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Bioanalytics

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Liane Tikkanen

English Pathology Material for Exchange Students

– for self study and use in cytology and histology training laboratory sessions

TURKU AMK
TURKU UNIVERSITY OF APPLIED SCIENCES

Bachelor’s Thesis | Abstract

Turku University of Applied Sciences

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2021 | 19+24+44

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English Pathology Material for Exchange students

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The production of English learning and instruction material for exchange students at Turku University of Applied Sciences as a part of the BioTriCK BLS Triangular Center of Knowledge project. The material is produced for the courses Clinical Cytology and Clinical Histology as part of Clinical Pathology and is tailored specifically to the practical laboratory work sessions.

Keywords:

Pathology, Histology, Cytology, laboratory training

Opinnäytetyö (AMK) | Tiivistelmä

Turun ammattikorkeakoulu

Bioanalytiikka

2021 | 19+24+44 sivua

Liane Tikkanen

Englanninkielinen Patologian Oppimateriaali vaihto-opiskelijoille

- kliinisen patologian laboraatiohin

Englanninkielinen oppimis- ja opetusmateriaalin tuottaminen vaihto-opiskelijoille Turun ammattikorkeakoulussa osana BioTriCK BLS Triangular Center of Knowledge -hanketta. Aineisto tuotetaan kliinisen patologian kurssille, erityisesti käytännön laboratoriotyön koulutukseen eli laboraatioihin.

Asiasanat:

Patologia, Histologia, Sytologia, laboraatiot

Content

List of abbreviations **5**

1 Introduction **6**

2 Pathology **8**

2.1 Cytology 8

2.2 Histology 9

3 Exchange students at Turku UAS **11**

3.1 Learning material and laboratory work instructions 11

4 Practical implementation of the thesis **13**

4.1 Methodological basis of the thesis 14

4.2 Ethical basis of the thesis 15

5 Conclusion **16**

References **18**

Appendices

Appendix 1. Histology Material

Appendix 2. Cytology Material

List of abbreviations

TUAS Turku University of Applied Sciences

BioTriCK BLS Triangular Centre of Knowledge, Biomedical Laboratory Science

1 Introduction

Pathology is that field of science and medicine concerned with the study of diseases, specifically their initial causes (etiologies), their step-wise progressions (pathogenesis), and their effects on normal structure and function (Funkhouser 2018).

The course “Clinical Pathology is part of the Degree Programme in Biomedical Laboratory Science. Students learn the handling process of histological specimen in the laboratory and master the tissue embedding technique, the tissue sectioning and the use of a microtome. Students are familiarised with immunohistochemical staining, they identify tissue components, carry out histological and cytological staining and make a cytocentrifuge preparate. Students can tell apart benign cell changes from normal findings (Turku UAS, 2020). As part of that course, students have practical training in the schools pathology training laboratory. To ensure safety and sufficient learning, students (also exchange students) need work instructions and study material they can understand.

Exchange student programmes at Turku UAS and BioTriCK BLS

Turku University of Applied Sciences offers a wide range of courses and projects taught in English where international and local students study together (TUAS, 2021).

BioTriCK BLS Triangular Centre of Knowledge is a knowledge alliance which overall aim is to provide better quality of the Biomedical Laboratory Science education. During their education BLS students are given a theoretic background and training both for soft skills and technical and analytical skills necessary. However, gaps are experienced in the transformation between teaching theory, training skills in the school laboratory and in the more authentic and complex scenario in clinical practice (TUAS, 2020).

The purpose of this thesis is to create English learning material and work instructions for the non-finnish speaking BioTriCK BLS exchange students, that participate in the Clinical Pathology practical training laboratory sessions at TUAS. The existing Finnish material was translated and compiled into updated English material.

The aim of the thesis is to provide exchange students with information on the topics of cytology and histology for self-learning, and easy-to-follow guidelines for the practical laboratory work training. Students will benefit from quality materials to enhance their learning experience. TUAS benefits from a wider range of English material for students, improving the quality of education they can provide.

The study material contains both theoretical information and work instructions related to the sample processing steps. In addition to text, photographs and drawings are used to perceive and understand the material better, especially the work instructions contain many pictures. Students can choose to either browse through the digital version of the material or print it out and bring it to the laboratory training sessions.

2 Pathology

Pathology is that field of science and medicine concerned with the study of diseases, specifically their initial causes (etiologies), their step-wise progressions (pathogenesis), and their effects on normal structure and function (Funkhouser 2018). The Department of Pathology is involved in diagnosing patients diseases and planning treatments. As a rule, samples of living patients are examined in the field, but autopsy is also part of pathology. Pathology is divided into two parts, histology and cytology. (Karttunen, Soini & Vuopala 2005; Mäkinen & Lehto 2012.)

Clinical pathology involves the laboratory analysis of body fluids, ie. cytology and bodily tissue, ie. histology for the diagnosis of disease (Mandal, 2019). Pathology is used to determine the patient's illness and to devise a treatment plan based on the findings (Jari Huhtakallio 1995). An important part of the goals of the Clinical Pathology course at Turku University of Applied Sciences is the processing of histological and cytological samples and the processing steps that the samples go through.

2.1 Cytology

Cytology is the study of individual cells of the body, as opposed to histology which is the study of whole human tissue itself. Strictly speaking, cytology is the study of normal cells and cytopathology is the examination of cells in the context of disease. There are two main branches of cytology, generally referred to as gynaecological cytology and non-gynaecological or diagnostic cytology. (British Association for Cytopathology, 2021.). Cytology often examines a single cell type, found in fluid specimens (like urine, sputum, pleural, peritoneal, pericardial, cerebro-spinal and synovial fluid). It is mainly used to diagnose or screen for cancer. It is also used to screen for fetal abnormalities, pap smears, to diagnose infectious organisms, and in other screening and diagnostic areas (Johns Hopkins Medicine, 2021). Cytological examination methods are often

cheap, fast, and non-invasive. For example, the processing of cytological specimen is simpler compared to a histological tissue sample, because the specimen does not have to go through tissue processing or does not have to be embedded in paraffin or cut into sections with a microtome. Although new methods are emerging alongside the Pap smear, such as liquid Pap, immunocytochemistry methods, and HPV tests, traditional pre-screening work by biomedical laboratory scientists will still be needed in the future. (Nieminen & Timonen 2014; Kholová 2015.).

The cytology study material contains information about basic cytology, relevant anatomy and cell structure, gynecological and non-gynecological exfoliative cytology, the principles of fixation and staining, a staining result interpretation guide, sample taking instructions, practical work instructions (eg. for cover slipping or preparing a cytocentrifuge preparation), as well as a pictured reference guide to the most common findings.

2.2 Histology

Histology is the study of cells, tissues and organs as seen through the microscope. Histology also includes cellular detail down to the molecular level that can be observed us-ing an electron microscope. The importance of histology is that it is the structural basis for cell, tissue and organ biology and function (physiology) and disease (pathology) (Sorenson, 2014).

In addition to these, the student must be familiar with the basic terms and histologic stains (HE and VG) and be able to recognize pathological conditions/changes present in the samples. (Turku University of Applied Sciences 2017.)

A histology laboratory examines tissue samples, specimens taken at various procedures, tumors removed during surgery, or other tissue samples such as warts or moles. Tissue samples are processed by different tissue processing and staining processes into a form that can be examined and diagnosed by a pathologist. The work in the histology laboratory is partly automated in terms of

tissue processing and staining, but mostly the work still requires good eye-hand coordination. (Association of Biomedical Laboratory Scientists in Finland, 2021).

The histology study material contains information about the basics of histology, the histological specimens laboratory process, tissue processing, embedding, sectioning and basic histological stains, and includes also practical work instructions for embedding and microtome sectioning, containing pictures of every work step.

3 Exchange students at Turku UAS

In the spirit of internationalization, TUAS receives over 300 foreign exchange students every year. (Turku UAS, 2021). The common language used by the exchange students and their peers and their teachers is English.

Internationalization significantly influences healthcare education through an increase in the mobility of students (Garone and Van de Craen, 2017).

Educational internationalization has grown rapidly. As a result of increased global movement of students, universities all over Europe and increasingly Asia, have implemented English-medium options for international students in a variety of different areas, including medicine and the sciences (G. Slethaug, J. Vinther, 2016). Furthermore the development of educators' and students' global competence in higher education is increasingly important due to internationalization (Abdul-Mumin, 2016).

Even if students are originally non-english speakers, in an earlier study students stated, that they had great improvement in their English skills, even though they were from a country where people did not speak English (Kurnaz, 2020)

3.1 Learning material and laboratory work instructions

Instructional materials provide the core information that students will experience, learn, and apply during a course. They hold the power to either engage or demotivate students (University of Wisconsin – Madison, 2021).

Exchange students need learning material they can understand, designed for their specific courses.

Especially in the natural sciences, students work in laboratories, learn how to apply certain procedures and use the required equipment. In this way, they learn how to perform scientific methods (e.g. analyzing chemical Substances or the use of tests) (Universität Göttingen, 2015).

Instructional materials are the content or information conveyed within a course. These include the lectures, readings, textbooks, multimedia components, and other resources in a course. Instructional materials provide the core information that students will experience, learn, and apply during a course. They hold the power to either engage or demotivate students. Therefore, such materials must be carefully planned, selected, organized, refined, and used in a course for the maximum effect. The planning and selection of instructional materials should take into consideration both the breadth and depth of content so that student learning is optimized. (University of Wisconsin – Madison, 2021).

When creating study material and evaluating the finished study material, it must be taken into account the aims, benefits, content and comprehensibility of the study material, the target group and its starting level, costs,layout and availability.

The instructions for practical laboratory work in the pathology training laboratory, histologic and cytologic, existed thus far only in finnish (Petra Vilonen, 2017 and Helena Vaastela, 2016).

The cytology as well as histology laboratory self learning study material and work instructions were carefully translated, updated and uploaded to the Optima learning platform, so exchange students can benefit from them and use them to complete the laboratory training sessions.

There were no costs for the author or TUAS.

The layout was kept simple and structured as clearly as possible. Many pictures were added to make it easier to envision the processes and work steps.

The material is available online for students and exchange students of Turku University of Applied Sciences in the Optima-learning platform. The Cinical Pathology-course teacher has the rights to the material and can make it available to students also anywhere else in the future, as well as update it or add additional content.

4 Practical implementation of the thesis

The topic for this thesis came from Turku University of Applied Sciences in the spring of 2021. After the topic was clarified, it was already known that the thesis would not cause any costs to Turku University of Applied Sciences. The assignment agreement was applied for in the summer of 2021.

When the assignment agreement and the research plan were approved in the summer of 2021, the writing of the thesis began.

The purpose of the thesis output was to create a concise and easily accessible and understandable information package based on the curriculum of the histology and cytology laboratory training lessons for exchange students at TUAS. The output was largely based on the laboratory processes of histological and cytological specimen performed during school laboratory training classes. However, some of the processing stages are impossible to complete in the schools laboratory, but these steps are included in the learning material so that the sample’s processing in the laboratory is pictured in its entirety. The output was also created to supports the students' own learning.

The same kind of study material has already been made for the finnish students, so with this product, the course in Clinical Pathology has good study materials for exchange students as well.

The writing of the thesis started in the spring of 2021 by translating the existing finnish study material. The content was updated and checked for its usability. Additional pictures, taken of equipment used in the TYKSLAB pathology department, were added.

The sources selected for the task, were literature on cytology, histology, pathology and medicine, as well as various articles and online dictionaries (Elsevier: Science Direct, Finto.fi, MOT, RefWorks). Most of the sources were in English and some in Finnish.

Lastly, ethical and methodological bases and a reflection on the making of the study material was written.

The completed study material was given to the teacher of the pathology course and she was given editing rights to the material. The students can access the learning material in the Optima workplatform as a Word file, from where it can be printed or used digitally.

4.1 Methodological basis of the thesis

A functional thesis is often a development project related to working life or otherwise a professional environment.

As a result of a functional thesis, a product is created, which can be, eg. a model, guide, brochure, orientation folder or process description (Salonen 2013). In most cases, the output created is study material, a book, a poster or an exhibition. It is essential that the work produces some kind of the above-mentioned output, but in addition, the correct reporting of the work is also important. The text of the report should be written in factual style, using the correct word and time formats. The report must show what, why and how things have been done, as well as the presentation of the results and their reflection. From the report, the reader is able to assess whether the thesis has been successful. (Vilkka & Airaksinen 2004; Virtual University of Applied Sciences 2006.)

This thesis is a functional thesis, as the output was histology and cytology study material for biomedical laboratory science exchange students to promote their studies for the course of pathology.

The output describes firstly, the laboratory process of histological specimen in its entirety and introduces the two most common basic histologic stains and secondly, the laboratory process of cytological specimen, including both gynecological exfoliative cytology and urine exfoliative cytology in its entirety, to contribute to better student learning.

4.2 Ethical basis of the thesis

The writing of this thesis was done according to Finnish standards and guidelines for Finnish students.

