_2O_5$ | C.I. 45380

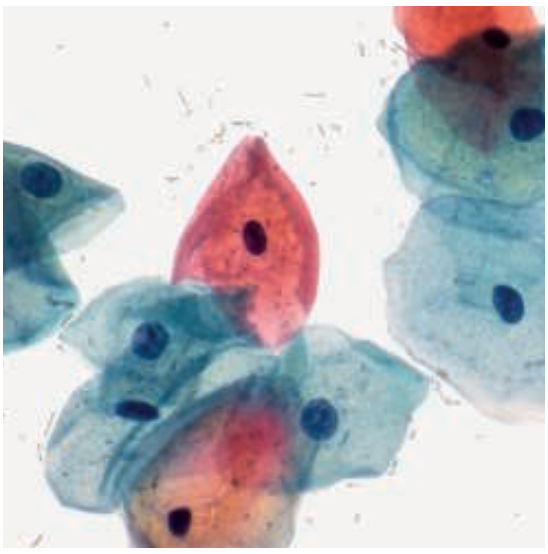


Light green SF $C_{37}H_{34}N_2Na_2O_9S_3$ | C.I. 42095



Bismarck brown (vesuvine) $C_{18}H_{20}N_8Cl_2$ | C.I. 21000





Papanicolaou stain with polychrome EA 50, Pap smear

Product	Package size	Cat. No.
For gynecological material		
Papanicolaou's solution 3a, polychromic solution EA 31	500 ml, 2.5 L	109271
Papanicolaou's solution 3b, polychromic solution EA 50	500 ml, 1 L, 2.5 L	109272
For non-gynecological / clinical material		
Papanicolaou's solution 3c, polychromic solution EA 65	100 ml	109270
Papanicolaou's solution 3d, polychromic solution EA 65	100 ml, 2.5 L	109269

Progressive and regressive nuclear staining techniques in Papanicolaou staining

Two methods can be distinguished. In the progressive hematoxylin method, staining is performed to the end point, followed by the blueing step in tap water to fix the dye permanently. In the regressive method hematoxylin is used to overstain, with the excess dye being removed again in acidic differentiating steps. Here, too, tap water is used for blueing and to fix the dye permanently. The structures of nuclei are more differentiated and rendered more visible by the regressive method.

Preparation

1. **Hematoxylin solution acc. to Harris**
   Dissolve 5 g Hematoxylin Certistain® or cryst. in 50 ml of ethanol while heating in a water bath. Dissolve 100 g of aluminium potassium sulfate (potassium alum) in 950 ml of distilled water while stirring and heating. Pour the hematoxylin solution into the hot alum solution while continuously stirring and heat to boiling. Remove the solution from the source of heat. Add 370 mg of sodium iodate while stirring and cool rapidly in a water bath. Add 4 ml of glacial acetic acid. Filter into bottles and close tightly. Filter prior to use.

2. **Hematoxylin solution acc. to Gill II**
   Dissolve 2 g of hematoxylin, 0.2 g of sodium iodate and 17.6 g of aluminium sulfate (x18 H_2O) in a solution of 250 g of ethylene glycol and 730 ml of distilled water. Add 20 ml of glacial acetic acid. Stir for one hour at room temperature. Filter prior to use.

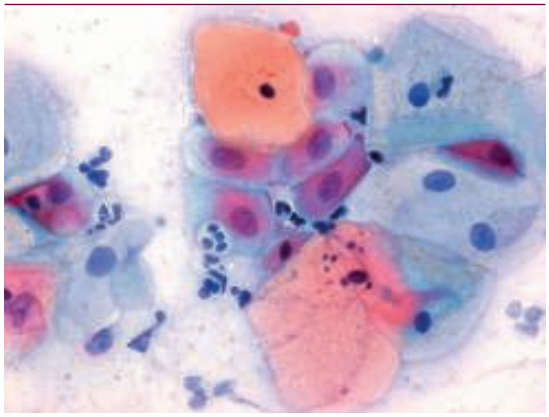
3. **0.1% aqueous HCl solution**
   Make 27.5 ml of HCl 1 N up to 1 L with distilled water.

4. **Sodium hydrogen carbonate solution 1.5%**
   Dissolve 15 g of $NaHCO_3$ in 1 L of distilled water.

Progressive staining

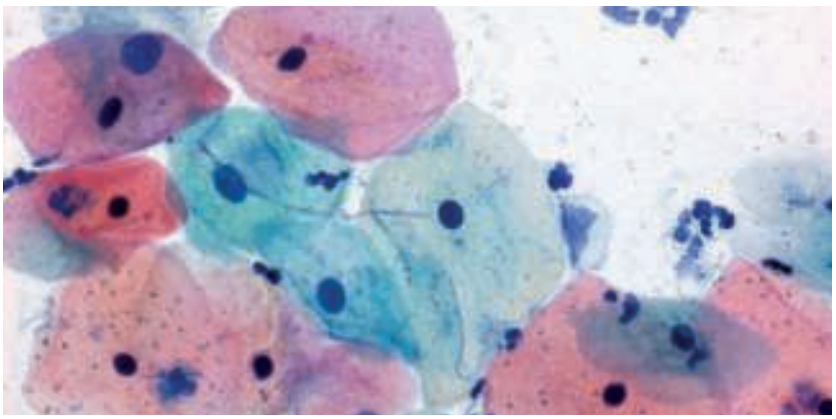
1.	Wash with 96% alcohol*	
2.	Wash with 80% alcohol*	
3.	Wash with 70% alcohol*	
4.	Wash with 50% alcohol*	
*** If M-FIX™ is used, steps 1 – 4 can be dropped.**		
5.	Wash with distilled water	
6.	Stain in hematoxylin solution	
	Harris' hematoxylin solution	3 min
	hematoxylin solution S	2-3 min
	hematoxylin solution acc. to Gill II	3 min
7.	Rinse under running tap water	3 min
8.	Wash with 70% alcohol	
9.	Wash with 80% alcohol	
10.	Wash with 96% alcohol	
11.	Stain in Orange G solution or Orange II solution	3 min
12.	Wash with 96% alcohol	
13.	Wash with 96% alcohol	
14.	Stain in Polychromic solution EA 31 or EA 50	3 min
15.	Dehydrate with 96% alcohol	
16.	Dehydrate with 96% alcohol	
17.	Dehydrate in absolute alcohol	5 min
18.	Dehydrate with absolute alcohol / Neo-Clear® 1:1 or absolute alcohol / xylene 1:1	2 min
19.	Clear with Neo-Clear® or xylene	5 min
20.	Clear with Neo-Clear® or xylene	5 min

Mount with Neo-Mount® (for Neo-Clear®) or Entellan® new (for xylene).



Papanicolaou stain, progressive. *Papanicolaou stain with Gill II, Orange G and polychrome EA 50, Pap smear*

Regressive staining		
1.	Wash with 96% alcohol*	
2.	Wash with 80% alcohol*	
3.	Wash with 70% alcohol*	
4.	Wash with 50% alcohol*	
*** If M-FIX™ is used, steps 1 – 4 can be dropped.**		
5.	Wash with distilled water	
6.	Stain in hematoxylin solution	
	Harris' hematoxylin solution	6 min
	hematoxylin solution S	5 min
	hematoxylin solution acc. to Gill II	5 min
7.	Rinse in distilled water	10 sec.
8.	Rinse in HCl 0.1%	10 sec.
9.	Rinse in distilled water	10 sec.
10.	Rinse in sodium hydrogen carbonate solution 1.5%	1 min
11.	Rinse under running tap water	3 min
12.	Wash with 70% alcohol	
13.	Wash with 80% alcohol	
14.	Wash with 96% alcohol	
15.	Stain in Orange G solution or Orange II solution	3 min
16.	Wash with 96% alcohol	
17.	Wash with 96% alcohol	
18.	Stain in Polychromic solution EA 31 or EA 50	3 min
19.	Dehydrate with 96% alcohol	
20.	Dehydrate with 96% alcohol	
21.	Dehydrate in absolute alcohol	5 min
22.	Dehydrate with absolute alcohol / Neo-Clear® 1:1 or absolute alcohol / xylene 1:1	2 min
23.	Clear with Neo-Clear® or xylene	5 min
24.	Clear with Neo-Clear® or xylene	5 min
Mount with Neo-Mount® (for Neo-Clear®) or Entellan® new (for xylene).		

