Cytology
staining method
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.
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 examinations 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 minutes. 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 minutes in ethanol to preserve the fine structures of the cell material.

Ethanol absolute, for analysis. Ethanol absolute with about 4% isopropyl alcohol.

DdFixx 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 DdFixx spray fixative so that the specimen is evenly wetted, but not drenched, with 0.3 to 0.6 ml of DdFixx. 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 DdFixx spray fixative evaporates, leaving a protective film of polyethylene glycol on the specimen. After approximately 10 minutes 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 approximately 10 second in order to remove the polyethylene glycol film. The descending alcohol series before the haematoxylin step in the Papanicolaou method can be omitted.

Advantages of DdFixx 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 approximately 10 minutes
• 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 code 22-112 - 22-113
Package 10x150 ml - 10x50 ml
Staining method

Papanicolaou stain

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

<table>
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<th>Orange stain</th>
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<tr>
<td>Haematoxylin stain</td>
<td>+</td>
<td>Orange stain</td>
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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.

Nuclear staining

Nuclear staining is accomplished using the natural dye, haematoxylin. Haematoxylin dye is mixed with a trivalent metal salt to produce a so-called haematoxylin 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 (Al³⁺, Fe³⁺, Cr³⁺) 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 haematoxylin 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 haematoxylin, i.e. hematein, present and that the oxidized dye is available in sufficient quantity throughout its entire declared useful life. The most commonly used staining solutions are listed below.

Harris’ haematoxylin 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 haematoxylin is just the same as using mercury. The staining solution must be filtered before use. Its applications are in cytology and histology using progressive and regressive methods.
**Gill's haematoxylin solutions**

contain various amounts of haematoxylin and use iodate as oxidizer, so they are more environment-friendly. Haematoxylin solution according to Gill II is suitable for use in cytology, stains delicately cell nuclei.

The staining solution should only be used for the progressive method. Gill haematoxylin modified solution stains more intensely, faster and has a more durable stain activity.

**Gill's solutions do not have to be filtered before use.**

EA50 polychromatic solution code 09-266
EA50 normal solution code 09-266-2
OG6 polychromatic solution code 09-2635
OG6 normal stain code 09-263-2
Harris haematoxylin solution code 09-182-1
Harris haematoxylin Liscia stabilized 09-181
Gill modified solution code 09-178-1
Gill II solution code 09-178-II
Carazzi haemallum solution code 09-146
Mayer haematoxylin normal solution 09-168
Mayer haematoxylin modified solution 09-168-B
Polychromatic cytology haematoxylin 09-270

**Packaging**

500-1000-2500-5000 ml or on request
Staining with orange solution

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 colours the target structures reddish-orange.

Orange II polychromatic solution code 09-264
Orange II normal solution code 09-264-2
OG6 polychromatic solution code 09-263
OG6 normal stain code 09-263-2
The second staining step for cytoplasm is staining with a polychromatic mixture of Eosin G, Light Green SF.

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:

EA31 and EA50, while EA65 is preferred for mucous material such as sputum, bronchial secretions and other non-gynecological material. 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 been stained with Light Green SF.

EA50 polychromatic solution code 09-266
EA31 polychromatic solution code 09-265
EA65 polychromatic solution code 09-267
Two methods can be distinguished. In the progressive haematoxylin 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 haematoxylin is used to over stain, 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.
Liscia & De Marchi fast cytology stain

**Liscia & De Marchi stain**

is a review and speed up of a method that is operating with different principle but is having the same result. In the first staining step the nuclei are quickly stained (one minute maximum time), with solution one. Nuclei are stained blue, dark violet to black.

The second step (solution two) is a cytoplasmic staining, for demonstration of mature and keratinized cells. The target structures are stained orange in different intensities, at the same time squamous cells are differentiated.

**Sample material**

Gynaecological and non-gynaecological specimens such as sputum, urine, FNAB, body effusions, lavages.

**Method**

Smear fixed to air step one

1) Deep rapidly 10-15 times for one-second section into physiologic solution just opened.
2) Alcohol 96°C deep rapidly 10 - 15 times slides for one-second.

Note: smear fixed with DdFixx (code 22-112), start from step two.
3) Stain slides into solution one from 30 to 60 second gently agitates.
4) Water tap to remove excess 10 - 12 second.
5) Blue water 20 - 30”.