Good scientific and ethical practices were followed in the writing of the thesis. Good scientific practice includes e.g. honesty and diligence in scientific practice, ethically and scientifically sound methods of obtaining information, research methods or reporting methods, and transparency and honesty in the publication of results. Obtaining the required research permits is also a good scientific research principle. A research permit is granted if the research plan has to be approved and it is the researcher's responsibility to ensure that the research is carried out in accordance with the plan. (Hirsjärvi et al. 2009; Leino-Kilpi & Välimäki 2014.)

The subject of the research must be such that it does not offend anyone. The same principle applies to all other phases of the research. Persons participating in the study must not suffer any inconvenience or damage as a result of participating in the study and their rights must be respected. (Leino-Kilpi & Välimäki 2014.)

In this thesis, study material was prepared for biomedical laboratory science students, so the study did not cause any harm or inconvenience to anyone. The samples used when testing the cytology learning material, were obtained from the students themselves (urine samples). Pictures were taken of various findings with a microscopic camera on gynecological and urine exfoliative cytology specimens. There is no patient data written on the school’s microscope slides, so it is impossible to identify patients from the slides. The samples used when testing the histology material, was obtained from former students during work practice in the pathology laboratory of TYKS, originated from patients, but did not contain any patient information (human tissue samples for embedding and microtome sectioning).

5 Conclusion

When creating the study material, it was important to keep in mind the target group. The material is aimed at exchange students learning the basics of histology and cytology, so the text needed to be consistent and clear. English was chosen as language, because it is the most commonly taught foreign language in most of European countries and widely used in the scientific community. The material focused on the basics of histology and cytology, so too extensive and in-depth knowledge was cut out, making the learning material suitable for beginners.

The theoretical content of the study material followed the goals set for the course of Clinical Pathology at Turku University of Applied Sciences.

The study material became suitable in length and its pictures were taken at the school's pathology teaching laboratory and at the pathology department of TYKS. The pictures helped make the study material easier to understand. Patient samples were used to take the images, but it was ensured that patient data was not visible in the images.

The content of the study material became a logical and cohesive whole, in which the whole process of pathological specimen was described. Attempts were made to make the text parts of the study material easier to read with the layout of the text and pictures, but it was sometimes difficult to understand due to the theoretical content of the text, medical terms and foreign vocabulary.

The strength of this study material is that its functionality has been tested as Finnish version before implementation. The benefit of testing was that the learning material could be further modified to be as clear and relevant as possible.

The English study material was then tested by four exchange students from Greece and their feedback was used to improve the product further. More pictures were added upon their suggestion. They considered the material as

helpful and comprehensive, but had minor difficulties understanding some of the scientific terms.

The subject of further research of this thesis could be to make a broader study material on histology, which could include more histological staining, for example. In the cytology field, a more extensive and comprehensive cell findings guide could be prepared as a thesis, because the findings guide of this study material is quite narrow. Another topic could be learning material related to immunohistochemistry.

Professional growth in this thesis has been reflected in the fact that the completion of the entire thesis, especially the translation, has required responsibility and commitment and has vastly improved the authors English vocational vocabulary.

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Learning material and practical laboratory training guide

Liane Tikkanen
2021

CLINICAL HISTOLOGY

Laboratory process of histological specimen

Content

1 Histology	2
1.1 Tissue Samples	3
2 The histological laboratory process	5
2.1 Sample Login	6
2.2 Placing the samples in cassettes	7
2.3 Tissue processing	9
2.4 Embedding of the sample in paraffin	10
2.5 Slicing of the sample - sectioning	14
2.6 Basic histologic stains	20
3 Sources:	23

1 Histology

The histology laboratory mainly handles tissue samples. They allow the pathologist to look at how the cells relate to each other and how malignant cells grow in their surrounding tissue. The pathologist examines the tissue sample under a light microscope and diagnoses the changes with a pathological anatomical diagnosis, or PAD. From the tissue samples one can detect and classify everything, from benign tumors to infections, degenerative diseases, and metabolic diseases.

In the histology laboratory, microscope specimens are prepared by using various tissue processing and staining processes. The work is partly automated, but it still involves a lot of handwork, so good eye-hand coordination is important.

The most common tasks of a biomedical laboratory scientists include logging in tissue samples into the system on arrival, transferring samples from their containers into cassettes, using tissue processing equipment, embedding of the samples in paraffin, microtome slicing, and staining. There are also tissues arriving at the laboratory every day, that need a faster response. From such tissues, biomedical laboratory scientists (bioanalysts) prepare a frozen section, that the pathologist examines and diagnoses. The whole frozen section process, from the arrival of the sample in the laboratory to the pathologist's response, takes about 10-20 minutes.

1.1 Tissue Samples

The tissue samples are used to identify functions and status of tissues and are taken when their examination has influence on the choice of medical or surgical treatment. They can be used to monitor the development of the disease and the response to treatment. An indication may be, for example, a suspicion of breast cancer. In addition to visible changes, specimens are also taken from areas that look normal, as not all diseases are visible to the naked eye. When making a diagnosis, the quality and representability of the sample must be kept in mind, because a negative finding does not exclude the possibility of a malignant change, if the sample has not been obtained from the correct site. In conflicting situations where the clinical finding and the pathologist's response do not align, further examination should be performed.

Tissue samples can vary a lot in size. The smallest are 1-2mm and weigh a few milligrams and the largest are whole limbs, organs, parts of organs or organ groups. Small samples include skin biopsies of moles, or other skin samples as well as other tissues removed under local anesthesia. In addition to the previously mentioned, the most common surgically removed specimens, like the gallbladder and appendix, are commonly among the small specimens. Large surgical samples include parts of organs, whole organs or groups of organs. The origin of the sample, its status and the completeness of the removal are assessed from every sample.

Most of the removals are performed because of various tumors, inflammation, or changes that interfere with function. The size of the tissue sample determines how much of it is taken to be examined. The smallest samples are usually taken as whole,

4

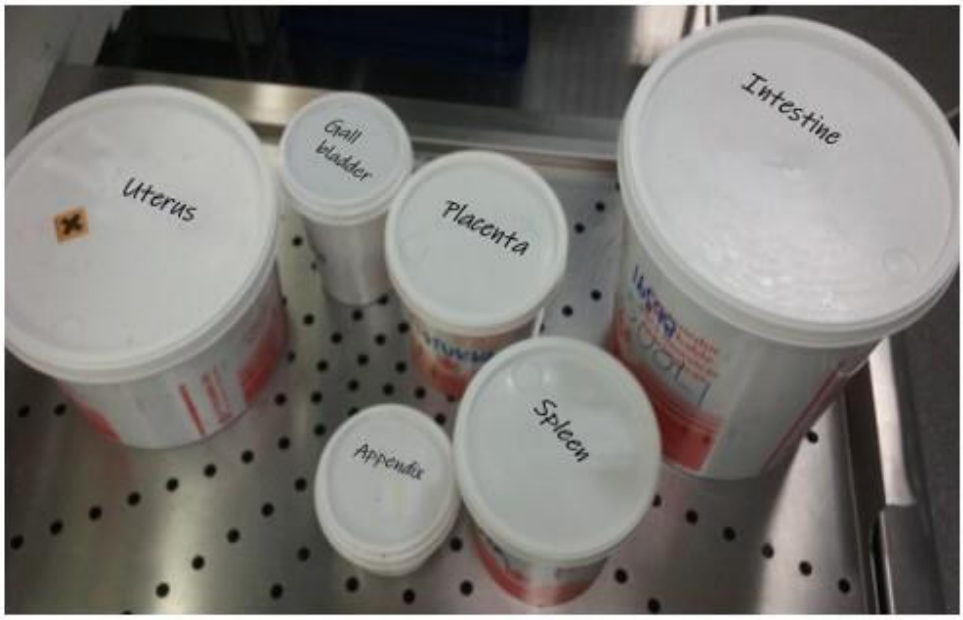
but from larger resected samples, usually a few tens of grams are taken as sample material and the rest of the tissue is conserved. In this case, the selected sample material represents only a small part of the tissue sent to the laboratory. Therefore, it is important that the referring physician accurately describes the tissue and the removal site at the time of removal, so that the pathologist can understand where the sample has been removed anatomically and what its removal margins are.

Opened gallbladder

5

2 The histological laboratory process

The time to completely process a normal sample from arrival in the laboratory to the pathologist's opinion is about 3-5 days. Usually, within one working week, the response can be sent to the department that sent the sample. Diagnosing samples that require more work, like softening, i.e., decalcification (e.g., samples containing bone), usually takes longer.

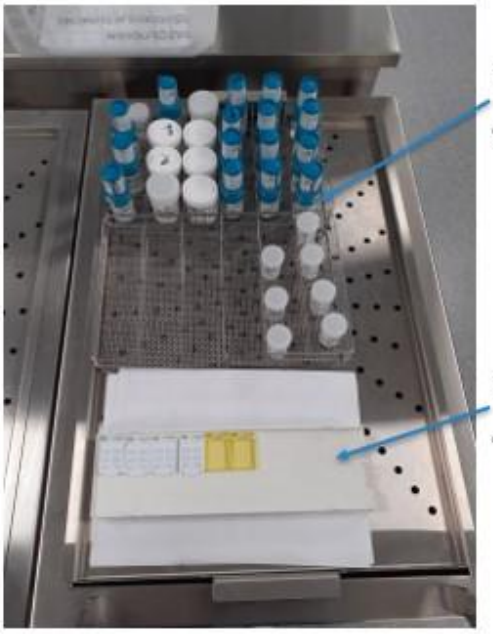


Sample containers

6

2.1 Sample Login

Upon arrival of the sample container at the laboratory, ensure that it is in the correct fixative and that the entire sample is well covered and submerged in the fixative. The most common fixative is 10% buffered formalin and must be at least 10 times more than the amount of tissue. The function of fixation is to prevent the lysis of tissue cells, i.e., autolysis. The fixative also helps the tissue to solidify, making it easier to process further. All tissue samples entering the laboratory must have an electronic referral made by a doctor. When the sample arrives in the laboratory, the referral information is checked. If the sample has been taken and fixated correctly and the referral information is sufficient, the sample can be logged in, numbered and embedding cassettes made. To identify samples, following information is required: the laboratory's unique identifier, sample type, year, serial sample number, and embedding cassette numbers.



Logged-in samples in their original containers.

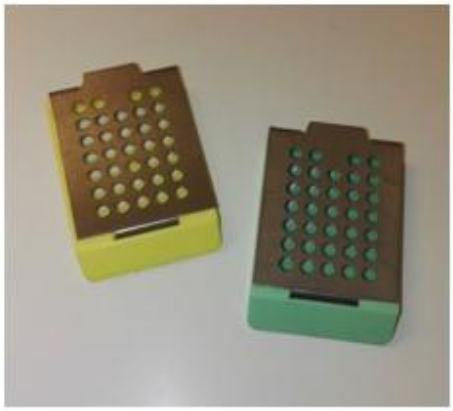
Ready printed referrals and embedding cassettes

7

2.2 Placing the samples in cassettes

Smaller samples such as punch biopsies of skin lesions and gynecological scraping samples are usually placed in cassettes as whole, if necessary, filtered into a small bag and continue as such to tissue processing. Samples going directly to the tissue processing must be small enough to fit in the perforated embedding cassette, but not completely fill the cassette to allow the substances used in tissue processing to penetrate the tissue completely.

Larger specimens such as surgically removed organs require preparation. At preparation, the pathologist examines the sample macroscopically and takes representative pieces of tissue from it into embedding cassettes. Samples are usually photographed or drawn, measured, and additional information written down in the referral, so that they can be identified later, if necessary, also from a different pathologist.



Embedding cassettes

8

Ready cassettes containing samples for tissue processing

2.3 Tissue processing

The purpose of tissue processing is to harden tissue structures and improve the storability of the sample. Tissue processing consists of two parts, dehydration and sample clarification, after which it is ready for paraffin infiltration. Tissue processing is automated and executed by tissue processors.

Dehydration is achieved with a series of ethanol (alcohol) solutions of increasing concentration. Most often, it is started with 50% or 70% ethanol and the concentration is slowly raised to absolute alcohol. There are other dehydrating agents such as methanol and acetone, but ethanol is the most widely used. The disadvantage of ethanol is its ability to shrink tissue and its cost. Excessive dehydration hardens, shrinks, and makes the tissue brittle, and insufficient dehydration prevents the absorption of solvents into the tissue, leaving it soft.