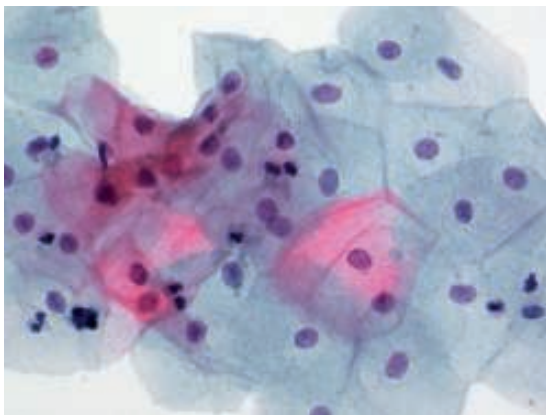


Papanicolaou stain, regressive. *Papanicolaou stain with Harris hematoxylin, Orange G and polychrome EA 31, Pap smear*

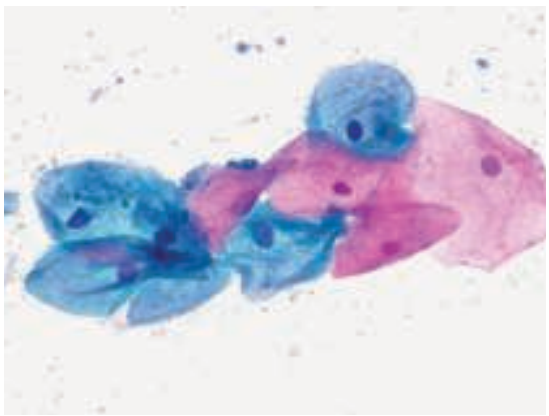
Result	gynecological	
Staining with:	**3a / EA 31**	**3b / EA 50**
Cytoplasm cyanophilic (basophilic)	intense green	blue-green
Cytoplasm eosinophilic (acidophilic)	pink	pink
Cytoplasm keratinized	pink-orange	pink-orange
Erythrocytes	red	red
Nuclei	blue, black, dark violet	
Microorganisms	grey-blue	
Trichomonads	grey-green	

Modified Papanicolaou stain with Cytocolor® and Neo-Cytocolor®

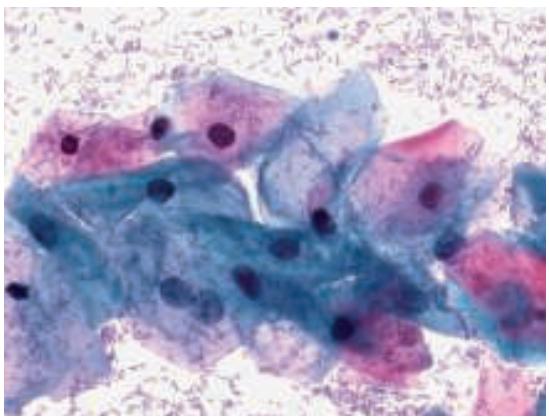
The modified Papanicolaou stain developed by Szczepanik is used predominantly for early detection of cervical carcinoma. In the modified Papanicolaou staining method, staining is achieved with modified hematoxylin solution and modified polychromic solution; the orange staining step is omitted. The structures that are normally stained with orange solution are stained here by Eosin G from the polychromic solution, and appear pink instead of orange. The intensity of the pink color depends on the degree of keratinization of the cells. Within approximately 3 minutes and with 10 staining and differentiating steps Cytocolor® produces a stained slide that provides complete information on dignity, hormonal status and vaginal flora.



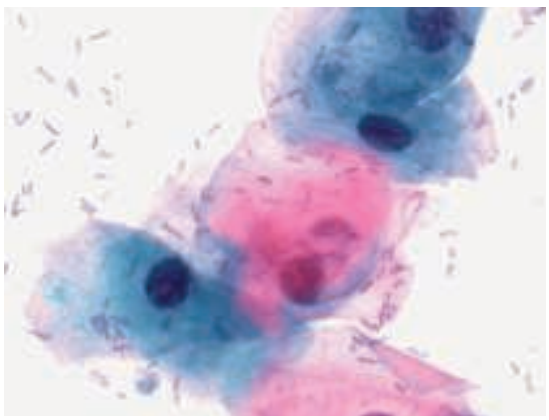
Cytocolor®, Pap smear



Cytocolor®, Pap smear



Neo-Cytocolor®, Pap smear



Neo-Cytocolor®, Pap smear

Cytocolor® contains all the reagents needed for staining in a set. The kit contains not only hematoxylin and polychromic solution but also alcohol, 2-Propanol and xylene. The reagents are contained in coplin jars which should be covered after staining in order to minimize evaporation of the solvents. Immerse and agitate the slides in the solutions. Simple immersion produces unsatisfactory stains.

Preparation

Fix specimens while still moist with spray fixative (M-FIX™) or for 30 minutes in ethanol.

Procedure

1.	Distilled water	10 x 1 sec.
2.	Modified hematoxylin solution	1 x 1 min
3.	Rinse under running tap water	1 x 5 sec.
4.	2-Propanol	2 x 1 sec.
5.	Modified polychromic solution	1 x 1 min
6.	80% 2-Propanol	5 x 1 sec.
7.	2-Propanol	5 x 1 sec.
8.	2-Propanol	5 x 1 sec.
9.	Xylene	5 x 1 sec.
10.	Xylene	5 x 1 sec.

Mount the xylene-moist specimen immediately with M-GLAS™ (without a coverslip) or with Entellan® new in conjunction with a coverslip.

Result

Nuclei	blue, dark violet, black
Cyanophilic (basophilic) cytoplasm	blue-green
Eosinophilic (acidophilic) cytoplasm	pink
Keratinized cytoplasm	bright pink
Microorganisms	blue-violet
Trichomonads	grey-blue, grey-green
Erythrocytes	red

Neo-Cytocolor® is based on the same principle as Cytocolor®. Xylene is replaced by Neo-Clear®. With Neo-Cytocolor® will be accomplished the same speed and reproducibility for the application in connection with user- and environmental-friendliness of Neo-Clear® and Neo-Mount® at the end.

Preparation

Fix specimens while still moist with spray fixative (M-FIX™) or for 30 minutes in ethanol.

Procedure

1.	Distilled water	10 x 1 sec.
2.	Modified hematoxylin solution	1 x 1 min
3.	Rinse under running tap water	1 x 5 sec.
4.	2-Propanol	2 x 1 sec.
5.	Modified polychromic solution	1 x 1 min
6.	80% 2-Propanol	5 x 1 sec.
7.	2-Propanol	5 x 1 sec.
8.	2-Propanol	5 x 1 sec.
9.	Neo-Clear®	5 x 10 sec.
10.	Neo-Clear®	5 x 10 sec.

Mount the Neo-Clear®-moist specimen immediately with Neo-Mount® and a coverslip.

Result

Nuclei	blue, dark violet, black
Cyanophilic (basophilic) cytoplasm	blue-green
Eosinophilic (acidophilic) cytoplasm	pink
Keratinized cytoplasm	bright pink
Microorganisms	blue-violet
Trichomonads	grey-blue, grey-green
Erythrocytes	red

Product	Package size	Cat. No.
Cytocolor® kit	6 x 500 ml	115355
Neo-Cytocolor® kit	6 x 500 ml	101971

Gynecological results classification

Findings are classified using the Munich nomenclature II, which has been used since December 1989 and is based on the Munich nomenclature I of 1975 by Soost und Droese. The Munich nomenclature II follows the Bethesda nomenclature, which was introduced in 1989 by the International Academy of Cytology in the USA.

According to the latest Munich nomenclature, a clear text description and / or evaluation of all cytological findings is obligatory. The diagnostic groups quoted are useful not only for classifying findings but also for raising statistical data and for quality assurance.

First, **A.** an assessment is provided of the quality of the smear:
1. Satisfactory
2. Satisfactory but with limitations
3. Unsatisfactory

Reasons are to be stated in the case of smears that are satisfactory but with limitations, such as:
- Too little cell material
- Inadequate fixing
- Severe degenerative cell changes
- Intense inflammation
- Bloody smear
- No endocervical cells

Furthermore, **B.** the degree of proliferation, stated acc. to A. Schmitt, is described as well as **C.** the microorganisms found, such as:
- Döderlein flora with or without cytolysis
- Mixed bacterial flora
- Coccoid flora / Gardnerella
- Fungi
- Trichomonads
- Others

The classification of cytological findings is according to the group definition

Group I
Normal cell picture, consistent with age, including mild inflammatory and degenerative changes, as well as bacterial cytolysis.

Group II
Inflammatory changes in cells of the squamous epithelium and cylindrical epithelium of the cervix, regenerative cells, immature metaplastic cells, more intense degenerative changes, para- and hyperkeratinizing cells, normal endometrial cells even after the menopause.
Also, special cell pictures such as follicular cervitis, cell changes through IUP (intrauterine pessary), signs of HPV infection without any significant nuclear changes, signs of herpes or cytomegaly virus infection.

Group III
Unclear finding
- severe inflammatory, degenerative or iatrogenic changes of the cells where benignity or malignancy cannot be diagnosed with certainty.
- abnormal cells of the glandular epithelium whose carcinomatous nature cannot be excluded with certainty; if possible with an indication of the endometrial, endocervical or extra-uterine origin of the cells.

Group IIID
Mild to moderate dysplasia
(signs of HPV infection should be specially mentioned).

Group IVa
Severe dysplasia or carcinoma in situ
(signs of HPV infection should be specially mentioned).

Group IVb
Severe dysplasia or carcinoma in situ,
invasive carcinoma not excluded.

Group V
Malignant tumor
- squamous epithelial carcinoma (keratinizing or non-keratinizing)
- adenocarcinoma, indicating if possible the endometrial, endocervial or extra-uterine origin of the cells
- other malignant tumors.

Findings report

The differentiated cytological finding should be recorded prospectively according to the result classification in the Munich nomenclature:

Group	Finding
III	Non-classifiable cytological pictures
III D	Mild to moderate dysplasia
IV	Severe dysplasia or carcinoma in situ, invasive carcinoma not excluded
V	Cervical carcinoma, uterine carcinoma or other malignant tumor

All cytological slides and results must be kept for 10 years.

A non-physician carrying out microscopic examination of slides may examine max. 10 slides within an hour. Their IDs must be documented in writing.

The level of training received by staff must be checked, and internal (e.g. laboratory conferences with discussion of problem cases and positive results) and external training (advancement events with lectures and practical exercises with the microscope) must be carried out. Microscopic examination must be performed using properly working light-microscopic systems using staining equipment meeting the latest standards and with regular changing of staining solutions. The slides must be long-term stable.

Recommendation
Cytological control if necessary, time interval dependent on clinical finding (with or without anti-inflammatory or hormonal treatment).

Recommendation
Short-term cytological control or immediate histological clarification depending on the clinical and colposcopic finding.

Recommendation
Colposcopic / cytological control in 3 months.

Recommendation
Colposcopic / cytological control and histological clarification.

Recommendation
Colposcopic / cytological control and histological clarification.

Recommendation
Colposcopic / cytological control and histological clarification.