If manually method is used, keep jar on the top of a white paper to see the virages.
6) Water taps 5 - 6 deep of one second each.
7) Alcohol 96°C 8 - 10 immersion 1 second each.
8) Counterstain with solution 2 for 30 to 60 second gently agitates
9) Alcohol 96°C deep rapidly 10 - 15 times slides 1 second.
10) Alcohol absolute 10 - 15 deep of 1 second each.
11) Xylene 10 deep of one second.
12) Mount with DdMount

**Technical’s note:** staining time vary according to age, types of solutions, thickness of sections, et.

When Gill (code 09-178) modified solution is used, get the best result, staining time (maximum 1-5 minutes), for best change in colour, wash quickly in tap water, and then in Scott acidulated solution, (code 00-136).

**Result**

Cytoplasm:

- Cyanophilic (basophilic) blue green intense
- Eosinophilic (acidophil) pink
- Keratinized pink-orange
- Erythrocytes red
- Nuclei blue black, dark violet
Gynecological results classification

The classification of cytological findings is according to the group definition. Classifications is according the Bethesda nomenclature, which was introduced in 1989 by the International Academy of Cytology in the USA. Accordingly, 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, 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, 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

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, 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.

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.

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

**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.

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

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

Recommendation
Colposcopic-cytological control in 3 months.

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

Recommendation
Colposcopic-cytological control and histological clarification.

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

Recommendation
Colposcopic-cytological control and histological clarification.

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

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 DdClear, a D-Limonene based solvent.

**Greensolv**
belongs to the group of so-called isoparaffins, is virtually odourless and can be used in the same way as xylene.

**DdMount low viscosity**
mounting medium is used for mounting. This gives excellent optical results and is so dry after less than 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 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.
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.

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.

Procedure
1. Stain in Shorr staining solution 1-3 minutes
2. Rinse in 70% ethanol 10 x 1 second
3. Rinse in 80% ethanol 10 x 1 second
4. Rinse in 96% ethanol 10 x 1 second
5. Rinse in absolute ethanol 10 x 1 second
6. Clear with xylene or DdClear 30 second
Mount with DdMount or L-Mount

Result
Cytoplasm cyanophilic (basophilic)
  blue-green
Cytoplasm eosinophilic (acidophilic)
  bright red
Nuclei  brown-red
Non-gynecological clinical cytology

Cytological investigation of biotic 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.

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.

• Pulmonary carcinomas can be diagnosed through examination of sputum and bronchial lavages material. Specimen material from peripheral pulmonary tumours 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 gastro endoscope 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 tumours 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 tumours as opposed to reactive processes.
• In the central nervous system, leukemias and lymphomas as well as inflammation and metastatic tumours can be detected in cerebrospinal fluid.
• Degenerative-inflammatory processes of the skeletal system can likewise by clarified by FNAB.

Staining of non-gynecological - clinical material

In addition to the Papanicolaou stain, haematological 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 Fast-color 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.

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).
Giems stain

**Giems stain**
Very versatile is also eminently suitable for non-gynaecological clinical material.
Nuclei and cytoplasm are stained in such a way that structural details are very apparent and differentiation is simplified.

**Giems solution for manual staining**
Dilute 10 ml Giems with 190 ml buffer solution, mix well, leave to stand for 10 minutes, and filter if necessary.

**Staining rack - Coplin jars**
Methanol 3-5 minutes
Giems 5-10 minutes
Rinse with buffer solution 2 x 1 minutes
Drying

**Staining in a stainers**
Methanol 3 minutes
Giemsa 10-15 minutes
Buffer solution 1 minutes
Running water (wash) 2 minutes
Drying 3 minute

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

Pappenheim stain

**May-Grünwald and Giems, enabling excellent results to be obtained for non-gynecological - clinical material.**

**Staining rack**
Cover the smear with 1 ml of May-Grünwald’s 3 minutes. Add 1 ml buffer solution, mix and stain 3-5 minutes. Cover with diluted Giems’s solution and stain 5-10 minutes.
Rinse with buffer solution - Dry

**Staining in coplin jars**
May-Grünwald’s solution 3-5 minutes
Giemsa 5-10 minutes
Rinse with buffer solution 2 x 1 minutes - Dry

**Result**
Nuclei   purple to violet
Cytoplasm  blue to dove-blue
Erythrocytes reddish
Results with Sorenson’s buffer pH 6.8
Wright’s stain can likewise be used for non-gynaecological clinical material.

Diluted Wright solution for manual staining
Add 20 ml of buffer solution and 150 ml of distilled water to 30 ml of Wright’s solution.