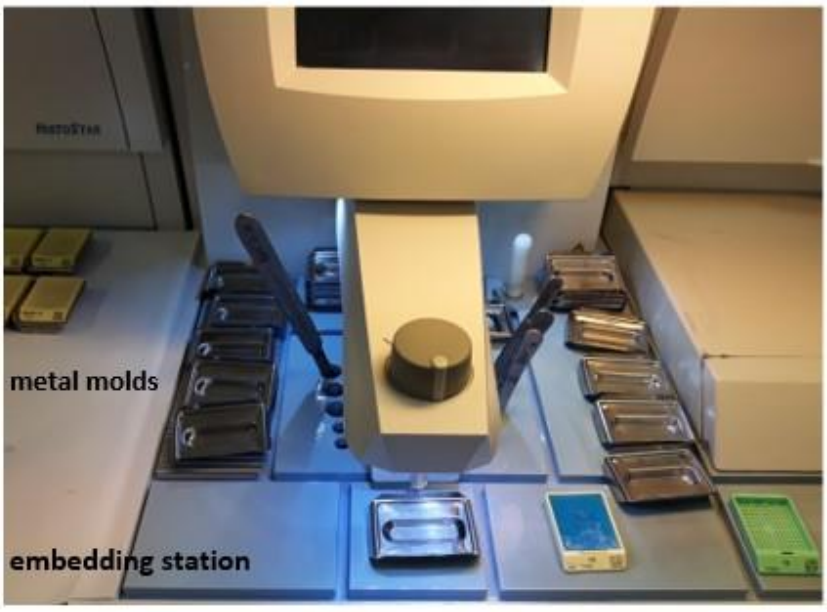
After absolute ethanol, the alcohol must be removed from the tissue, i.e., the sample is clarified. Ethanol must be removed before paraffin infiltration because paraffin is not soluble in ethanol. Most often, xylene is used for this, which clarifies the sample and makes it translucent. However, the sample should not be in xylene for too long or the tissue may start to shrink.

Tissue processor

10

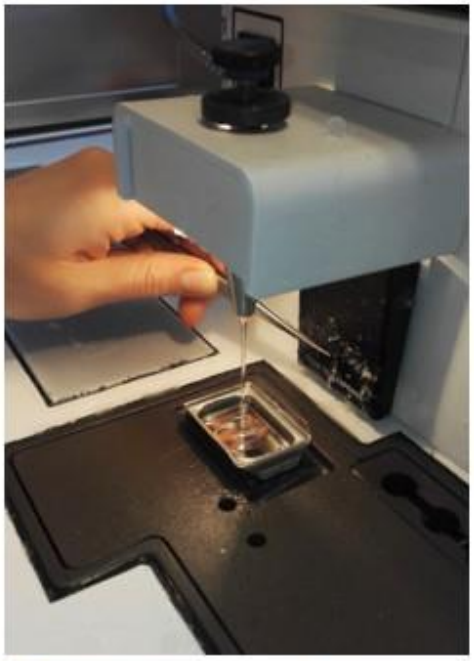
2.4 Embedding of the sample in paraffin

Once in the tissue is processed, the samples can be cast in paraffin. Paraffin supports the tissue and thus allows very thin sections to be cut with a microtome. In embedding, the sample is transferred from the cassette to a casting mold on a hot plate and molten, liquid paraffin is poured over it. Different samples are oriented in the casting mold in different ways, but as a general rule, the sample is cast cutting surface facing down. Skin samples must be poured so that all layers of skin are visible on the microscope slide. Once the sample is properly placed in the casting mold, the entire mold is transferred to a cold plate where the hardening paraffin solidifies the sample into the correct position. The original cassette, which reads the sample identification information, is placed on top and paraffin is poured until the entire mold is filled. The mold is then transferred to a cold plate to solidify the paraffin block for microtome cutting.

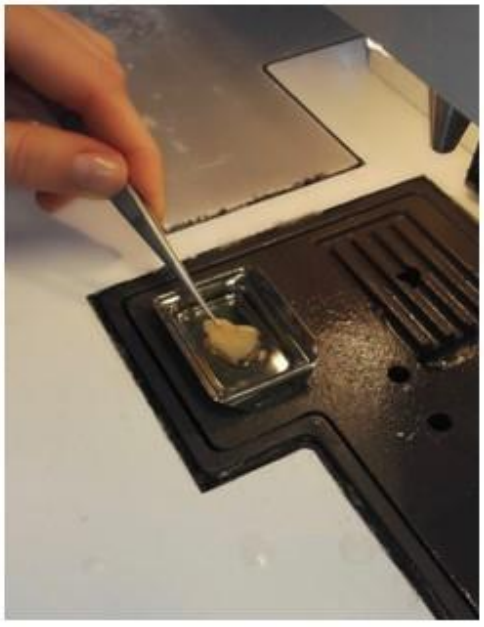


11

Embedding the sample in practice:



1. Pour a little amount of liquid paraffin in a suitably sized mold.



2. Position the tissue in the mold on a hot plate.

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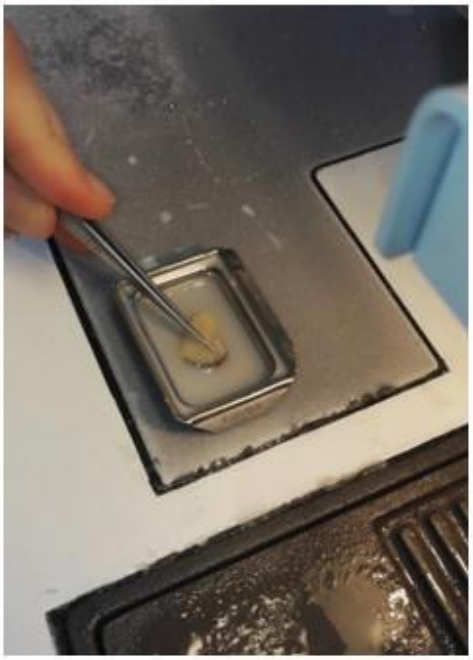
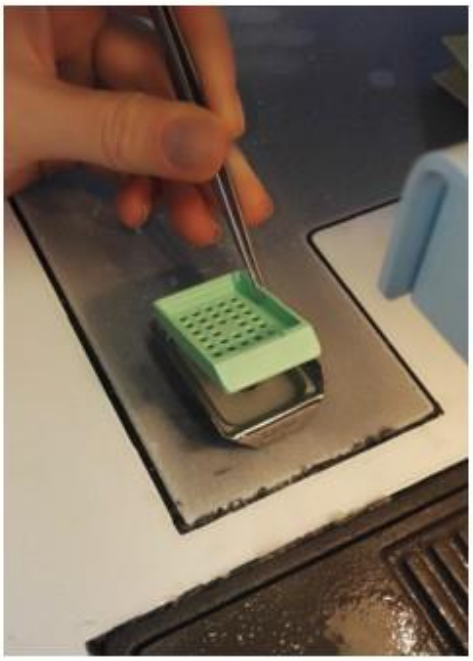


3. Press the tissue evenly down onto the bottom of the mold on the coldplate.



4. Place the cassette containing sample identification information on top of the sample.

13

5. Fill the mold with molten paraffin and transfer it to the cold plate to solidify.

Ready cassettes on the cooling plate

2.5 Slicing of the sample - sectioning

Thin slices are cut from the paraffin block with a microtome (i.e., sectioning) There are two types of traditional microtomes, sledge microtomes in which the paraffin block is in place and the knife moves towards the block at an angle of about 40 °; and rotary microtomes in which the paraffin block moves vertically towards the knife, which remains in place. Prior to cutting, the block is cooled on a cold plate for better cleaner cutting sections and is then attached to a microtome. Excess paraffin is trimmed off the surface of the block, until the entire tissue structure of the sample is exposed. From the block, 2 to 10 µm thick slices are cut from several sample levels. Intact, clean sections are transferred with brushes to a cold-water bath (+20° C), from which they are then picked up with a microscope slide and transferred into a warm water bath (+45° C) for straightening. The slides are then allowed to dry for a short time in an upright position, then placed on a hot plate (+60° C) so that the sections adhere well to the slide before staining.

The newest microtome is a rotary water slide microtome. It is basically a rotary microtome with an inbuilt waterslide to transfer the cut sections from the block into a water basin. Such microtomes also have a built-in block cooler in the block holder and the hot water basin is attached to the device. With the water slide microtome, the sections slide directly into a hot water basin, where they straighten up immediately, allowing them to be picked up directly on the microscope slide.

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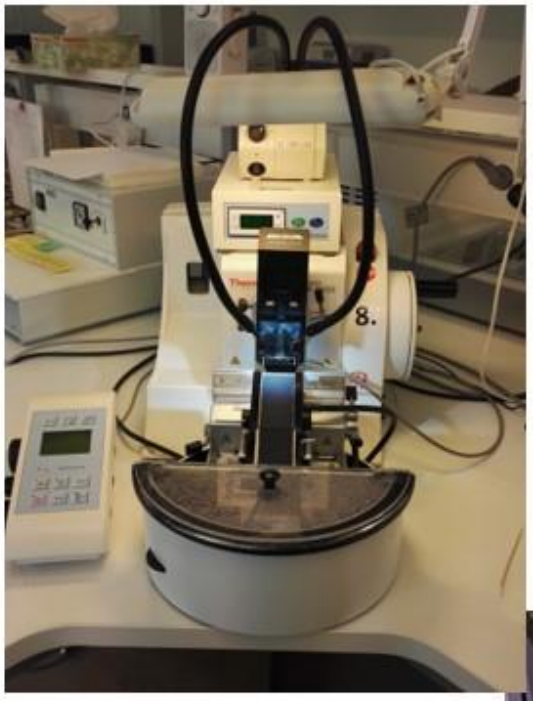
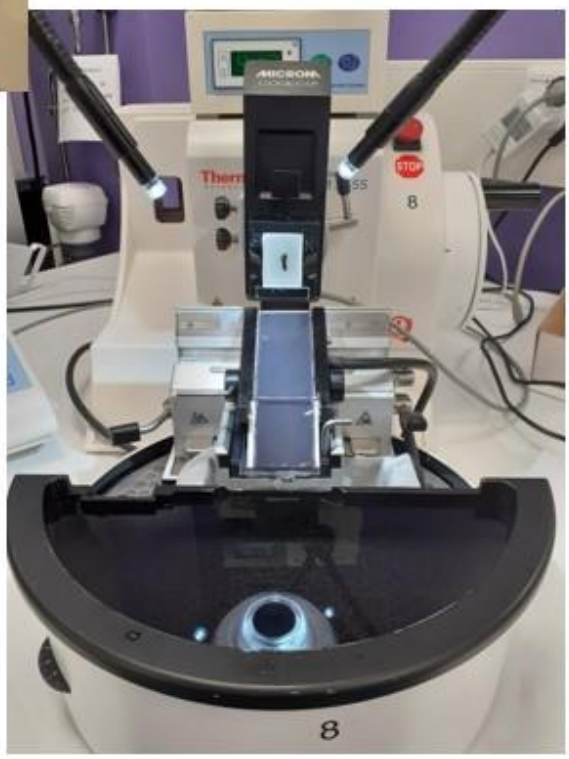


Rotary waterslide microtome



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Cutting sections of samples in practice:

1. Trim the block until you can see the entire tissue.

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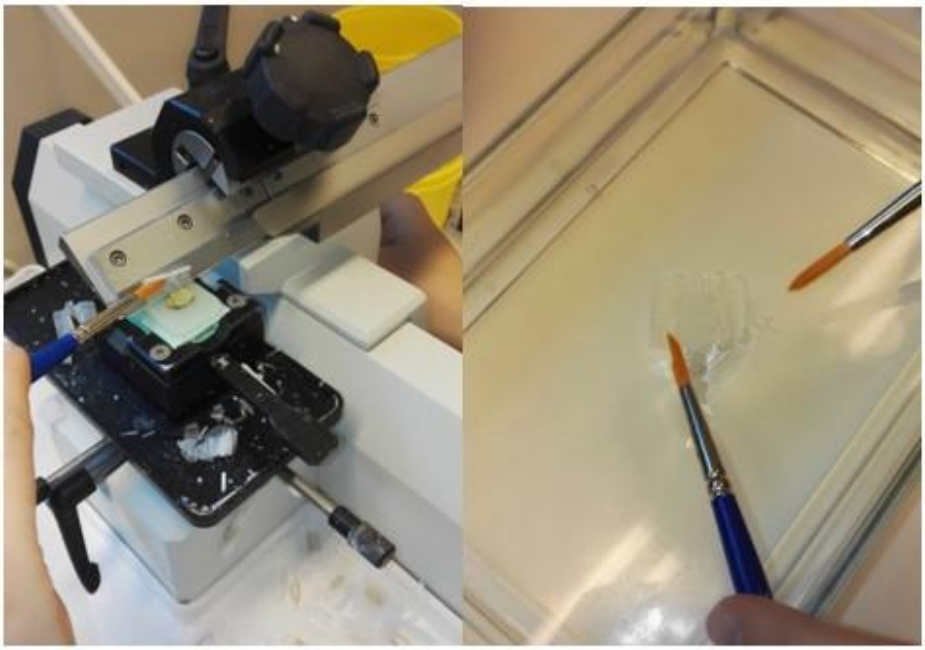

2. Using brushes, transfer the section to the cold-water basin and straighten it if necessary.

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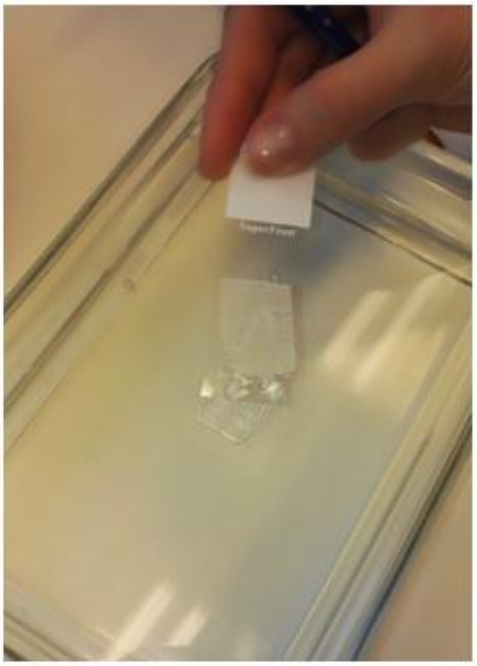


3. Collect the section onto a labeled microscope slide.

4. Using the slide, transfer the section into a hot water bath to straighten, pick it up again and then...

19

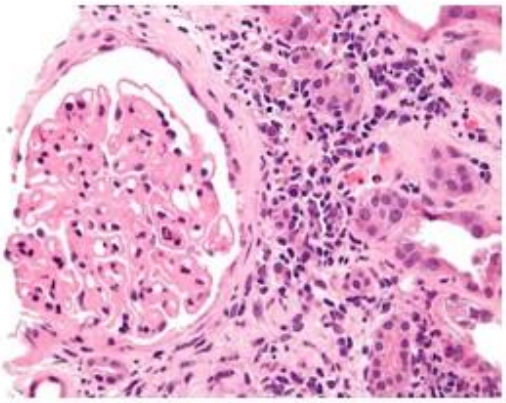
5. Place the slide on a hot plate, so the section adheres to the glass.