Technical information on Papanicolaou staining

Xylene is the most used solvent for clearing. For clearing in the Papanicolaou staining technique, results of similar quality are achieved with Neo-Clear®, a non-aromatic solvent. Neo-Clear® belongs to the group of so-called isoparaffins, is virtually odorless and can be used in the same way as xylene. Neo-Mount®, a mounting medium based on Neo-Clear® is used for mounting. This gives excellent optical results and is so dry after 30 minutes that the slide is ready for microscopic examination.

It is recommended that the staining solutions be filtered once daily in order to remove any loose cells / cell components.

It is important to renew alcohol baths (ethanol 96% and 100%) regularly in order to achieve good differentiation and stain transparency. For specimen documentation it is especially important that the last alcohol, xylene or xylene-substitute baths (Neo-Clear®) be absolutely clean and free of water. Any water remaining can lead cause the slide to become decolored as a result of oxidation. Alcohol quality has a significant negative effect on staining results; traces of other solvents and water can spoil the result. Denaturants such as pyridine or toluene also impact negatively on the staining effect. Alcohol denatured with methyl ethyl ketone has no effect on the staining result. Our denatured 99,5% alcohol is available under Cat. No. 100974.

Hormonal diagnosis – Shorr's stain

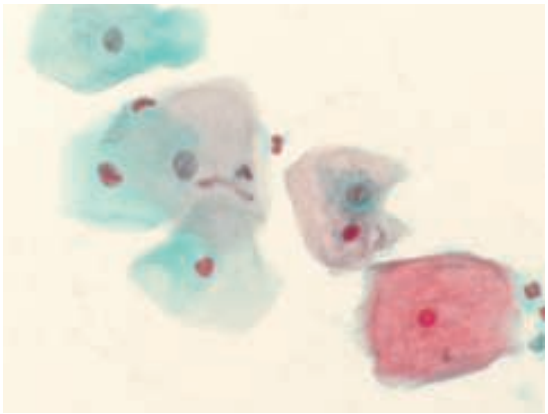
During the menstrual cycle sex hormones induce characteristic changes in the vaginal epithelium. The current hormone status can be assessed with the aid of stained vaginal smears. Shorr staining solution is a solution used to diagnose hormonal dysfunctions. With Shorr's stain it is possible to differentiate easily between epithelial eosinophilia and cyanophilia. The ratio of eosinophilic to cyanophilic cells makes it possible to assess follicular hormone and luteinizing hormone status. The number of eosinophilic cells rises with follicular hormone, and of cyanophilic cells with luteinizing hormone.

Procedure

1.	Stain in Shorr staining solution	1-3 min
2.	Rinse in 70% ethanol	10 x 1 sec.
3.	Rinse in 80% ethanol	10 x 1 sec.
4.	Rinse in 96% ethanol	10 x 1 sec.
5.	Rinse in absolute ethanol	10 x 1 sec.
6.	Clear with xylene or Neo-Clear®	30 sec.
Mount with Entellan® new (for xylene) or Neo-Mount® (for Neo-Clear®)		

Result

Cytoplasm cyanophilic (basophilic)	blue-green
Cytoplasm eosinophilic (acidophilic)	bright red
Nuclei	brown-red



Shorr stain, Pap smear

Product	Package size	Cat. No.
Shorr staining solution	500 ml	109275

Technical information

The Shorr staining solution is ready to use. Dilution of the solution is not necessary and might reduce the staining result and useful life.

Additional diagnostic methods

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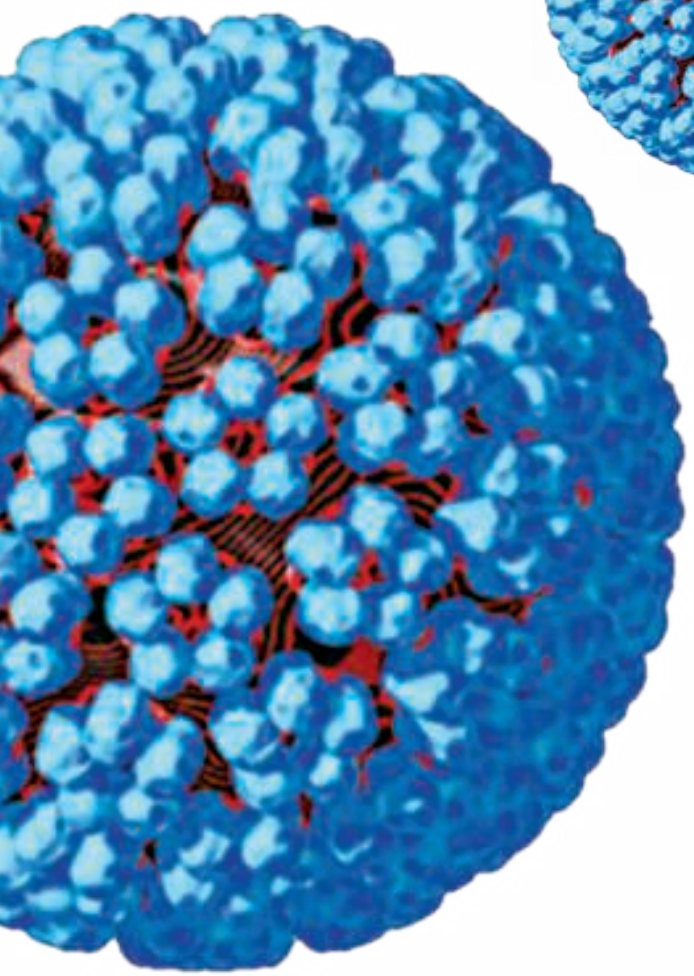
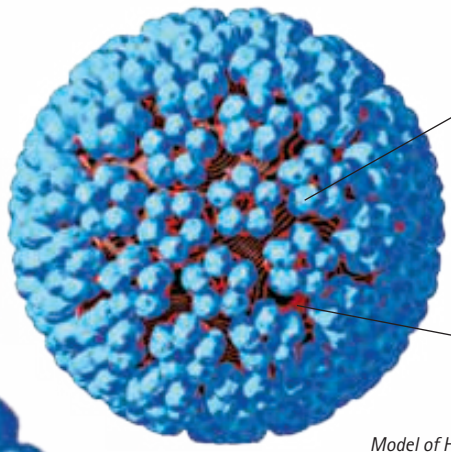
Beside the Papanicolaou staining will be used other diagnostic methods, a few will be assumed here.





Model of Human Papilloma Virus

Human Papilloma Virus – HPV

The discovery of Human Papilloma Virus (HPV) has led to remarkable progress in the diagnosis of cervical carcinoma. The HPV group, which now includes more than 100 subtypes, was first described by Meisels in the Nineteen-Eighties. More than 98% cervical squamous intraepithelial lesions (SIL) contain HPV DNA, which is PCR-detectable.

Low grade cervical SILs contain approximately 75% HPV of types 6, 11, as well as 16, 31, 33 and 35, while the remaining 25% contain various HPV types in decreasing frequency, including type 18. There are started prevention programs worldwide to prevent HPV infections. Young girls will be vaccinated against the especially aggressive types of HPV.

In contrast to this, types 16 and 18 are detectable in more than 80% of cervical carcinomas. In patients with mild, moderate or severe dysplasia (CIN I, II, III, CIN = Cervical intraepithelial neoplasia), clinical progression of the condition is diagnosed when persistent HPV infection occurs in association with a high-risk virus type, while patients in which no high-risk virus types have been detected do not develop cervical carcinoma. Cervical carcinoma is the first demonstrably virus-induced female carcinoma. The International Agency for Research on Cancer (IARC) has classified HPV types 16 and 18 as carcinogenic and types 31, 33 and 35 as potentially carcinogenic. Early contact with HPV may be the cause of a growing incidence of cancer. Detection of HPV DNA is closely associated with the age of the population being screened. Peaks of high-risk virus types are found in 20-25% of women aged 20-24 years, while in patients aged more than 35 years these high-risk virus types are only found in 4-5% of cases.

The use of an additional and objective test that is less susceptible to error can improve detection of severe dysplasia and the standardization of cytological results. Patients with cervical carcinoma have positive test results in 80% of cases when testing is done for subtypes 16, 18, 31 and 35. The aforementioned high-risk virus types pose an approximately 40% higher risk of the disease progressing than other HPV types.

The combination of cytology and HPV test is reported to help stop patients falling through the cervical carcinoma screening net. Screening is necessary, as 33 out of every 100,000 women died of cervical carcinoma. Tests are available for molecular HPV determination. The system provides method standardization and high result reproducibility. In the test 18 HPV types are qualitatively detected by a chemiluminescence method through hybridization in cervical specimens.

There are two standard HPV DNA groups.

- **Low risk HPV types:** 6, 11, 42, 43, 44
- **High risk HPV types:** 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 68

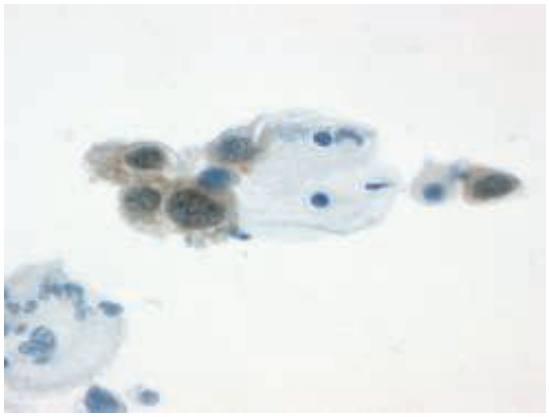
The test offers a simple and reliable procedure that is reproducible and standardizable and provides good sensitivity. A combination of cervical screening and HPV test detects 90% of abnormalities while, with cervical screening alone, only 75% of abnormalities are reportedly found, as studies in the UK and Europe have shown.