Diluted Wright 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 solution. Staining rack
Covering with Wright stock solution 1 minute
Buffer solution (1 ml) add, mix and stain 4 minutes
Rinse with buffer solution 2 x 1 minutes. Dry

Staining in coplin jars
Wright’s solution 3 minutes
Diluted Wright solution 6 minutes
Rinse with buffer solution 2 x 1 minutes. Dry

Staining in a stainers
Wright solution 3 minutes
Diluted Wright solution 6 minutes
Buffer solution 1 minute
Running water (wash) 2 minutes.
Dry 3 minutes

Result
Nuclei red to violet
Cytoplasm grey-blue
Erythrocytes reddish
Staining method

Fast color kit

Application.
Rapid method for differentiates stain of blood figured elements. The method can be used for staining slides of: cytological, fine needle, cytocentrifuge, malaria, and supplying a chromatic picture of the traditional method May Grünwald Giemsa.

Principle.
Fast color is a fast-acting variation of May Grünwald Giemsa stain. In hematology, it allows differential staining of blood smears (white blood cell count, morphological erythrocyte analysis and testing of parasites). It is ideal for emergencies and for studying smears in specimens showing as positive on cell counter machine. In cytology, it allows cytological examinations of fluid and of fine-needle punctures. In pathology, it allows emergency examination of frozen tissue section completed during surgical operation. Stains present in this kit are the same utilized in traditional May Grünwald Giemsa. In this case, the rapidity with which the coloration process is completed is due to the fast dissociation of the tyazinic and others stain contained that they rented extremely rapid the absorption of cellular structures.

Method

Blood smear
Dip the slides 5 times for 1 second into solution 1. Drain the surplus onto filter paper. Dip the slides 5 times for 1 second into solution 2. Drain the surplus onto filter paper. Dip the slides 5 times for 1 second into solution 3. Drain the surplus onto filter paper. Briefly wash in running water to remove excess and dip into alcohol 5-6 time.

Malaria (Plasmodium)
Dip the slides 3 times for 1 second into solution 1. Drain the surplus onto filter paper. Dip the slides 2 times for 1 second into solution 2. Drain the surplus onto filter paper. Dip the slides 2 times for 1 second into solution 3. Drain the surplus onto filter paper. Briefly wash in running water to remove excess and dip into alcohol 5-6 time.

Cytological of fluid and fine-needle punctures
Dip the slides 5 times for 1 second into solution 1. Drain the surplus onto filter paper. Dip the slides 5 times for 1 second into solution 2. Drain the surplus onto filter paper. Dip the slides 10 times for 1 second into solution 3. Drain the surplus onto filter paper. Wash carefully in running water to remove excess and dip into alcohol 5-6 time.

Histological slides
Dip the slides 10 times for 1 second into solution 1. Drain the surplus onto filter paper. Dip the slides 15 times for 1 second into solution 2. Drain the surplus onto filter paper. Dip the slides 15 times for 1 second into solution 3. Drain the surplus onto filter paper. Dehydrate and mount with DdMount.
Staining method

**Pneumocystis carinii Cryptosporidia Leishmania Cryptococcus Microfilaria Toxoplasma**

Dip the slides 10 times for 1 second into solution 1. Drain the surplus onto filter paper.
Dip the slides 10 times for 1 second into solution 2. Drain the surplus onto filter paper.
Dip the slides 10 times for 1 second into solution 3. Drain the surplus onto filter paper.
Briefly wash in running water to remove excess and dip into alcohol 5-6 time.

**Frozen tissue section**

Dip the slides 10 second in solution 1. Drain the surplus.
Dip the slides 15 second in solution 2. Drain the surplus.
Dip the slides 20 second in solution 3. Drain the surplus.
Dehydrate and mount with DdMount.

**Blood cells result:**

- **Nuclei:** chromatin: purple
- **Leucocytes:**
  - cytoplasm with out ARN: light pink
  - eosinophilic granules: orange brown
  - basophilic granules: dark purplish blue
  - neutrophilic granules: purple ± intense
- **Lymphocytes:**
  - cytoplasm with ARN: blue
  - cytoplasm with out ARN: light blue
  - azurophilic granules: red
- **Monocytes:** cytoplasm: grey-blue
- **Erythrocytes:**
  - cytoplasm: light red
- **Platelets:**
  - chromomer: purplish red
  - hyalomere: bluish
- **Blood parasites:**
  - nucleus (Malaria) red
  - cytoplasm blue

**Please note**

Staining time may change according to thickness, and dryness’ of slides.

**Reagent:**

- **Fixative** 100 ml
- **Eosin D.D.K. modified** 100 ml
- **Blue D.D.K. modified** 100 ml

**Or on request**

**Literature**

- Papanicolaou Technique; Proposed Guideline; NCCLS, Vol. 9, No. 15, 1989.