20

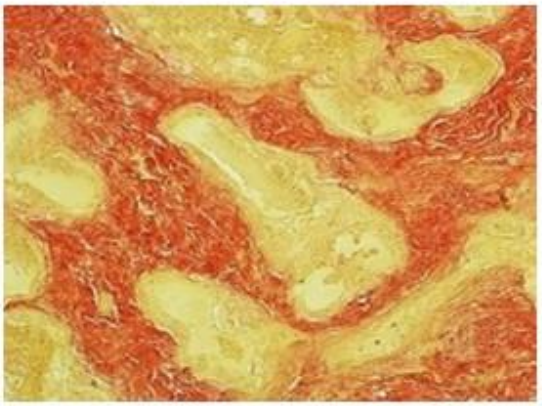
2.6 Basic histologic stains

Most sample sections are completely colorless. The section must be stained to be examined under a microscope. Different colors make different parts of the cell tissue visible. The most common stains are done with an automatic stainer, but some less frequently used stains are done by hand. Prior to staining, paraffin is removed from the sections with xylene and then xylene is removed with absolute alcohol. The section is then rehydrated, i.e., water is soaked back in, so that it is ready for staining. After staining, the sections are dehydrated with ethanol, then the ethanol is removed with xylene. Finally, a coating agent is pipetted onto the sections and a coverslip is placed on top, so that the slide can be viewed under a microscope.

Basic stains used in histology are hematoxylin-eosin staining (HE) and Weigert Van Gieson staining (VG).



HE stain



VG stain

21

HEMATOXYLIN-EOSIN STAINING

Two different dyes are used for hematoxylin-eosin staining. In this staining, the pH of the tissue affects how it stains. In HE staining, hematoxylin is an alkaline dye and eosin is acidic.

- Hematoxylin stains the acidic parts of tissues, which are the nuclei of the tissue cells, as well as the RNA in the cytoplasm. Eosin stains alkaline parts of tissues such as fibrous, connective and muscle tissue and eosinophils.
- Structures that do not stain are glycogen and mucus.
- In HE staining, the nuclei stand out as blue-black, while the cytoplasm stains in shades of light and dark red.

The advantages of this staining are clear nuclear staining, which helps in the assessment of nuclear atypia, and a good shelf life.

22

WEIGERT VAN GIESON STAINING

Weigert Van Gieson is a staining method for the detection of elastic fibers, connective tissue, collagen and nuclei.

- Weigert iron hematoxylin is used to stain the nuclei of the cells.
- Counterstaining is performed with a van Gieson solution containing picric acid and acid fuchsin.
- Picric acid stains cytoplasm, muscle tissue, and red blood cells yellowish.
- Acidic fuchsin stains the collagen fibers of connective tissue red.

As a dye, VG is not as long-lasting because the van Gieson dye fades over time.

Automated stainer

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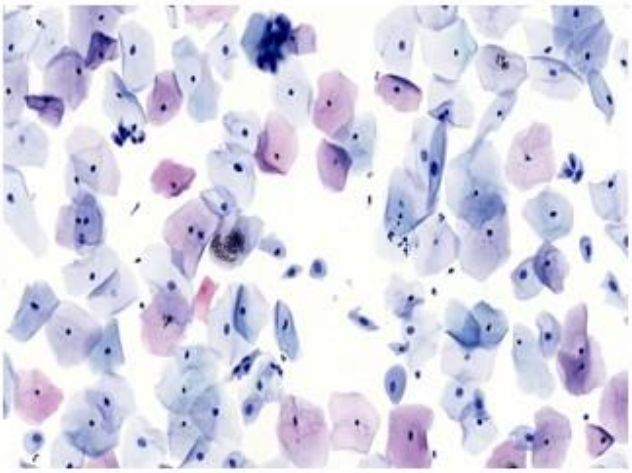


CLINICAL CYTOLOGY

Gynecological and Urine Exfoliative Cytology

Learning material and practical laboratory training guide

Tikkanen Liane
2021

Content

1 Cytology 2
2 Exfoliative Gynecologic Cytology 3
2.1 Referral 5
2.2 Taking a Pap smear sample 6
2.3 Fixation 8
3 Exfoliative urine cytology 9
3.1 Taking a urine sample 10
3.2 Fixation 12
3.3 How to produce a Cytocentrifuge preparation 12
4 Papanicolaou stain 19
4.1 The principle of staining 19
4.2 Staining instructions 21
4.3 Staining result 23
5 Covering the microscope slides - coverslipping 23
6 Pre-inspection/Screening 25
7 Interpretation 26
7.1 Gynecological cytologic specimens 26
The Bethesda system 28
A small guide to identifying findings 30
7.2 Urine cytology or exfoliative urine test 38
8 Sources 42

1 Cytology

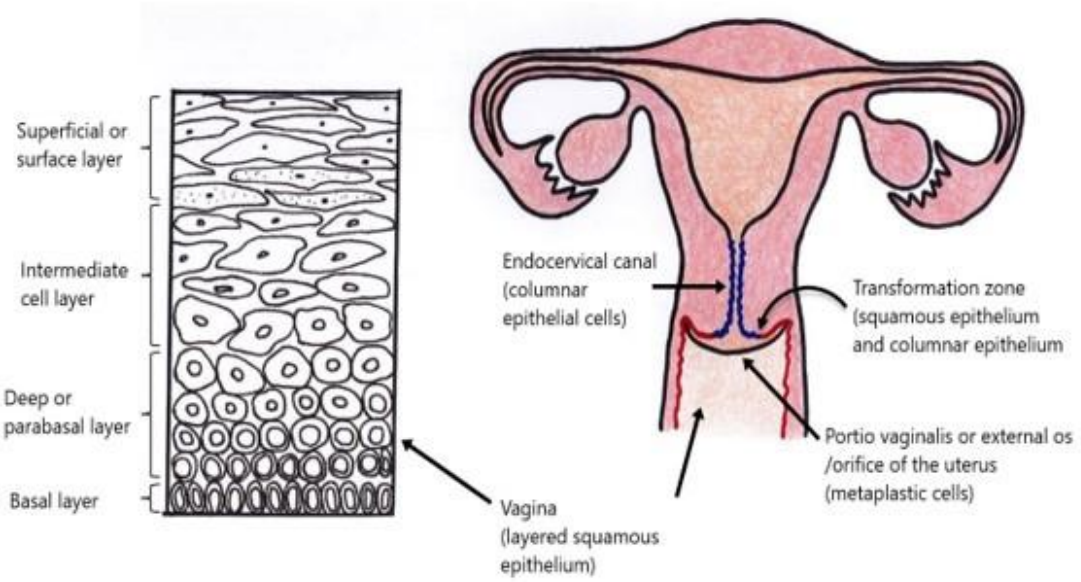
Cytology is the medical and scientific study of cells and refers to a branch of pathology. Cytologic exams are performed to study cells, including their variations and mutations, that may be linked to a certain disease or tumors. Cytologic examinations may be performed on body fluids, (exfoliative cytology) (examples are blood, urine, and cerebrospinal fluid) or on material that is aspirated (aspiration cytology), (drawn out via suction into a syringe = fine needle biopsy) from the body. Exfoliative cytology also can involve examinations of preparations that are scraped or washed (irrigated with a sterile solution) from specific areas of the body. The most common samples are taken from the bronchi/lungs (sputum cytology), abdominal cavity, bladder (exfoliative cytology of urine) and female genitals (Pap smear, cervical smear). Compared to histologic samples, the processing of cytologic samples is often easier, faster and cheaper. Cytological examinations also allow sampling of sites where a histological sample cannot be taken or where there are too many risks associated with taking a piece of tissue. Cytologic samples are usually examined and diagnosed by pathologists. Pathologists who specialized in the examination of exfoliative cytologic samples can be referred to as cytologists. Also, gynecologists can be cytologists. Pathologists are responsible for the final interpretation and diagnosis of samples, but in Finland also biomedical laboratory scientists (bioanalysts) partake in the examination of loose cell samples as pre-examiners/screeners.

2 Exfoliative Gynecologic Cytology

Exfoliative gynecologic cytology, or Pap smear, is a research method developed for the detection of cervical cancer and its precursors. The method was developed by Dr. George Papanicolaou in the 1920s and 1930s. In Finland, the method was introduced in the 1950s. Today, the Pap test is used as a screening tool for cervical cancer. Indications for a Pap smear include mass screening, diagnosis of gynecological infections, investigation of persistent recurring inflammation or unexplained bleeding, monitoring previous findings and the effect of treatments, and suspicion of genital tumors. The Pap smear is no longer used to assess a woman's hormonal status, because hormone changes appear in the squamous epithelial cells of the vagina with a few days delay. Blood tests are a better way to determine the hormonal situation.

Stages of a Pap smear:

1. Verification of the referral and correct sampling
2. Sample fixation on the slide
3. Papanicolaou staining
4. Covering of the sample slide
5. Pre-inspection/Screening


4
Superficial or surface layer
Intermediate cell layer
Deep or parabasal layer
Basal layer
Endocervical canal (columnar epithelial cells)
Transformation zone (squamous epithelium and columnar epithelium
Portio vaginalis or external os /orifice of the uterus (metaplastic cells)
Vagina (layered squamous epithelium)

2.1 Referral

Before taking the sample, it is necessary to always check the patient's referral first. A Pap smear always requires a referral from a physician (gynecologist, pathologist), which makes the examination of the sample easier and more reliable. In the patient's referral must be mentioned the following things:

- Patients' identification (name, birthday, social security number or similar)
- Length of menstrual cycle and start date of previous menstruation
- Possible hormone therapy/treatment
- Results of previous Pap smears
- Other gynecological diseases and related treatments
- Reason why a Pap smear is taken now (i.e., control sample, mass screening, unexplained discharge or suspicion of infection)

Sometimes a Pap smear is taken much later than the referral was issued, so it is vital to confirm that all the patient's information (i.e., menstrual cycle) is still correct.

2.2 Taking a Pap smear sample

The correct sampling is vital, so the specimens can be examined and diagnosed correctly. In a Pap smear samples are taken from three different sites of the internal female genital organs. A typical cell image varies in different parts of the uterus, so a high-quality sample contains cells specific to all three sampling sites. A Pap smear is preferably not taken during menstruation, because the bloodiness of a sample impairs the reliability and accuracy of its interpretation. Bloody discharge is however not an obstacle or precludes sampling, especially if the sample is to be examined for cellular changes suggestive of cancer or its precursors. In the sample taking situation, attention must be paid to the tactful, polite treatment of the patient, as the situation is very intimate and can be unsettling for many women. It would be optimal for the patient to feel relaxed, otherwise sampling may cause pain or discomfort.