Molecular marker – p16

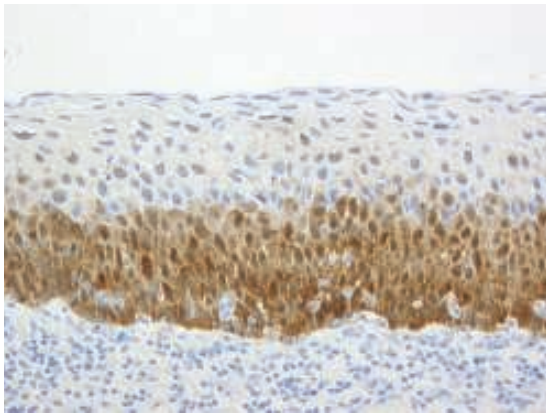
Protein p16 is considered to be a possible diagnostic marker for cervical carcinoma. p16 is an inhibitor of CDK4 and CDK6, a cyclin-dependent kinase inhibitor that plays an important role in cell cycle control, in which it phosphorylates the retinoblastoma product (pRb). p16 employs its anti-proliferative effects in binding to, and suppressing the action of, CDK4 and CDK6. The INK4a gene sits on chromosome loci 9p21, a region which is frequently exposed to hemi- and homozygotous disturbances in malignant diseases.

In the normal cell cycle, p16 expression is closely controlled. Expression of p16 has been observed in the pathogenesis of a number of malignomas, including cervical carcinomas, and is linked to HPV-positive and HPV-negative adenocarcinomas. Pronounced p16 overexpression is observed in cells of the cervix that are changed by high-risk types of Human Papilloma Virus. The HPV oncogens E6 and E7 are required to induce and sustain this transformation.

Overexpression of $p16^{INK4a}$ increases in cells with cervical dysplasia CIN I to CIN III and invasive carcinomas. The various stages are heterogenous with regard to $p16^{INK4a}$ expression. A number of CIN III lesions and invasive carcinomas are negative for $p16^{INK4a}$; reactive and inflammatory lesions are always negative. p16 detection is suitable for clarifying cases where differential diagnosis is difficult and for checking persistent dysplastic changes.



Portio smear, positive $p16^{INK4a}$ reaction in a typical cells of a moderate dysplasia CIN II



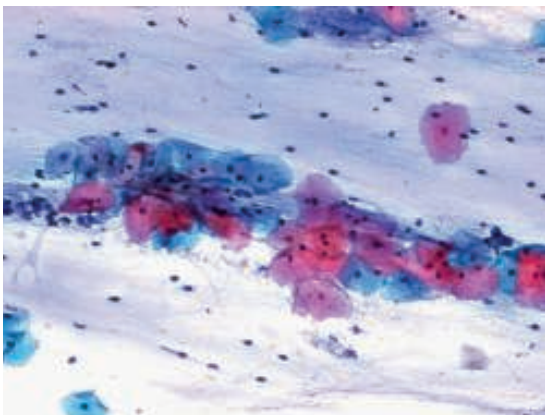
Paraffin section portio conus, positive $p16^{INK4a}$ reaction in deeper layer of dysplastic squamous cells

Automation in gynecological diagnosis

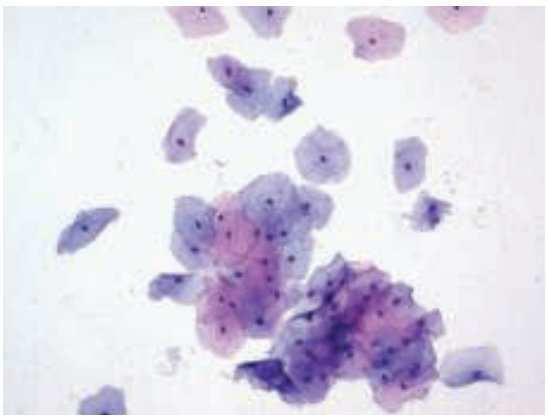
Since the Nineteen-Nineties there has been a trend towards the use of automated systems for screening gynecological specimens in cytology. These reportedly optimize the number of hits in conventional cytology. The systems based on optical systems and sophisticated PC calculation software. Also, special sample processing systems are available for preparing monolayer specimens.

The specimen will be scanned and permit 128 abnormal regions with 64 individual cells plus 64 abnormal cell groups to be localized. Approximately 20% (in the USA 10%) of all screened specimens are resampled for quality control, making this type of system particularly useful. Some systems need the specimens to be prepared by the monolayer method. The cell material is placed into a container with the preserving medium. The specimen material is fixed and stabilized by the preserving medium, representative cell material is placed on the slide using filter technology, and abnormal cells are investigated. The specimens are stained by the Papanicolaou method.

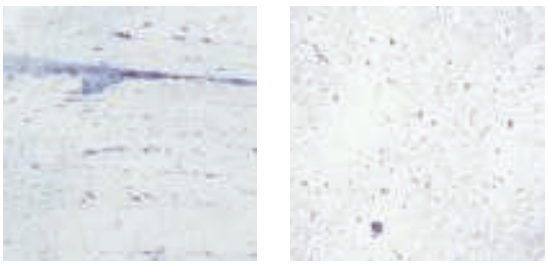
Not only gynecological but also clinical-cytological specimens such as urine sputum, lavages, fine needle aspiration biopsies, effusions and (for DNA cytometric analyses) monolayer specimens can be prepared and screened.



Papanicolaou stain – standard, Pap smear

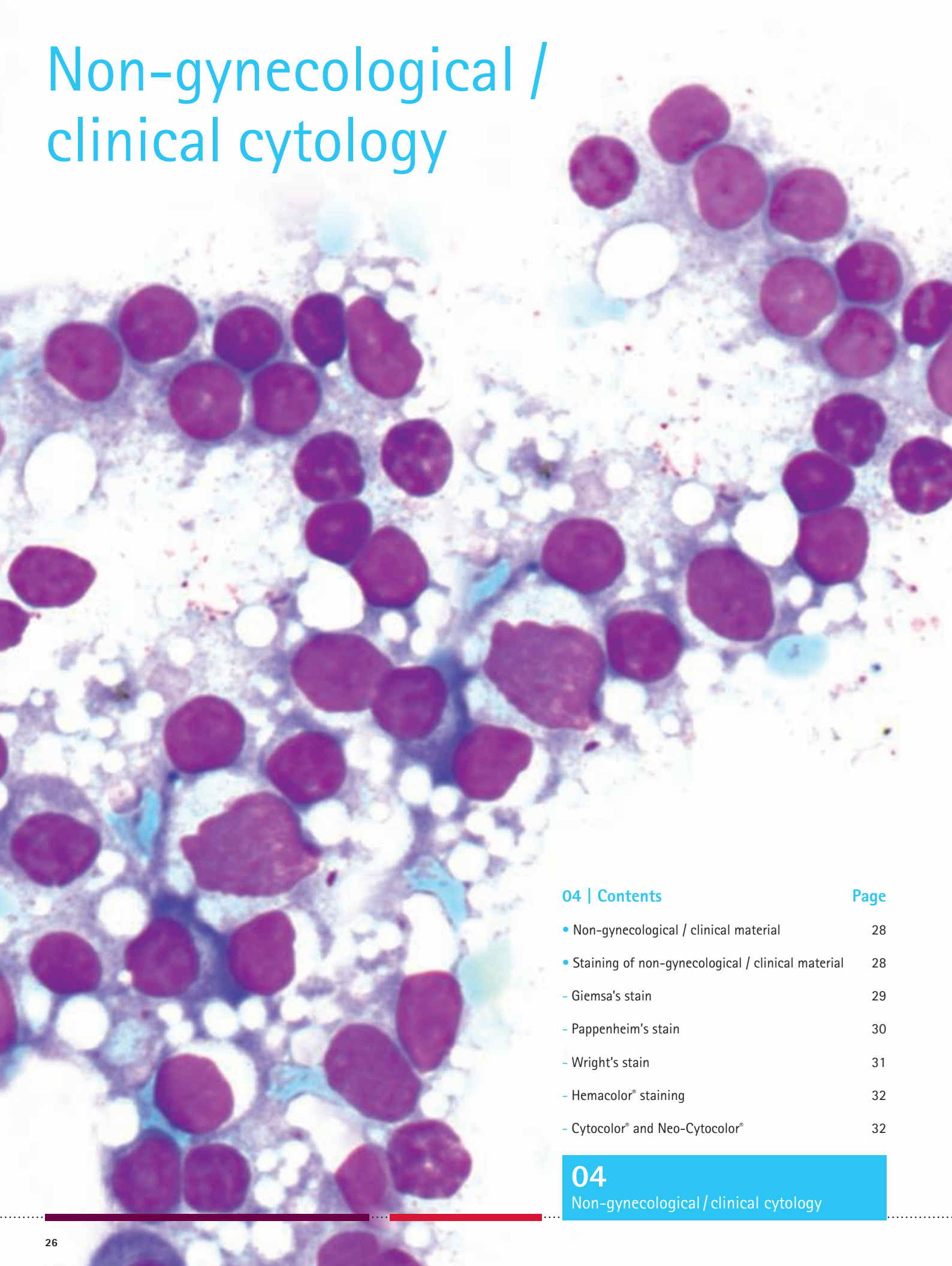


Papanicolaou stain – liquid based cytology, Pap smear



Papanicolaou stain – comparison of standard and liquid based cytology, Pap smear

Non-gynecological / clinical cytology



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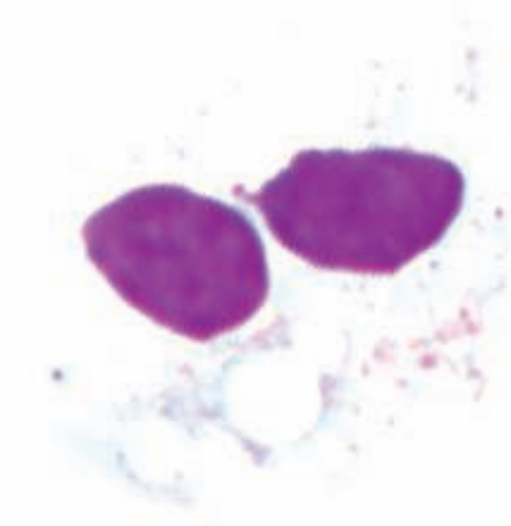
Introduction

Cytological investigation of bioptic material is very important for diagnosis. Virtually all locations and all organs can be reached by image-assisted or simple fine needle aspiration biopsy (FNAB) and any changes clarified diagnostically.