Necessary equipment:

- Examination gloves
- Microscope slide, labeled with the patient's identification (with pencil)
- Speculum
- Cotton swabs, to remove excess discharge
- Sampling tools: round-headed spatula, indented head spatula (Aylesbury spatula) and cell brush/endocervical brush
- Saline solution (to moisten the speculum)
- Fixative spray

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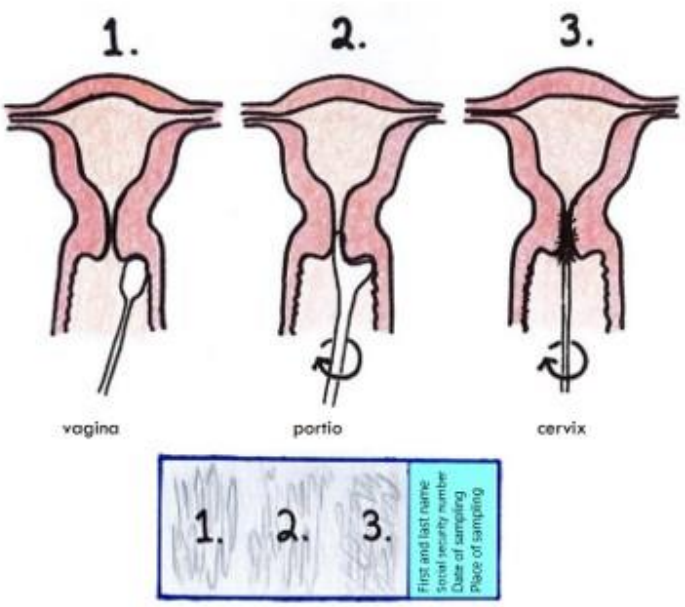


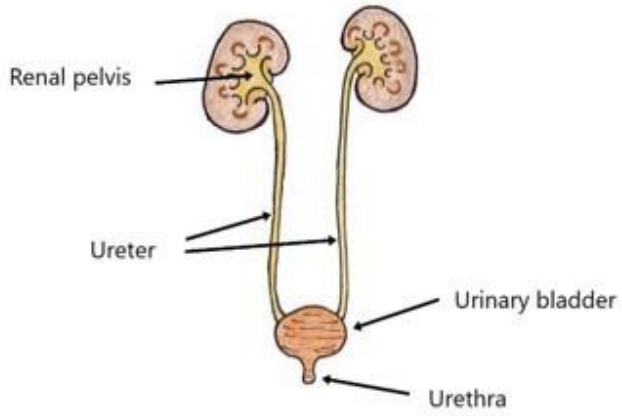
1. The first sample (vagina) is scraped with the round headed spatula from the vaginal wall near the cervix. The sample is brushed on the correctly labeled glass slide, avoiding too much pressure.
2. The ecto-cervical (portio) sample is taken with the indented head spatula from the outer opening of the cervix and brushed on the slide, avoiding too much pressure.
3. The last cervical sample is taken with the cell brush, inserted into the cervix and rolled into the endocervical canal. The brush is rotated a few times and removed. The sample is applied to the slide by gently rotating the cell brush on the surface of the slide.

2.3 Fixation

Immediately after sampling, the specimen applied to the slide are preserved, i.e., fixed. The fixation stops all cell's activities and the details of the cell's appearances remain intact. Without fixation, the cells will dry on the slide, which would prevent reliable interpretation of the sample. To fix the pap smear sample, either a commercial fixative spray is used or the sample glass is immersed in 95% ethanol for at least 15 minutes. Although the fixation spray is the more common method of fixation, immersion in alcohol is often preferred, because it yields a better staining result. After fixation, the sample must dry properly. The next steps in the Pap test are staining of the sample by the Papanicolaou method, coating of the microscope slide, and pre-inspection/ screening.

3 Exfoliative urine cytology

The main indication for urine cytological examination is the suspicion of a malignancy in the urinary tract. In addition, a microscopic cell examination can determine the cause of hematuria (blood in the urine) or persistent inflammation for which no definitive cause can be found. Also, the effects of therapeutic treatments of urinary tract diseases can be monitored with this exam. The cells in the urine sample are largely derived from the lower urinary tract, e.g., the bladder, urethra, ureters, and renal pelvis (picture below). A few individual cells from the kidneys or in men from the urinary tract side organs, such as the prostate or seminal vesicles and ducts, can also end up in the sample. However, the presence of cells from these organs in a cytologic urine sample is quite rare and they are usually poorly preserved.



Stages of a urine cytological examination:

1. Correct sample taking
2. Fixation of the sample in 70% ethanol (1:1)
3. Making a cytocentrifuge/cytospin preparation
4. Papanicolaou staining
5. Covering the microscope slide
6. Pre-inspection/Screening

3.1 Taking a urine sample

For cytological urinalysis examination, the samples are usually taken in a series of 3-5 consecutive days, but it is also possible to take and examine a single sample. Different kinds of urine samples, taken in different ways, can be used for the examination. Different sample types include midstream urine sample, catheter sample, bladder lavage (flushing) and brushing samples. A midstream urine sample is the most common and easiest method of sampling. The method of sampling must always be mentioned in the cytological urine examination referral, to avoid misunderstandings during the examination of the specimen. The catheter sample, as well as the bladder lavage and brushing samples are more cell-rich than the standard midstream urine sample. Lavage and brushing samples are taken by physicians during bladder endoscopy (cystoscopy) procedures, and physiological saline solution is used as rinsing agent. The midstream urine sample used for the cytologic urine examination, is obtained in the same manner as midstream urine samples for other tests, e.g., chemical evaluation using urine test strips (U-KemSeul, urine chemical screening). However, in this examination, attention must be paid to the preparations and the timing of sampling. Following a description how to prepare for sampling and how to take a sample.

Preparations:

- ✓ A high-quality sample is obtained after ingesting fluids. Urine that has been in the bladder for more than four hours or the first morning urine are not suitable samples. The sample would then be rich in cells, but the cells are disintegrated and broken.
- ✓ Preparation for sampling starts with emptying the bladder, then followed by drinking about half a liter of fluid.
- ✓ The sample is taken earliest 1-2 hours after the bladder was emptied.

Sampling:

- Wash hands with soap
- Wash urogenital area with water
- Pass a small portion of urine into the toilet. Without interrupting the stream, midway through urination, move a clean container into the stream of urine to collect. A sufficient amount is 15 ml (according to the Hospital District of Southwest Finland's instructions, the amount for samples may vary in different hospital districts).

3.2 Fixation

After sampling the specimen has to be preserved, i.e., fixated. There are two ways to fixate a urine sample. The sample taken at home is fixated by pouring the collected urine into a separate sample jar containing a fixative solution of 70% ethanol (TYKS, Turku University Hospital). Pour as much urine into the sample jar as there is fixative in it, i.e., half urine and half ethanol, equal parts. The other method of fixation is to centrifuge the sample in the laboratory as it is, after which only the sediment of the sample is fixated with ethanol.

3.3 How to produce a Cytocentrifuge preparation

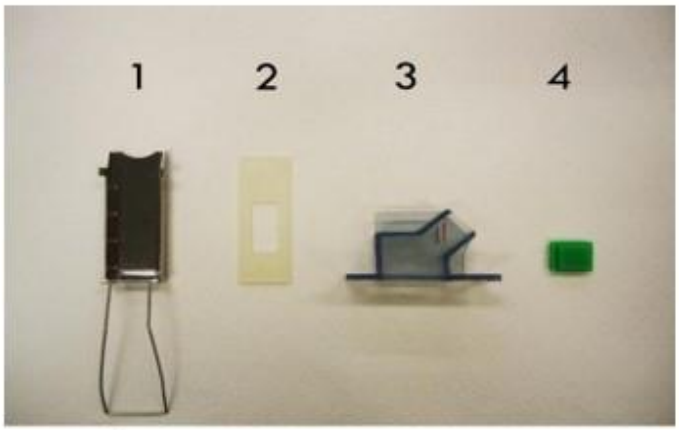
For the cytologic microscopic urinalysis the urine sample is processed in the laboratory by a cytocentrifuge and then stained by Papanicolaou staining. Cytocentrifugation is the most commonly used sample preparation technique in the cytology laboratory. This technique can be used to make preparations from all liquid and fixated samples, in addition to urine, e.g., flushing and brushing samples taken from the lungs. With a cytocentrifuge, the cells in the specimen are pressed onto the slide, forming a single thin layer of cells. Microscope slides pretreated with poly-1-lysine can be used in the preparation of the cytologic urinalysis samples. Poly-1-lysine is a binder that enhances cell-to-cell adhesion. The work is done in a fume cupboard, because samples could potentially be infectious.

Needed equipment:

- Lab coat and disposable examination gloves
- Protective surface cover sheets
- Tissues or paper towels
- Fixated sample
- Microscope slides
- Cytocentrifuge (Sakura Cyto-Tek)
- sample cuvette parts: metal holder, stopper, cuvette and cap

14

Cytocentrifuge and rotor



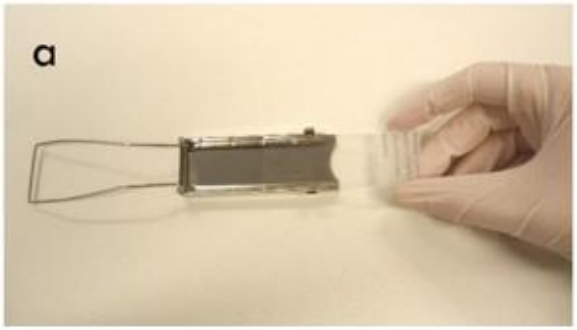
sample cuvette parts:

1. metal holder 2. stopper 3. cuvette 4. cap

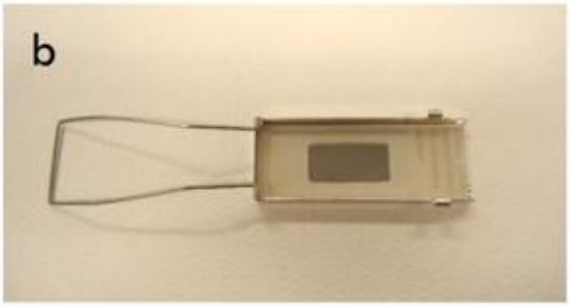
Execution:

1. Write on the microscope with a pencil, so that you can identify your own sample
2. Assemble the Cyto-Tek sample cuvette.

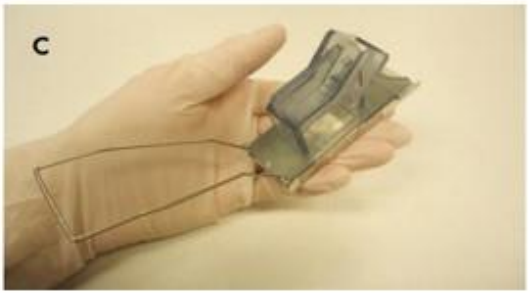
a. Place the slide with the labeled end facing up in the metal holder.



b. Place the rubbery limiter on the slide. Note that the opening is not completely in the center of the limiter. The limiter is placed on the slide so that the shorter end comes closer to the bracket of the metal holder, and the longer end covers the labeled part of the slide.

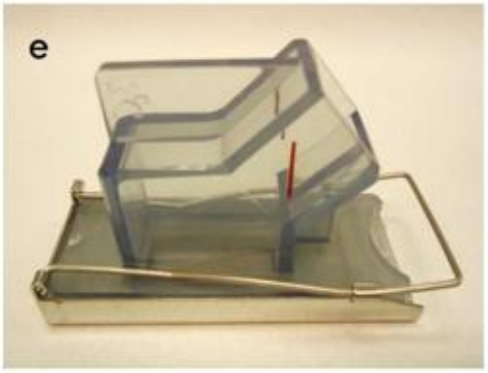


c. Place the cuvette on top of the limiter.



d. Attach the cuvette to the metal holder by pressing the bracket under the hooks of the holder. Make sure that the cuvette is properly in place and that the openings of the limiter and the cuvette are aligned.

e. Assembled sample cuvette.

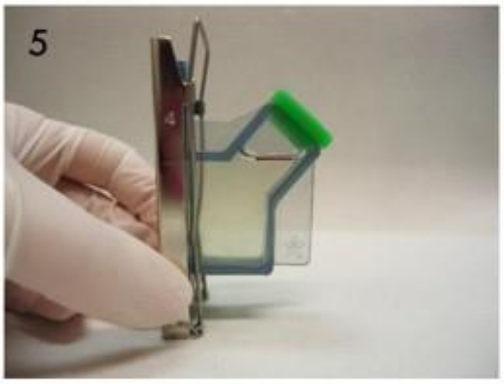


3. Approximately 0.5 ml of 50% PEG solution can be pipetted into the cuvette using a Pasteur pipette, because it helps the cells to adhere better to the slide.

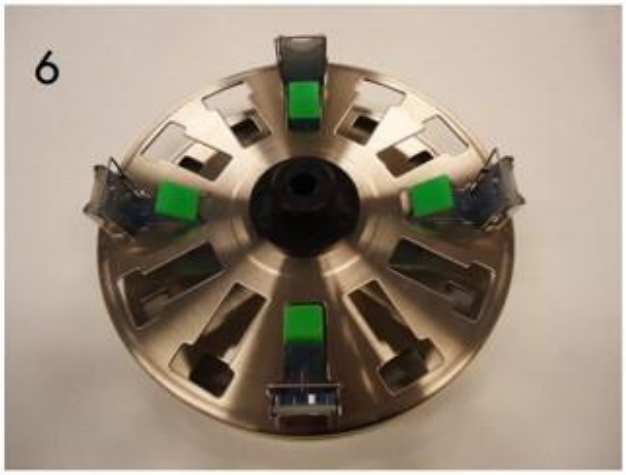
4. Mix the urine sample thoroughly and pipette the specimen with a Pasteur pipette up to the mark line of the cuvette. If the sample is not sufficient and the cuvette is not filled to the line, add saline solution up to the mark. For

centrifugation, all cuvettes must contain the same amount of liquid. Dilution of a very cloudy sample is done by pipetting only a small amount of the sample into the cuvette, and the rest of the cuvette is filled with saline solution.

5. Close the sample cuvette with the green rubber cap. Mix the sample by gently inverting the cuvette a few times.



6. Place the sample cuvettes in the rotor of the cytocentrifuge. Remember to balance! Centrifuge for 5 min, 2000 rpm.



7. After centrifugation, open the rubber cap of the sample cuvette, and pour out the sample with a quick turn of the wrist. Leave the sample cuvette upside down to dry for a while in a fume cupboard on tissues or paper towels.
8. Carefully take the sample cuvette apart. Leave the limiter still on and allow the slide to dry in the fume cupboard for about 30 minutes.
9. After drying, the preparations are stained with Papanicolaou stain.

4 Papanicolaou stain

Papanicolaou stain is the basic staining in cytology. The staining emphasizes the nucleus and its chromatin. In addition, the cytoplasm can be easily separated from the nucleus and the cytoplasmic structure can be successfully evaluated. The dye stain was developed by Dr. Papanicolaou in 1942. Many different variations of the original staining method have been developed over the years. Nowadays, most laboratories use commercial dye reagents for staining. Laboratories can also modify staining to suit their own needs, so there are differences in staining methods between laboratories. For example, the intensity of cell coloring can be affected by changing staining times.

4.1 The principle of staining

Papanicolaou staining dyes are hematoxylin, OG 6 (orange green) and EA 50 (eosin azure). Hematoxylin is used for staining of the nucleus and OG 6 and EA 50 to stain cytoplasm. As a result of staining, the nuclei turn dark blue or almost black and the cytoplasms turn different shades of pink, blue, or green. At different stages of dyeing, different rinses and treatments are performed, e.g., with water and ethanol. The first step in Pap staining is the rehydration of the sample in a series of descending grade ethanol. The purpose of rehydration is to get the sample in a water based medium, because the hematoxylin solution contains water. After rehydration, cell nuclei are stained with hematoxylin. Hematoxylin itself is not a dye, but the hematein in hematoxylin, which is an oxidation product of hematoxylin, acts as a dye. After staining the nuclei, the next step is differentiation. Nuclear staining often uses a regressive method, i.e., in regressive staining, samples are deliberately overstained, then

further differentiated (e.g., with dilute acid), until the optimal endpoint is reached. For this stage, either dilute hydrochloric acid or ammonia-alcohol solution can be used. The way the differentiation step is performed varies widely between laboratories. With the staining kit available in school the excess nuclear dye is rinsed off with water only. The next step is bluing. In the bluing step, the sample is rinsed in an alkaline liquid, giving the nuclei their final blue-black color. Ordinary tap water is alkaline enough to produce the color change. After bluing, the sample is dehydrated in a series of ascending grade ethanol for cytoplasmic staining, because the cytoplasm dyes contain ethanol. The dehydration step is also "simplified" in schools staining kit. Two different dyes are used to stain the cytoplasm: OG-6 (orange-G-6-phosphotungstic acid) and EA 50 (eosin azure). OG-6 stains superficial squamous epithelial cells, eosinophil granules, and cell keratin in orange. EA 50 dye contains two colorants: eosin Y and light green. The abbreviation number 50 refers to the composition of the color and the ingredients it contains. Eosin Y stains superficial squamous cells, erythrocytes, and cilia pink. Light green, on the other hand, turns e.g., squamous epithelial middle layer cells, columnar epithelial cells and metaplastic cells green. The acidity of cytoplasmic dyes greatly influences which dye stains the cell cytoplasm more intensely. Finally, the sample slide is rinsed in absolute alcohol and lastly clarified in xylene. The slide is then ready to be covered with a coverslip.

4.2 Staining instructions

The school's cytology lab practical training exercises use a commercial Papanicolaou staining kit (Empire Genomics Papanicolaou (PAP) Stain Kit). The dyes in the kit are in dropper bottles, from which the dye is pipetted onto the sample slides. Because there are many different variations of Papanicolaou staining, the dyeing instructions for the staining kit do not follow exactly the same formula as the dyeing principle described previously. Dyeing is always done in a fume cupboard, because the substances required for dyeing are harmful and toxic.

Needed equipment:

- ✓ Lab coat and disposable examination gloves
- ✓ Protective surface cover sheets
- ✓ Tissues or paper towels
- ✓ Sample microscope slides
- ✓ Glass staining bowls and lids
- ✓ Sample tray
- ✓ Stopwatch
- ✓ Staining kit (Empire Genomics Papanicolaou (PAP) Stain Kit)
- ✓ Laboratory water
- ✓ Tap water
- ✓ Ethanol (70%, 96% and absolute)

Step	What to do?	Time/duration
1	Place the sample slide in 96% ethanol	5 mins
2	Place the sample glass in 70% ethanol	5 mins
3	Place the sample glass in distilled water	2 mins
4	Pipette Mayer hematoxylin (Hematoxylin, Mayer's) from the dropper onto the slide so that the whole specimen is covered and incubate the slide. Note! Do not let the dye solution to evaporate completely. If necessary, add a few drops of dye	5 mins
5	Rinse the slide by immersing it in distilled water a few times to remove excess color	a few dips & waving the glass in the liquid
6	Rinse the slides gently in the staining bowl under running tap water	2 mins
7	Rinse the slide 2 times in distilled water	2 x a few dips & waving the glass in the liquid
8	Dip the slide several times in 96% ethanol and pour the excess onto tissues or paper towels	Several dips
9	Pipette the OG-6 dye solution from the dropper on the slide, so that the whole specimen is covered and incubate the slide. If necessary, add dye.	2 mins
10	Rinse the slide gently by dipping once in absolute ethanol	1 quick dip
11	Pipette the EA 50 dye solution from the dropper on the slide, so that the whole specimen is covered and incubate the slide. If necessary, add dye	3 mins
12	Rinse the slide gently by dipping once in absolute ethanol	1 quick dip
13	Rinse the slide 3 times in absolute ethanol	3 quick dips
14	Place the slides in xylene. After that, cover the slide with a coverslip	No time limit

4.3 Staining result

Nucleus – blue/almost black

Superficial squamous cells – light pink

Middle layer cells – (light) blue/green

Deep layer cells – blue/green

Strongly keratinizing cells – orange

Metaplastic cells – blue/green

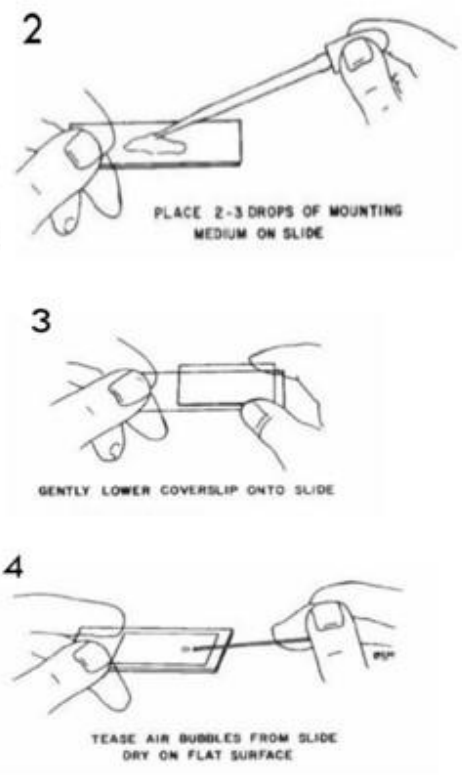
Erythrocytes – red/pink

5 Covering the microscope slides - coverslipping

Thin glass coverslips and a coating material are used to cover the slides. The purpose of covering the slides is to protect the sample from damage, drying and fading of the stain. The coating material must be compatible with the dye, so it does not dissolve the dye. The most commonly used coating material is a xylene-based coating agent. Covering the specimen by hand requires precision and practice. The aim is to minimize the usage of coating agent in the covering and to avoid air bubbles or artifacts on the slide. Too much coating agent messes up the glass and complicates microscopy. If the coating agent has time to solidify for too long before applying the coverslip, large dark brown pigment artifacts (the so-called “corn flake” artifacts) can be seen on the slide during microscopy.

Execution:

1. Take the slide out of the xylene bath.
2. Using a Pasteur pipette, pipette a little coating agent either onto the slides or the coverslips (in the picture, it is pipetted onto the slides, choose the way that suits you best). The amount to be pipetted depends on the size of the slide and the coverslip.
3. Place the coverslip carefully onto the slide. If the coating agent has been pipetted onto the coverslip, invert the coverslip and gently drop it onto the sample glass.
4. Remove air bubbles by gently pressing the coverslip with tweezers and, if necessary, wipe off excess xylene and coating agent from the slide.



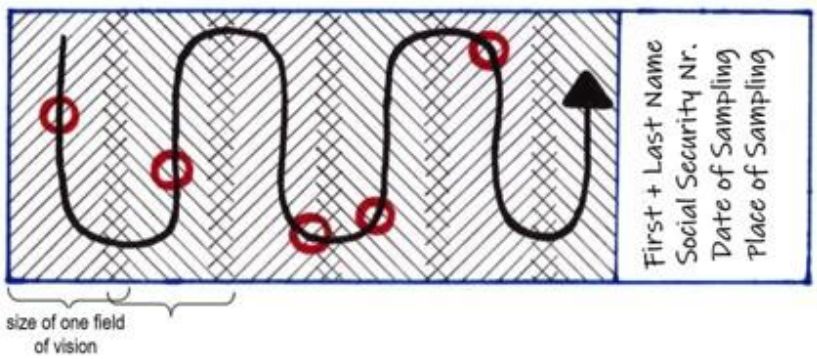
Method of coverslipping. Bales 2005.

If the coating is unsuccessful, the slide can be put back with the coverslip in the xylene bath to soak for a while. The xylene makes it easier for the coverslip to come off the slide. Once the old coverslip has been removed, the slide can be covered again.

6 Pre-inspection/Screening

Pre-inspection or "screening" means the preliminary examination of a cytological sample under a microscope. The pre-examiner screens through the entire sample slide, marks any abnormal findings and gives his or her own diagnostic proposal for the sample. The slide then reaches the pathologist, who goes through the abnormal findings marked by the pre-examiner and can then either confirm or change the pre-examiners proposed response. Lastly, the pathologist makes a formal diagnosis of the sample.

Preliminary examination of cytologic samples is performed with an optical microscope and the sample is examined with a 10x objective lens. A 20x or 40x objective lens is used to view single cells or details of other findings. When microscoping, the slide is always placed the same way: the labeled end of the slide is always either to the right or to the left. However, especially in the pre-examination of a Pap smear, it is important to always start with the vaginal specimen and then the ecto-cervical and cervical specimens are examined. The sample slide is screened through, one field of view at a time, from top to bottom, so that the entire sample is looked through. When moving the field of view to the left or right, it is good to partially overlap the adjacent fields of view, to ensure that all sections of the sample are viewed (picture). The findings in the sample are marked onto the sample slides using a ring marker in the microscope.



7 Interpretation

7.1 Gynecological cytologic specimens

Bethesda system

The diagnosis of Pap tests is given based on the Bethesda system. The Bethesda system was introduced at the turn of the 21st century. Prior to the Bethesda system, the Pap test diagnosis was given according to Pap class categories. The advantage of the Bethesda system over the Papa classification is that it tells whether there are cellular changes detectable in squamous cells or columnar epithelial cells.

Pre-examination of the pap smear always starts from the vaginal sample, from which we proceed to the ecto-cervical and cervical samples. First, the interpretability of the sample is assessed. A good quality and easily interpretable sample contains a lot of cells. It is also essential that columnar epithelial cells be found in the cervix sample, to ensure that sampling has been performed correctly. In addition, the presence of metaplastic cells in the ecto-cervical sample is an indication of a good quality sample. A very bloody sample or a really high number of inflammatory cells impairs the reliability of the interpretation. In assessing the interpretability, it is also worth paying attention to the quality of the staining and the identification markings on the microscope slide.

The general classification depends on whether there are atypical cells in the sample or not. If an abnormal microbe, such as Trichomonas vaginalis, is found in the sample, but there are no other findings, the sample can be considered to belong to the category “no epithelial cell atypia”. “Epithelial cell atypia” also includes changes in squamous or columnar epithelial cells, that are at risk of becoming malignant. Squamous cell atypia and columnar epithelial cell atypia are further specified separately by the Bethesda system's own international abbreviations and terms (see table on the next page). The “other atypia”

option can be used, for example, for cytolysis or atrophy. The descriptive diagnosis specifies in more detail, which finding is found in the sample.

The hormonal status is assessed by comparing the patient's biodata in the referral to the general normal cellular findings. In the biodata, it is worth paying special attention to the patient's age, possible method of contraception and information about the menstrual cycle. Nowadays, the use of a Pap test as examination method of the hormonal status, e.g., the evaluation of the maturation effect of estrogen, is quite limited.