Alongside imaging techniques such as x-ray imaging, computer tomography (CT), ultrasound, nuclear spin tomography, positron emission tomography (PET), all of which can indicate space-requiring changes, FNAB, which enables material resulting from these changes to be obtained, is an indispensable part of diagnosis. The material is processed and investigated using a cytological method. In addition to FNAB various other methods have a firm place in the collection of specimen material for cytology.

- Pulmonary carcinomas can be diagnosed through examination of sputum and bronchial lavage material. Specimen material from peripheral pulmonary tumors localized through x-ray imaging can be collected under visual control by FNAB.
- Mammary cytology is a suitable way of differentiating between benign adenomatous nodes and mammary carcinomas.
- Esophageal and gastric carcinomas can be discovered by brush biopsy through a gastroendoscope and enable a high healing rate to be achieved when they are detected in an early stage.
- Cytological testing of urine samples on 3 successive days enables bladder and urethral tumors to be detected.
- Cytological investigation of body fluids such as pleural or peritoneal effusions, obtained by aspiration, can cast light on the origin and type of primary tumors as opposed to reactive processes.
- In the central nervous system, leukemias and lymphomas as well as inflammation and metastatic tumors can be detected in cerebrospinal fluid.
- Degenerative-inflammatory processes of the skeletal system can likewise by clarified by FNAB.



Non-gynecological / clinical material

Specimen material such as sputum, urine, body cavity effusions and lavage material is centrifuged, and the sediment subsequently smeared onto a microscope slide. When cytocentrifuges are used, the material is applied to the slide during centrifugation. FNAB material from the breast, thyroid, lymph nodes, prostate, cerebrospinal fluid and other localizations are carefully smeared onto slides and, depending on the staining method that will be used, are either fixed immediately (Papanicolaou's stain) or thoroughly dried in air (prior to hematological staining).

If biopsy material undergoes cytological examination prior to histology, then one or more imprints of the fresh material can be made on a microscope slide and either fixed immediately or thoroughly dried in air depending in the proposed staining technique.

Staining of non-gynecological / clinical material

In addition to the Papanicolaou stain, hematological stains are also being used for non-gynecological specimens all according to the experience and background of the diagnostician. Giemsa's stain is widely used for FNAB specimens from lymph nodes, while Pappenheim's stain is used for urinary sediments, effusions, bronchial lavage material, FNAB material from various locations (breast, thyroid, cerebrospinal fluid), and Wright's stain also for non-gynecological material. Within less than a minute, the Hemacolor® staining set provides results equivalent to those achieved with Pappenheim's stain.

Procedure

To obtain material it is often necessary to filter or sediment the material or to collect cell material directly through fine needle aspiration biopsy. For the various stains thoroughly air-dried smears are used, and drying times should be not less than 30 minutes.

Giemsa's stain

The very versatile Giemsa's stain is also eminently suitable for non-gynecological / clinical material. Nuclei and cytoplasm are stained in such a way that structural details are very apparent and differentiation is simplified.

Preparation

1. Buffer solution
Dissolve 1 buffer tablet* in 1 L of distilled water.
*111374 or 109468 depending on the required reaction color

2. Diluted Giemsa's solution for manual staining
Dilute 10 ml Giemsa's azure eosin methylene blue solution with 190 ml buffer solution, mix well, leave to stand for 10 min, and filter if necessary.

3. Diluted Giemsa's solution for staining in an automated staining device
Slowly add 25 ml of Giemsa's azure eosin methylene blue solution to 275 ml buffer solution, mix and leave to stand for 10 min, and filter if necessary.

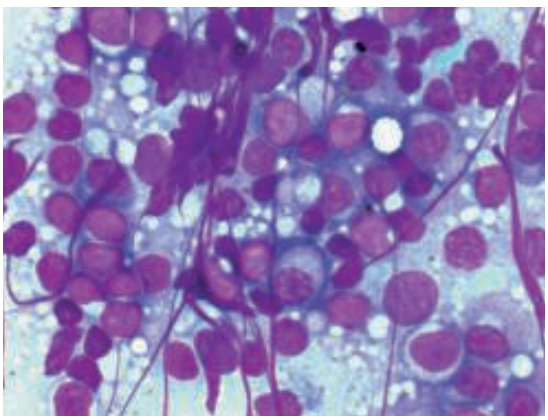
4. Giemsa's azure eosin methylene blue solution
Dissolve 0.76 g of Giemsa's azure eosin methylene blue in 50 ml of glycerol and heat for 3 h at 60°C on a water bath, add 50 ml of methanol, leave to stand for 5 days and filter.

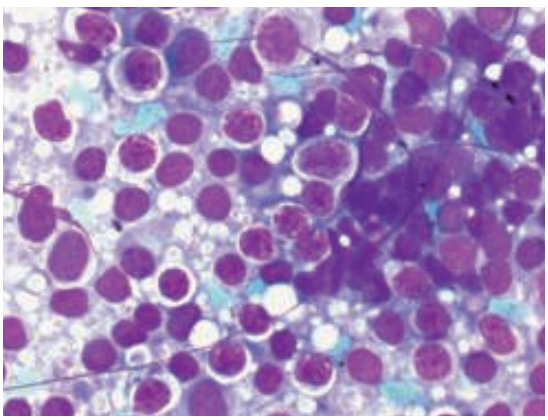
Staining rack / Coplin jars	
Methanol	3-5 min
Diluted Giemsa's staining solution	15-20 min
Rinse with buffer solution	2 x 1 min
Drying	

Staining in a stainer	
Methanol	3 min
Diluted Giemsa's solution	20 min
Buffer solution	1 min
Running water (wash)	2 min
Drying	3 min

Result	
Nuclei	red to violet
Cytoplasm	grey-blue, blue to dove-blue
Erythrocytes	reddish

Product	Package size	Cat. No.
Giemsa's azure eosin methylene blue solution	100 ml, 500 ml, 1 L, 2.5 L	109204
Giemsa's azure eosin methylene blue	25 g, 100 g	109203


Giemsa stain, tumor imprint


Giemsa stain, tumor imprint

Pappenheim's stain

Standard stain with May-Grünwald's solution and Giemsa's solution, enabling excellent results to be obtained for non-gynecological / clinical material.

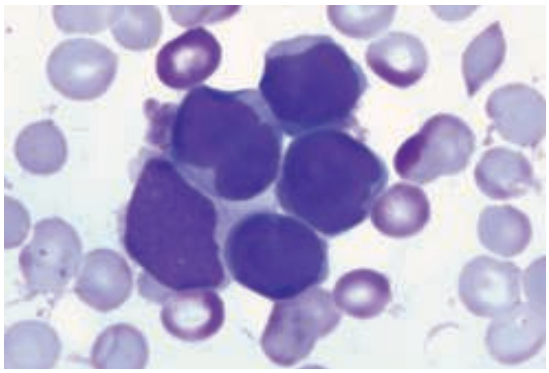
Staining rack	
Cover the smear with 1 ml of May-Grünwald's solution	3 min
Add 1 ml buffer solution, mix and stain	3-5 min
Cover with diluted Giemsa's solution and stain	15-20 min
Rinse with buffer solution	
Dry	

Staining in coplin jars	
May-Grünwald's solution	3-5 min
Diluted Giemsa's staining solution	15-20 min
Rinse with buffer solution	2 x 1 min
Dry	

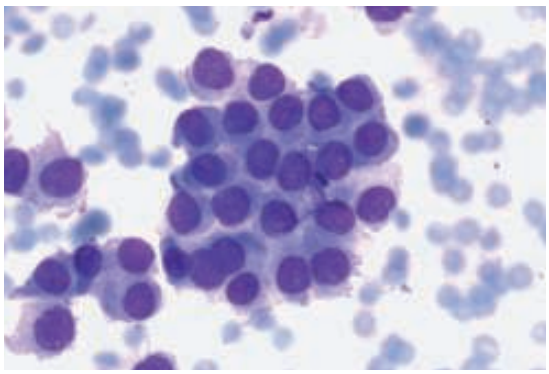
Result	
Nuclei	purple to violet
Cytoplasm	blue to dove-blue
Erythrocytes	reddish

Results with Weise's phosphate buffer pH 6.8

Product	Package size	Cat. No.
Giemsa's azure eosin methylene blue solution	100 ml, 500 ml, 1 L, 2.5 L	109204
May-Grünwald's eosin methylene blue solution	100 ml, 500 ml, 1 L, 2.5 L	101424



Pappenheim stain, smear, FNAB tumor



Pappenheim stain, smear, FNAB tumor

Wright's stain

Wright's stain can likewise be used for non-gynecological / clinical material.

Preparation

1. Buffer solution
Dissolve 1 Weise's buffer tablet* in 1 L distilled water.
*111374 or 109468 depending on the required reaction color

2. Diluted Wright's solution for manual staining
Add 20 ml of buffer solution and 150 ml of distilled water to 30 ml of Wright's eosin methylene blue solution.

3. Diluted Wright's solution for staining in an automated staining device
Add 30 ml of buffer solution and 220 ml of distilled water to 50 ml of Wright's eosin methylene blue solution.

4. Wright's eosin methylene blue solution
Dissolve 0.25 g of Wright's eosin methylene blue in 100 ml of methanol, warm gently on a water bath for 20-30 min or until the dye is dissolved, and filter before use.

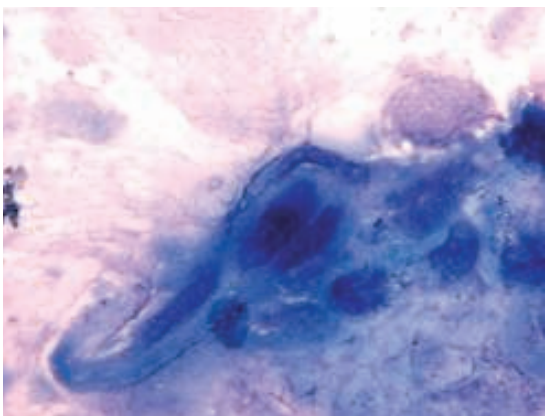
Staining rack	
Covering with Wright's stock solution	1 min
Buffer solution (1 ml) add, mix and stain	4 min
Rinse with buffer solution	2 x 1 min
Dry	

Staining in coplin jars	
Wright's solution	3 min
Diluted Wright's solution	6 min
Rinse with buffer solution	2 x 1 min
Dry	

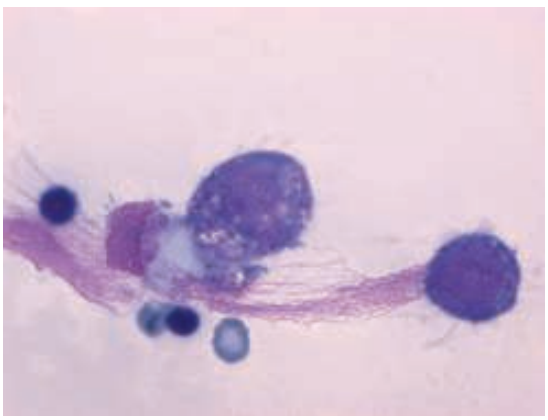
Staining in a stainer	
Wright's solution	3 min
Diluted Wright's solution	6 min
Buffer solution	1 min
Running water (wash)	2 min
Dry	3 min

Result	
Nuclei	red to violet
Cytoplasm	grey-blue
Erythrocytes	reddish

Product	Package size	Cat. No.
Wright's eosin methylene blue solution	500 ml, 1 L, 2.5 L	101383
Wright's eosin methylene blue	25 g	109278



Wright stain, smear, bronchial lavage



Wright stain, smear pleura effusion

Non-gynecological / clinical cytology

Staining of non-gynecological / clinical material

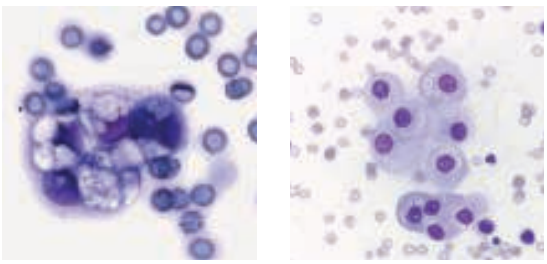
Hemacolor® staining | Cytocolor® and Neo-Cytocolor®

Hemacolor® staining

Within less than a minute, Hemacolor® provides results equivalent to those of Pappenheim's stain. The staining set contains the fixing solution and a red and blue staining solution plus phosphate buffer tablets acc. to Weise, pH 7.2, which additionally ensure that the results are highly stable. Staining should be done in coplin jars, and the specimens should be moved in the solution in order to permit the dyes to react optimally with the target structures in the material.

Staining in coplin jars	
Hemacolor® solution 1	5 x 1 sec.
Hemacolor® solution 2	3 x 1 sec.
Hemacolor® solution 3	6 x 1 sec.
Rinse in buffer solution pH 7.2	2 x 10 sec.
Dry	

Result	
Nuclei	red to violet
Cytoplasm	light grey, blue
Erythrocytes	reddish



Hemacolor® stain, smear body effusion

Product	Package size	Cat. No.
Hemacolor® staining set	3 x 100 ml, 3 buffer tablets	111674
Hemacolor® staining set	3 x 500 ml, 6 buffer tablets	111661

Cytocolor® and Neo-Cytocolor®

Cytocolor®, which is based on the modified Papanicolaou stain acc. to Szczepanik, is a very efficient way of rapidly staining non-gynecological / clinical material such as FNAB, tumor smears, urine, sputum, body effusions and bronchial lavage material for diagnostic purposes. Stained slides are available in less than 3 minutes. Cytocolor® contains all the reagents needed for staining in a set. The set contains not only hematoxylin and polychromic solution but also 2-Propanol and xylene. Slides are fixed immediately with M-FIX™ spray fixative or placed for 30 min in ethanol. The reagents are filled in the order stated into coplin jars which should be covered following staining. Immerse and agitate the slides in the solutions. Simple immersion produces unsatisfactory stains.

Procedure		
1.	Distilled water	10 x 1 sec.
2.	Modified hematoxylin solution	1 x 1 min
3.	Rinse under running tap water	1 x 5 sec.
4.	2-Propanol	2 x 1 sec.
5.	Modified polychromic solution	1 x 1 min
6.	80% 2-Propanol	5 x 1 sec.
7.	2-Propanol	5 x 1 sec.
8.	2-Propanol	5 x 1 sec.
9.	Xylene	5 x 1 sec.
10.	Xylene	5 x 1 sec.

Mount the xylene-moist specimen immediately with M-GLAS™ (without a coverslip) or with Entellan® new in conjunction with a coverslip.

Result	
Nuclei	blue, dark violet, black
Cytoplasm	pink, blue-green
Erythrocytes	red

Staining of non-gynecological / clinical material
Cytocolor® and Neo-Cytocolor®

Neo-Cytocolor®

Neo-Cytocolor® is based on the same principle as Cytocolor®. Xylene is replaced by Neo-Clear®. With Neo-Cytocolor® will be accomplished the same speed and reproducibility for the application in connection with user- and environmental-friendliness of Neo-Clear® and Neo-Mount® at the end.

Preparation

Fix specimens while still moist with spray fixative (M-FIX™) or for 30 minutes in ethanol.

Procedure

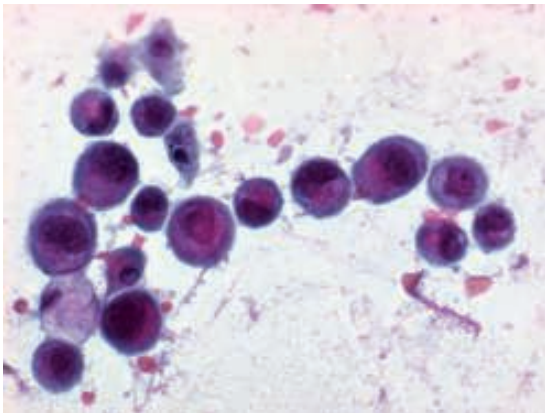
1.	Distilled water	10 x 1 sec.
2.	Modified hematoxylin solution	1 x 1 min
3.	Rinse under running tap water	1 x 5 sec.
4.	2-Propanol	2 x 1 sec.
5.	Modified polychromic solution	1 x 1 min
6.	80% 2-Propanol	5 x 1 sec.
7.	2-Propanol	5 x 1 sec.
8.	2-Propanol	5 x 1 sec.
9.	Neo-Clear®	5 x 10 sec.
10.	Neo-Clear®	5 x 10 sec.

Mount the Neo-Clear®-moist specimen immediately with Neo-Mount® and a coverslip.

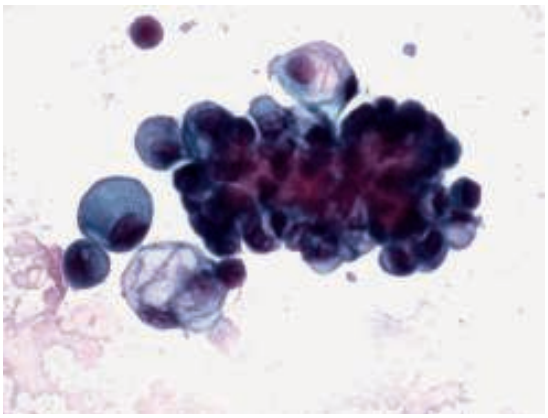
Result

Nuclei	blue, dark violet, black
Cytoplasm	pink, blue-green
Erythrocytes	red

Product	Package size	Cat. No.
Cytocolor® kit	6 x 500 ml	115355
Neo-Cytocolor® kit	6 x 500 ml	101971



Cytocolor® stain, smear body effusion



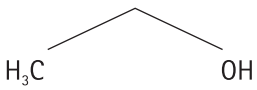
Cytocolor® stain, smear body effusion

Dehydration and clearing

Ethanol

Ethanol [C_2H_5OH], is among the most frequently used solvents in cytology. It is used in cytology, for the ascending and descending alcohol series employed in staining, for rinsing during staining as well as in numerous staining solutions. Baths must be exchanged often to prevent loss of quality seen as decolored or milky-turbid slides.

Ethanol C_2H_5OH



Being flammable, ethanol bears the F hazard symbol. Safety instructions and other information on how to handle ethanol without risk are contained in the safety data sheet which is available via the Web or upon request.