28

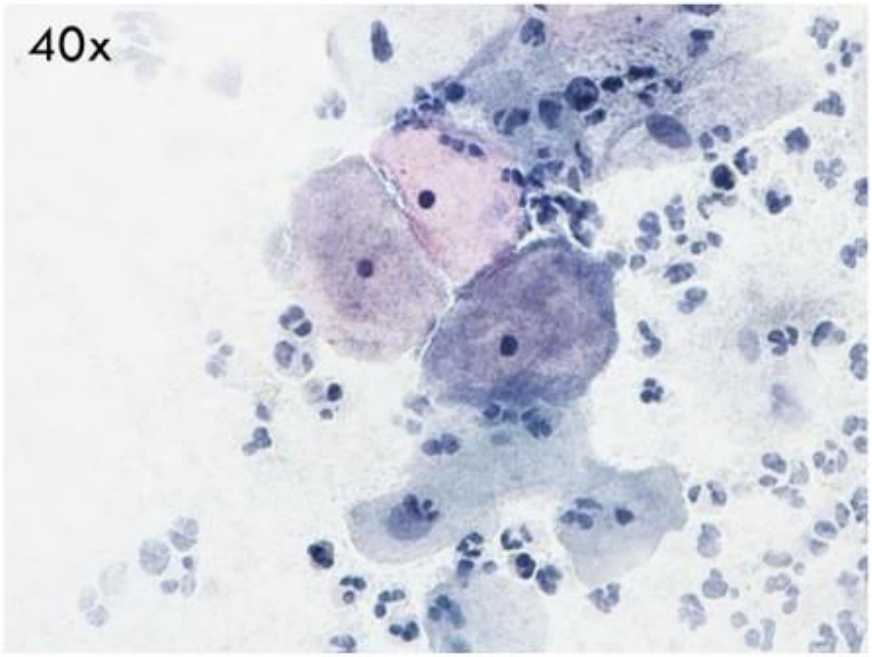
The Bethesda system

Bethesda	Pap system class
Interpretability of the sample • Sufficient/satisfactory • Sufficient, columnar epithelial cells are missing • interpretation uncertain because… • not interpretable because…	no suitable counterpart Class 0
General categorization • Negative for intraepithelial lesion or malignancy, no epithelial cell atypia • Epithelial cell abnormality • Other abnormalities	 Class I and II Class III and IV No suitable counterpart
Descriptive diagnosis Abnormal microbes • Shift in flora suggestive of bacterial vaginosis, Clue cells • Mixed bacterial flora present • Fungi/yeast (e.g., Candida species) • Trichomonas vaginalis • Actinomyces • Cellular changes associated with herpes simplex virus • Other (clarification in the diagnosis) Other non-neoplastic changes • Metaplasia • Cytolysis • Atrophy • Pregnancy associated changes • Endometrial cells (in a woman ≥ 45 years of age) Reactive changes • Inflammation • Regeneration • Changes due to radiation • Intrauterine contraceptive device changes	 Class I and II Class I and II No suitable counterpart Class I and II

• Other (clarification in the diagnosis)	
Epithelial cell abnormalities Squamous cell abnormalities	
• ASC-US (Atypical squamous cells of undetermined significance)	Class II
• ASC-H (Atypical squamous cells of undetermined significance, cannot exclude HSIL	Class III
• LSIL (Low grade squamous intraepithelial lesion)	Class II and III
• HSIL (High grade squamous intraepithelial lesion)	Class III and IV
• Squamous cell carcinoma	Class V
Glandular cell abnormalities	
• AGC-NOS (Atypical endocervical or endometrial glandular cells, not otherwise specified)	Class II and III
• AGC-FN (Atypical endocervical or endometrial glandular cells, suggesting neoplasia)	Class III and IV
• the origin cannot be determined	Class II – IV
• AIS (Endocervical adenocarcinoma in situ)	Class IV
• Adenocarcinoma	Class V
Hormonal effect • Corresponds to age and biodata • Does not correspond to age and biodata, because... • Cannot be determined, because...	No suitable counterpart
Interpretation / result • A more detailed description of the findings and possible treatment recommendations	

30

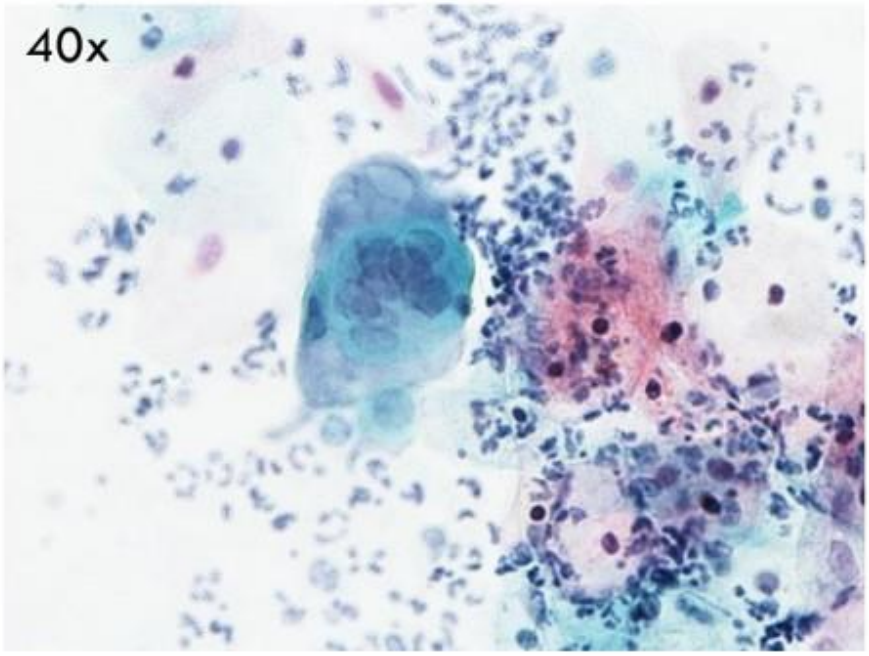
A small guide to identifying findings



Clue cell

- Gardnerella vaginalis causes non-specific bacterial vaginosis, treated if it causes symptoms
- these bacteria accumulate on the squamous cells → clue cells darker than other squamous cells and the cell surface is "granular"
- the background of the sample appears messy, because there are a lot of bacteria present

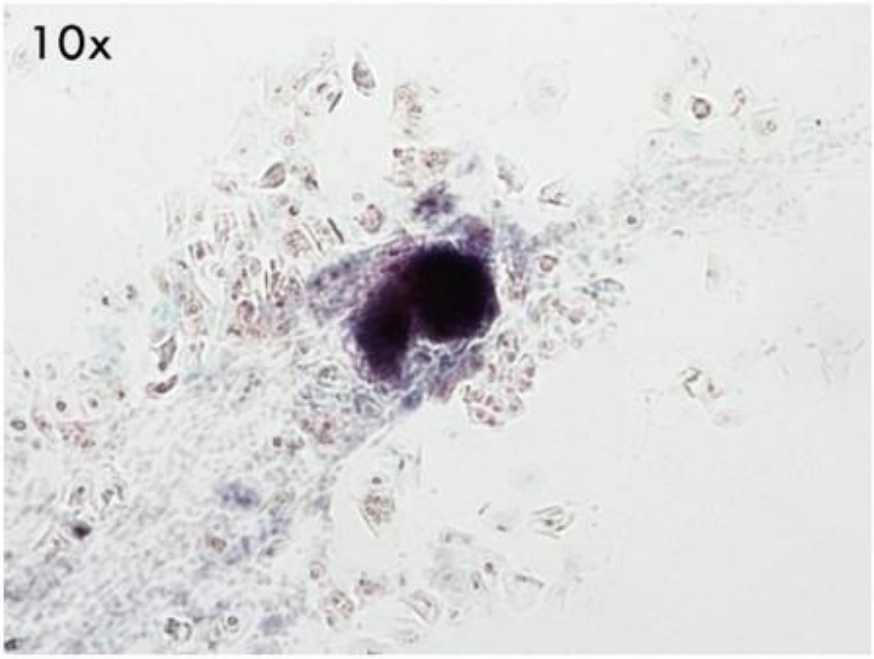
31



Herpes Simplex (Type 2)

- Multinucleated “cloudberry-cells”
- frosted glass nuclei = the structure of the nucleus is not clearly visible
- nuclear inclusions may appear sometimes (darker ‘eye’ inside the nucleus)
- coloring varies, blue or red

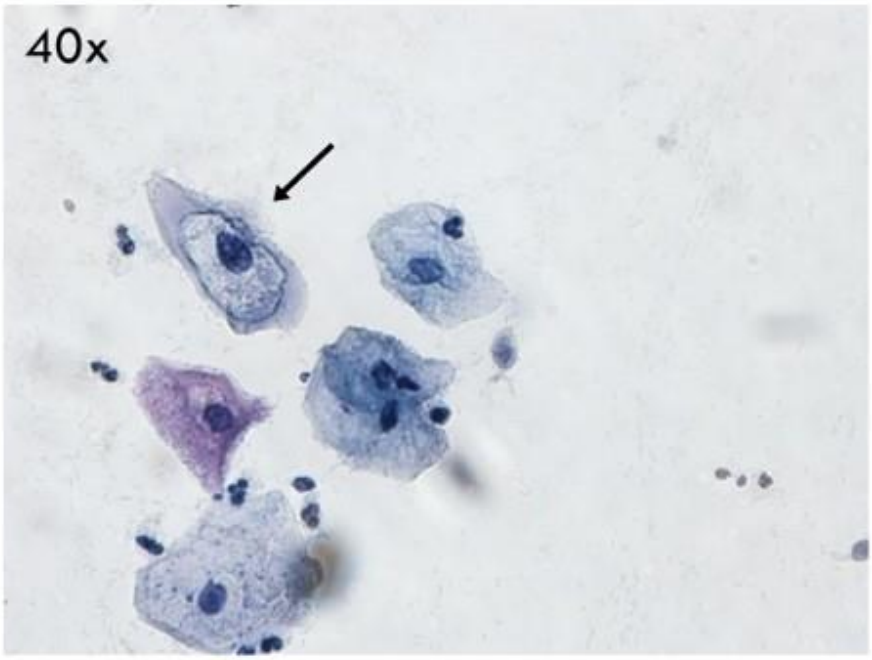
32



Actinomyces

- occurs very rarely in non- Intrauterine contraceptive device users
- a gram positive anaerobic filamentous bacillus
- Tangled clumps of filamentous organisms, often with acute angle branching, sometimes showing irregular wooly or cotton ball appearance, also called "dust bunnies" or "Gupta bodies"
- Dark purple in color
- abundant mixed flora in the sample

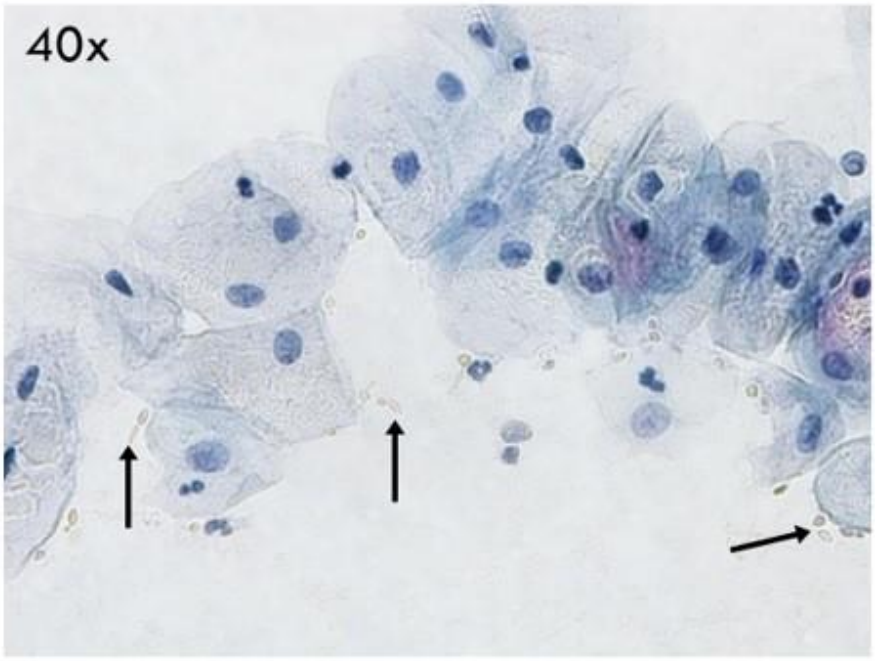
33



Koilocytes

- a cell finding typical of condyloma
- nucleus hyperchromatic, slightly enlarged
- lightly colored, well-defined area around the nucleus = "halo"
- Binucleation is typical