Ethanol is a solvent having the following physicochemical properties:

Chemical and physical data	
Ignition temperature	363°C (DIN 51794)
Solubility in water	(20°C) soluble
Melting point	-114.5°C
Molar mass	46.07 g/mol
Density	0.790-0.793 g/cm³ (20°C)
pH	7.0 (10 g/l, H_2O, 20°C)
Boiling point	78.3°C
Vapor pressure	59 hPa (20°C)
Explosive limits	3.5-15% (V)
Flash point	13°C c.c.
Refractive index	1.36

Ethanol is used in an undenatured grade with a purity of >/= 99.9% or else as a 96% solution. The undenatured grades (in Germany) have certain taxes imposed on them, making them quite pricey. Besides the undenatured grade there is also denatured ethanol, which contains about 1% MEK (methyl ethyl ketone). This denaturant has been used for many years and has proven so popular because it has no bearing on cytological results. Being denatured, it does not attract special taxes so that this grade should be given preference in routine applications.

In addition to concentrated ethanol solutions procedures often call for diluted ones. These can be prepared manually as required. For re- and de- hydrations 50%, 70% and 80% solutions are used. Ethanol comes, of course, in numerous grades ranging from for analysis right down to technical grade, and also as reworked solutions. As elsewhere, the rule is that using good solvent grades tends to prevent problems in the workflow and with the results. A realistic cost-benefit analysis should be conducted from time to time to enable changes to be made where necessary.

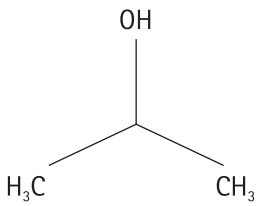
Product	Package size	Cat. No.
Ethanol absolute for analysis EMSURE® ACS, ISO, Reag Ph Eur	1 L, 2.5 L, 5 L	100983
Ethanol denatured with about 1% methyl ethyl ketone for analysis EMSURE®	1 L, 2.5 L	100974

2-Propanol

2-Propanol [C_3H_8O], is an alcohol used as a laboratory solvent. 2-Propanol can also be used for the alcohol series of ascending concentrations for rehydrating and dehydrating specimens. It acts more slowly than ethanol.

Product	Package size	Cat. No.
2-Propanol for analysis EMSURE® ACS, ISO, Reag. Ph Eur	1 L, 2.5 L	109634

2-Propanol C_3H_8O



Being flammable and irritant, 2-Propanol bears the F and Xi hazard symbols.

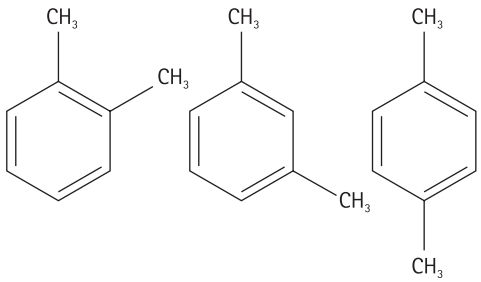
2-Propanol is a solvent having the following physicochemical properties:

Chemical and physical data	
Ignition temperature	425°C (DIN 51794)
Solubility in water	(20°C) soluble
Melting point	-89.5°C
Molar mass	60.1 g/mol
Density	0.786 g/cm$_3$ (20°C)
pH	(H_2O, 20°C) neutral
Boiling point	82.4°C (1013 hPa)
Vapor pressure	43 hPa (20°C)
Explosive limits	2-13.4% (V)
Flash point	17°C, open crucible
Refractive index	1.378
Water absorption	1,000 g/kg
Evaporation index	11

Xylene

Xylene is an aromatic solvent [$C_6H_4(CH_3)_2$], which has been used for decades in clarification applications. A mixture of the ortho-, meta- and para-isomers of xylene is used.

Xylene $C_6H_4(CH_3)_2$



Xylene is a solvent having the following physicochemical properties:

Chemical and physical data	
Ignition temperature	490°C (DIN 51794)
Solubility in water	0.2 g/l (20°C)
Saturation concentration (air)	35 g/m³ (20°C) air
Melting point	> -34°C
Molar mass	106.17 g/mol
Density	0.86 g/cm³ (20°C)
pH	(H_2O) not applicable
Boiling point	137-143°C
Vapor pressure	10 hPa (20°C)
Explosive limits	1.7-7.0% (V)
Flash point	25°C
Kinematic viscosity	0.85 mm²/s (201°C)
Evaporation index	13.5

The advantage of xylene is: it works very quickly and efficiently for dehydration.

Various grades of xylene are available ranging from for analysis to technical grade, but difficulties may be encountered when the less good grades are used. The stain and also its durability may be adversely affected when the xylene is of a grade that is too low. Xylene is a solvent that can be reworked. There are systems on the market that allow spent xylene to be processed, i.e. distilled, so it can be recycled and re-used. It should be borne in mind, however, that this type of reworking is always associated with a certain loss of quality and any xylene that has been recycled must be replaced before quality loss in the specimen material being treated can occur.

The problem with xylene, a hazardous substance with an Xn classification, is that it is flammable, harmful and an irritant, and has a high evaporation rate and a characteristic odor. The odor quickly reveals where xylene is being used. Laboratories must have good ventilation and any work involving xylene must be carried out under an extraction hood.

Safety instructions and other information on how to handle xylene without risk are contained in the safety data sheet which is available via the Web or upon request.

Aromatic solvents such as xylene are known to cause damage to the liver, hematopoetic organs and the lymphatic system as a result of intense exposure. The LD 50 oral dose for the rat is 2,840 mg/kg; LD 50 dermal for the rabbit is > 4,350 mg/kg.

Product	Package size	Cat. No.
Xylene for analysis EMSURE® ACS, ISO, Reag. Ph Eur	1 L, 2.5 L	108661

Neo-Clear®

Neo-Clear® is a solvent based on an aliphatic hydrocarbon. It is used for clearing in the Papanicolaou staining technique instead of xylene. The stained specimens, which must be anhydrous before being mounted, are normally cleared in xylene following dehydration with alcohol. Xylene, the gold standard for clearing in cytology / histology, is an aromatic solvent that can be absorbed through the lungs and skin and so impair metabolic functions and cause irreversible damage. Information on the classification of this substance is contained in the safety data sheet, available at www.merckmillipore.com or in Merck ChemDat.

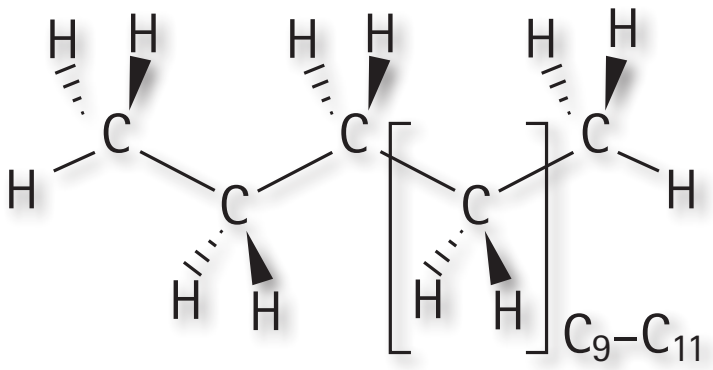
Neo-Clear® belongs to the group of so-called isoparaffins. Neo-Clear® also bears hazard warnings but is less harmful to the user than xylene. A prerequisite condition for the use of xylene substitute is that it can be used in the same way, that no major change in the method is required, and that results are identical.

The quality of the alcohol used is important as Neo-Clear® is water-immiscible and, also, water causes clouding and possibly to the formation of two phases (unmixing of the water-solvent mixture) in the Neo-Clear® bath. Consequently, technical grades should be used with caution, as the stated and actual alcohol concentrations are not always exact. Neo-Clear® can be used in existing protocols almost without change. See Papanicolaou's stain. In order to ensure that the usual optical brilliance is achieved, slides treated with Neo-Clear® must be mounted with the matching mounting medium, Neo-Mount®, which contains Neo-Clear® as solvent. Suboptimal results are obtained with mounting media based on xylene or toluene.

The advantages of Neo-Clear® are:

- Aliphatic hydrocarbon
- Virtually odorless
- Same results as with xylene
- No change or only marginal change in method required
- Low rate of evaporation
- No higher alcohol requirement
- Best optical results when mounting with Neo-Mount®
- Very stable stain

Product	Package size	Cat. No.
Neo-Clear®	5 L, 25 L	109843



Neo-Clear® is an aliphatic hydrocarbon mixture with a chain length of C_9-C_{11}

Mounting

Long-term specimens are mounted with mounting media that are first applied to the slide in dissolved form and then harden as the solvent evaporates. The refractive index of mounting media is approximately 1.5 and is close to that of glass. For optimum optical properties, transparency and brilliance of specimens it is important to ensure that a mounting medium is used that, as base, contains the solvent that was used for clearing.

Mounting with a coverslip

Cytological specimens must be completely dehydrated before they are mounted. In the final stage, xylene or Neo-Clear®, the xylene-substitute, should be used for clearing. To mount a specimen, use a glass rod to drop approximately 0.5 ml of an anhydrous mounting medium such as Entellan®, Entellan® new, Canada balsam, DPX new or Neo-Mount®* to the horizontally positioned microscope slide so as to fill the space between the slide and the coverslip. As soon as even distribution over the entire specimen is assured, carefully apply a clean coverslip in such a way as to exclude air bubbles. Then allow the slide to lie horizontally for approximately 30 minutes until it is dry and ready for microscopic examination. Slides prepared in this way are color-stable for 10 years.

*** Note: Allow Neo-Clear® to run off the slide prior to mounting by placing for 1–2 minutes on filter paper in order to prevent the formation of bubbles and slow drying caused by an excess of Neo-Clear®.**

Advantages of Neo-Mount® during mounting
- Aliphatic solvent
- Virtually odorless
- Only marginal change in procedure
- Optical brilliance

Advantages of mounting agents containing xylene / toluene during mounting
- Optical brilliance
- Good drying times
- No air bubbles under the coverslip
- Stained slides have excellent color stability

Product	Package size	Cat. No.
Neo-Clear®-based		
Neo-Mount®	100 ml, 500 ml	109016

Product	Package size	Cat. No.
Xylene-based		
Entellan® new rapid mounting medium	100 ml, 500 ml, 1 L	107961
Canada balsam	100 ml	101691
DPX new water-free mounting medium	500 ml	100579
Toluene-based		
Entellan® rapid mounting medium	500 ml	107960

Mounting without a coverslip

M-GLAS™ is used in cytology without a coverslip in order to achieve even coating of specimens. Apply a few drops of mounting medium to the specimen and make sure it is evenly distributed. The solvent evaporates to leave a solid protective film.

The M-GLAS™ layer is not resistant to immersion oil. In fact it softens on contact with immersion oil and loses its transparency at the point of contact. This can be rectified by wiping off the immersion oil and applying a new thin layer of M-GLAS™ or immersing the slide in xylene. M-GLAS™ can be completely removed and reapplied.

Advantages of M-GLAS™ during mounting

- Time saving
- Space saving in archive
- Good drying times
- No air bubbles under the coverslip
- Stained slides have excellent color stability

Product	Package size	Cat. No.
M-GLAS™	500 ml	103973

Mounting using an automated mounting instrument (coverslipper)

An automated coverslipper may be used to mount cytological specimens. The mounting medium Entellan® new for automated mounting instruments has a narrow viscosity range, so that when the specimen changes there is no time-consuming setting of the quantity of mounting medium to be applied.

Entellan® new for automated mounting instruments is suitable for commercial coverslippers that work with glass coverslips. The mounting medium Entellan® new for automated mounting instruments is used as instructed in the coverslipper manual. The optimum quantity of mounting medium is obtained in an initial run with blank coverslips and slides based on the size / thickness of the coverslip and checked when the bottle is changed.

Advantages of Entellan® new for automated mounting instruments

- Time saving at change-over owing to narrow viscosity range
- Good drying times
- No air bubbles under the coverslip
- Stained slides have excellent color stability

Product	Package size	Cat. No.
Entellan® new for automated mounting instruments	500 ml	100869

Reagents

The methods mentioned in this brochure require various reagents. They are listed here together with details of package sizes, etc.

Classic Papanicolaou stain

Product	Package size	Cat. No.
Papanicolaou's solution 1a, Harris' hematoxylin solution	500 ml, 1 L, 2.5 L	109253
Papanicolaou's solution 1b, Hematoxylin solution S	500 ml, 2.5 L	109254
Hematoxylin solution acc. to Gill II	500 ml, 2.5 L	105175
Hematoxylin solution acc. to Gill III	500 ml, 1 L, 2.5 L	105174
Hematoxylin monohydrate (C.I. 75290) for microscopy Certistain®	25 g, 100 g	115938
Hematoxylin cryst. (C.I. 75290) for microscopy	25 g, 100 g	104302
Hematein (C.I. 72590) for microscopy	25 g	111487
Papanicolaou's solution 2a, Orange G solution	500 ml, 1 L, 2.5 L	106888
Papanicolaou's solution 2b, Orange II solution	500 ml, 2.5 L	106887
Papanicolaou's solution 3a, Polychromic solution EA 31	500 ml, 2.5 L	109271
Papanicolaou's solution 3b, Polychromic solution EA 50	500 ml, 1 L, 2.5 L	109272
Papanicolaou's solution 3c, Polychromic solution EA 65	100 ml	109270
Papanicolaou's solution 3d, Polychromic solution EA 65	100 ml, 2.5 L	109269

Also required for Papanicolaou staining (nuclear staining)

Product	Package size	Cat. No.
Ethanol absolute for analysis EMSURE® ACS, ISO, Reag. Ph Eur	1 L, 2.5 L	100983
Aluminium potassium sulfate dodecahydrate for analysis EMSURE® ACS, Reag. Ph Eur	1 kg	101047
Acetic acid (glacial) 100% anhydrous for analysis EMSURE® ACS, ISO, Reag. Ph Eur	1 L	100063
Sodium iodate for analysis EMSURE®	100 g	106525
Aluminium sulfate-18-hydrate extra pure Ph Eur, BP	5 kg	101102
Ethylene glycol for analysis EMSURE® Reag. Ph Eur, Reag. USP	1 L	109621
Thymol blue indicator ACS, Reag. Ph Eur	5 g	108176

Modified Papanicolaou stain

Product	Package size	Cat. No.
Cytocolor®	1 kit with 6 x 500 ml bottles	115355
Solution 1: Modified hematoxylin solution	500 ml	
Solution 2: Modified polychromic solution	500 ml	
Solution 3: 2-Propanol for analysis	3 x 500 ml	
Solution 4: Xylene for analysis	500 ml	
Neo-Cytocolor®	1 kit with 6 x 500 ml bottles	101971
Solution 1: Modified hematoxylin solution	500 ml	
Solution 2: Modified polychromic solution	500 ml	
Solution 3: 2-Propanol for analysis	3 x 500 ml	
Solution 4: Neo-Clear®	500 ml	

Hormonal dysfunctions

Product	Package size	Cat. No.
Shorr staining solution	500 ml	109275

Non-gynecological / clinical methods

Product	Package size	Cat. No.
Giemsa's azure eosin methylene blue solution	100 ml, 500 ml, 1 L, 2.5 L	109204
Giemsa's azure eosin methylene blue	25 g, 100 g	109203
May-Grünwald's eosin methylene blue solution	100 ml, 500 ml, 1 L, 2.5 L	101424
May-Grünwald's eosin methylene blue	25 g, 100 g	101352
Wright's eosin methylene blue solution	100 ml, 500 ml, 2.5 L	101383
Wright's eosin methylene blue	25 g	109278
Methanol for analysis EMSURE® ACS, ISO, Reag. Ph Eur	1 L, 2.5 L	106009
Glycerol (about 87%) extra pure, Ph Eur, BP	1 L, 2.5 L	104091
Buffer tablets acc. to Weise pH 7.2	1 pack (100 tabs)	109468
Buffer tablets acc. to Weise pH 6.8	1 pack (100 tabs)	111374
Hemacolor® staining set	3 x 100 ml, 3 buffer tablets	111674
Hemacolor® staining set	3 x 500 ml, 6 buffer tablets	111661
Hemacolor® solution 1, fixing solution	2.5 L	111955
Hemacolor® solution 2, color reagent red	2.5 L	111956
Hemacolor® solution 3, color reagent blue	2.5 L	111957
Cytocolor® kit	6 x 500 ml bottles	115355
Neo-Cytocolor® kit	6 x 500 ml bottles	101971

Auxiliary reagents

Product	Package size	Cat. No.
M-FIX™	100 ml	103981
Entellan® new	100 ml, 500 ml, 1 L	107961
Entellan®	500 ml	107960
Canada balsam	25 ml, 100 ml	101691
DPX new water-free mounting medium	500 ml	100579
M-GLAS™	500 ml	103973
Entellan® new for automated mounting instruments	500 ml	100869
Neo-Mount®	100 ml, 500 ml	109016
Immersion oil	100 ml, 500 ml	104699
Immersion oil acc. to ISO 8036-1	100 ml	115577
Neo-Clear® (xylene substitute)	5 L, 25 L	109843
Xylene for analysis EMSURE® ACS, ISO, Reag. Ph Eur	1 L, 2.5 L	108661
Ethanol denatured with 1% methyl ethyl ketone for analysis EMSURE®	1 L, 2.5L	100974
Ethanol absolute for analysis EMSURE® ACS ISO, Reag. Ph Eur	1 L, 2,5 L	100983
2-Propanol for analysis EMSURE® ACS, ISO, Reag. Ph Eur	1 L, 2,5 L	109634

References

Abbreviations

Pap	Papanicolaou, Papanicolaou smear, Papanicolaou stain
CIN	Cervical intraepithelial neoplasia
CIN I	Cervical intraepithelial neoplasia grade I (slide / mild grade)
CIN II	Cervical intraepithelial neoplasia grade II (moderate grade)
CIN III	Cervical intraepithelial neoplasia grade III (high grade)
LGSIL	Low grade squamous intraepithelial lesion
HSIL	High grade squamous intraepithelial lesion
SIL	Squamous intraepithelial lesion
HPV	Human Papilloma Virus
HBV	Hepatitis B Virus
ASCUS	Atypical squamous cells of undetermined significance

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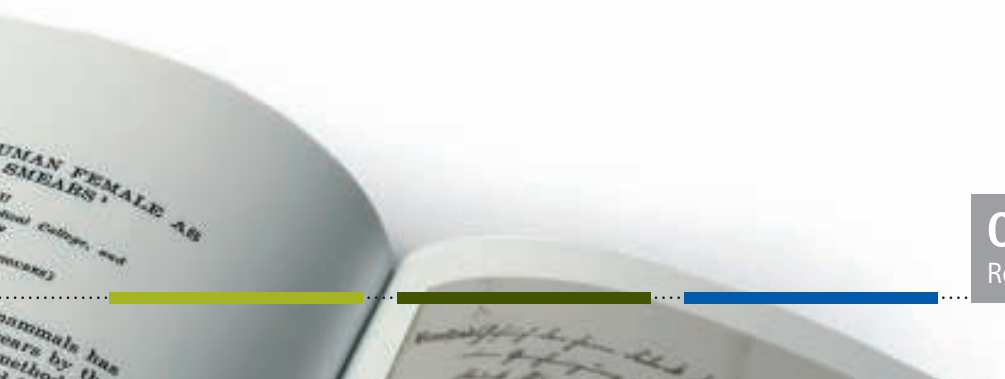

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