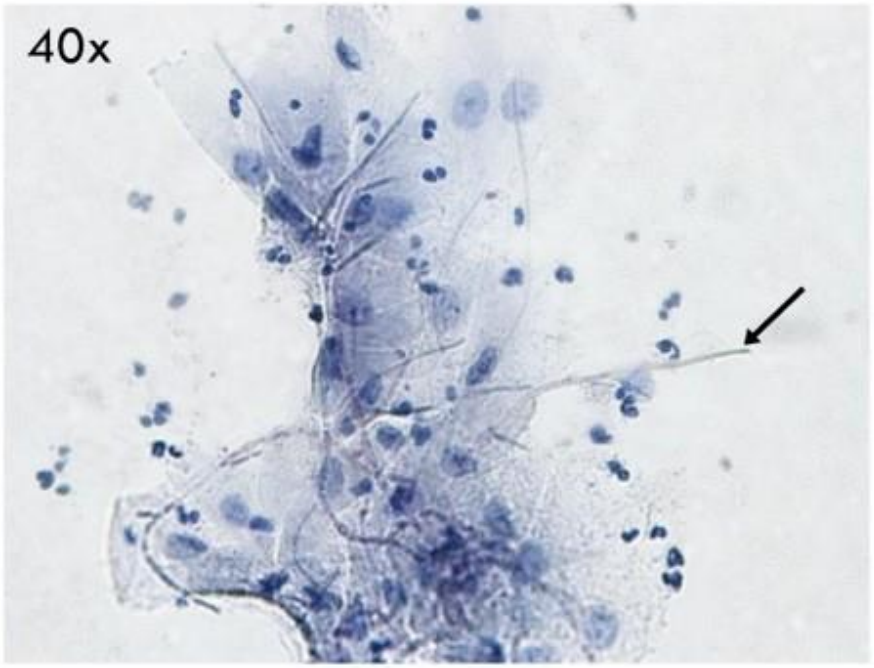
34



Yeast/fungus (yeast spores)

- Caused by Candida albicans species
- Small yeast cells are found at the edges of squamous epithelial cells
- Oval shape
- The yeast cell has a capsule of cellulose and chitin that can appear as a colorless "light yard" around the cell
- Pinkish, purple, dark blue or brownish in color

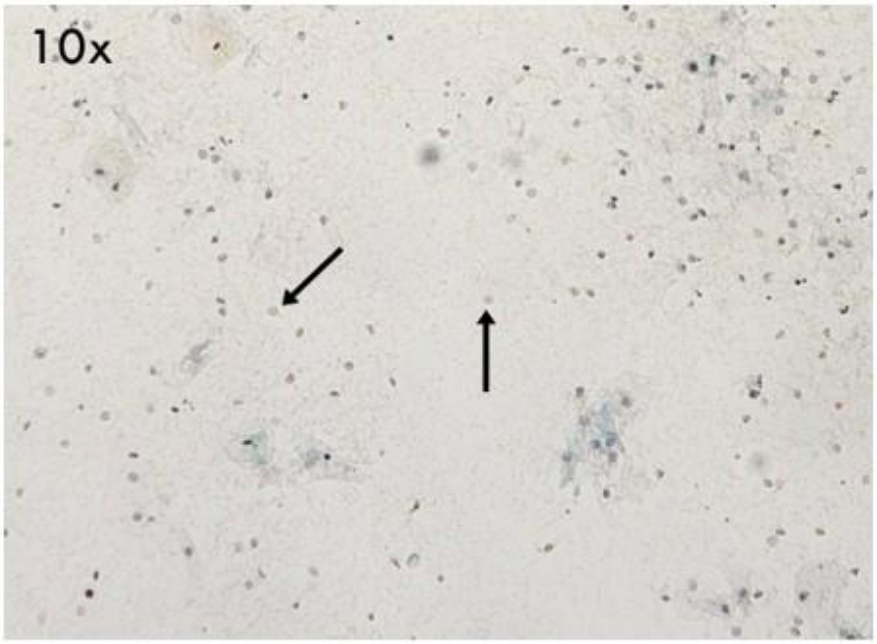
35



Yeast (fungal hyphae = threads)

- Caused by the same source as yeast cells
- In the threads or strands one can see segments
- The threads occur among squamous cell clusters
- Dark blue or purple in color

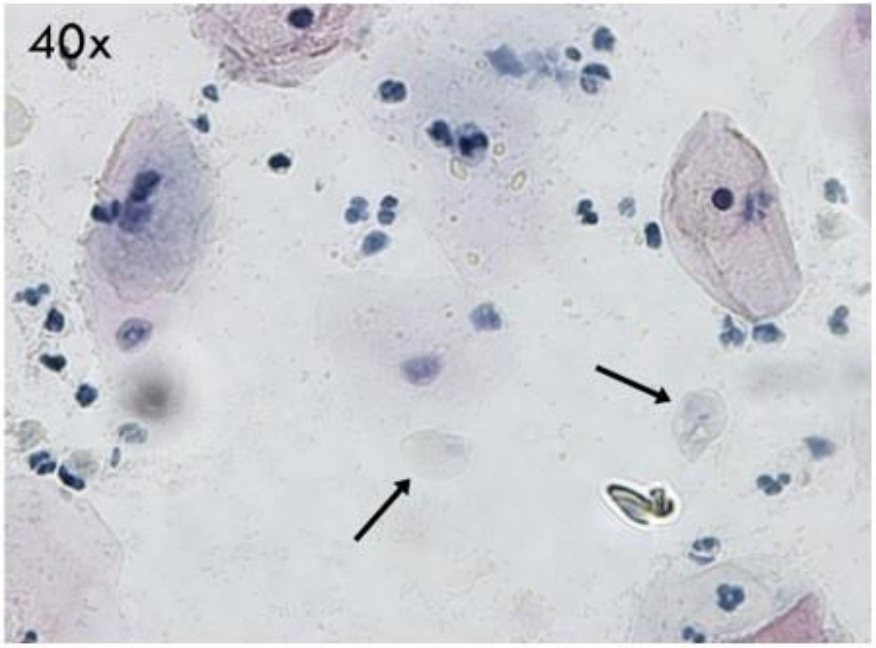
36



Cytolysis

- Döderlein rod-shaped bacillus (Lactobacillus) disintegrate the middle layer of squamous epithelial cells → the sample has a rich lactobacillus flora and bare mid-layer cell nuclei (plenty of naked nuclei in the picture)

37



Trichomonas vaginalis

- Round oval, teardrop-shaped, flattened. Significantly smaller in size than the superficial squamous cell
- Usually very light gray in color, sometimes slightly greenish. Rarely a small red granule can be seen
- The nucleus ('eye') is located on one side of the cell, also light gray in color, slightly darker than the rest of the cell
- The background of the sample is messy and inflammatory (mixed bacterial flora, lots of leukocytes)

7.2 Urine cytology or exfoliative urine test

The urine cytology test (or exfoliative urine test) result is given according to the Papanicolaou classes, and the result is supplemented by a cytologist's statement/diagnosis. Reliable interpretation requires sufficient biodata information in the referral and a high-quality sample.

Pap classes:

0 = insufficient sample

I = normal sample

II = benign sample, harmless cell atypia

III = pre-malignant sample, mildly suspicious

IV = pre-malignant or probably malignant sample, very suspicious

V = malignant, cancerous cells

Most cells in the urine cytologic sample (exfoliative urine sample) originate from the lower urinary tract, where the predominant cell type is the intermediate epithelium, or urothelium. There is also a non-keratinizing squamous epithelium at the very bottom of the urethra.

A sample from a healthy person is clean, with only a few single cells under a microscope (Figure 1). The sample contains mostly superficial squamous epithelial cells, more so in samples of women than in men's samples. There are normally very few intermediate epithelial cells.

Leukocytes and erythrocytes are normally very scarce. Abundant leukocyte counts indicate inflammation (Fig. 2). Catheter, lavage, and brushing specimens are characterized by a high number of intermediate epithelial cells and appear as uniform clusters.

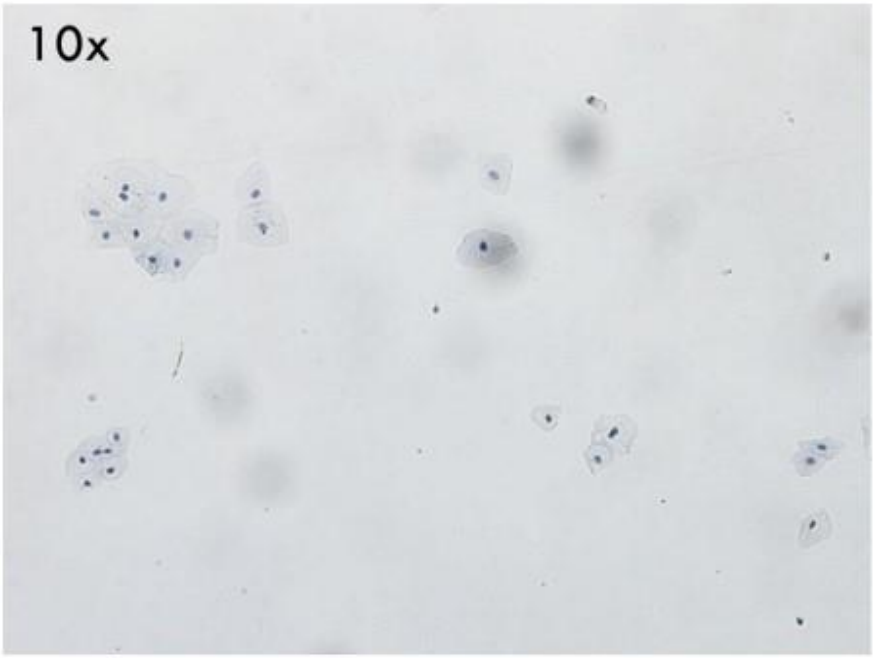


Figure 1.

Normal sample, poor in cells and clean. The figure shows squamous cells, which in the urine cytologic sample are very similar in appearance to the gynecological sample.

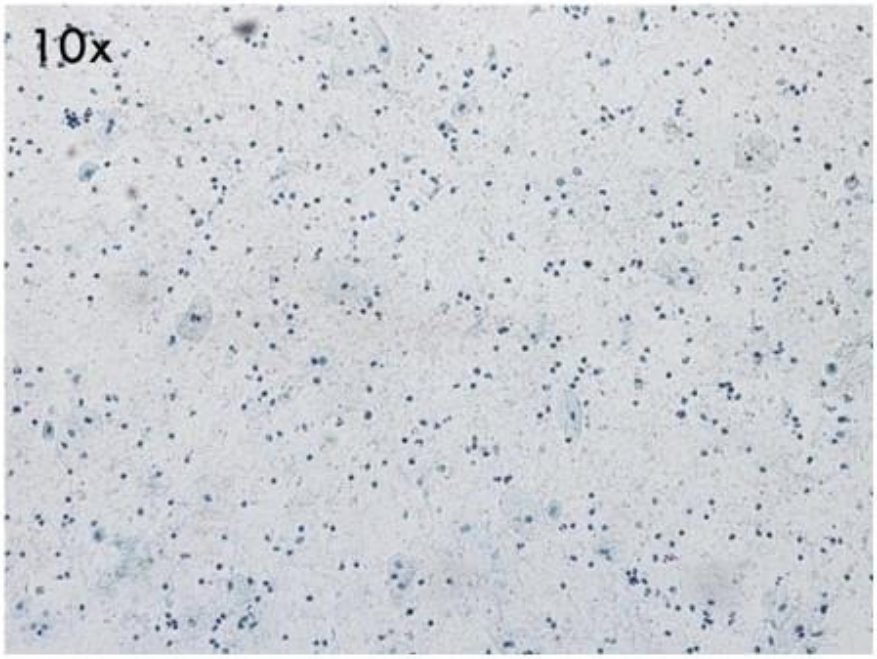


Figure 2.

There are a lot of leukocytes in the sample, which indicates inflammation

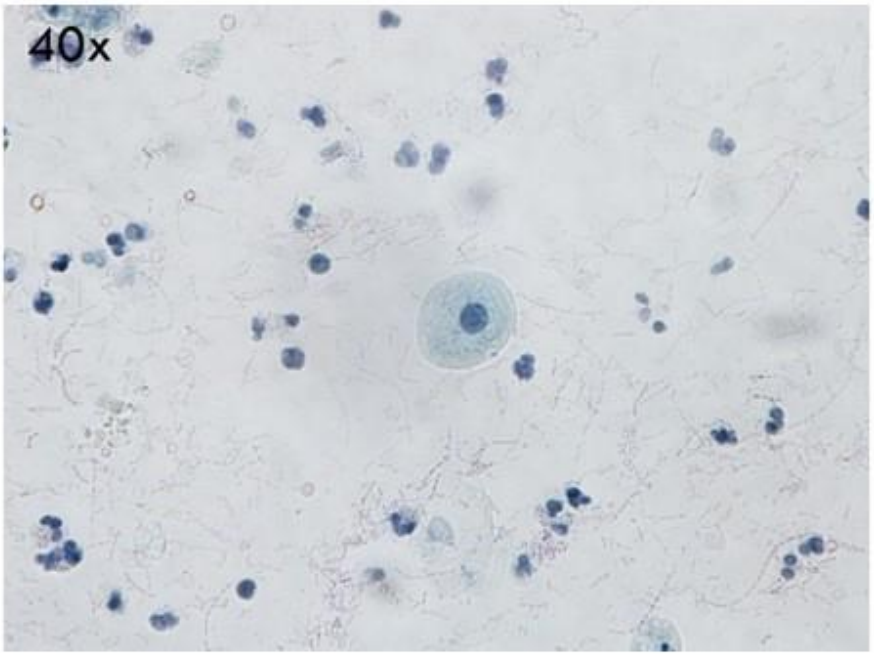


Figure 3.

Intermediate epithelial cell

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