

Searching for Microbes Part I.

Introduction to Diagnostics Microscopy of microbes I

e-learning version

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To practicals of VLLM0421c

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Hyperlinks to individual parts

Laboratory safety and practical advice

Clinical microbiology, definition and relations

Morphology of bacteria

Diagnostics of bacteria

Microscopy

Wet mount

Simple staining

Practical notes to microscopy

A tale...

- Once **and wolf, and tiger** and **HIV virus** met together.
- **The wolf** said: I wanted to bite a man, but he was armed, and so he would kill me. So I had to run away. But you, tiger, you are stronger than I am, maybe you could kill him.
- **The tiger** said: No, no, I can't, the man's arms are stronger than I am.
- Just now something laughed – something definitely invisible. „But I'll get him, you'll see!“

It was HIV virus...

Laboratory
safety &
practical
advices

Practical advice

- **Boxes for bags and outer coats** are the 1st, the 2nd and the 4th from window. **Do not use the 3rd box, even when open!** It is and box for secondary medical school.
- **Keys from boxes** should be let at the table next to the computer keyboard
- **On the wall next to the door** you can find and WC key. After coming back from WC, put it back to its place
- **It is forbidden to students to enter the space behind the practical's hall!**

Safety in the laboratory

- **Main risks of laboratory work** are risk of fire and risk of infection
- In the lab it is **forbidden to eat, drink and smoke**
- Students are obliged to **use the labcoats, but only labcoats of the Institute (NOT their own labcoats)**
- Students should **do only what they have to do** according to the protocols and the teacher's bid

Literature

- You need your **lab reports**. You will find them in Study Materials to VLLM0421c in is.muni.cz website. This week, exceptionally, I have printed some lab report forms for you.
- You will need and **textbook** – Greenwood's one, Murray's one or any like these can be used. The textbook should have virological, mycological and parasitological parts – not all textbooks have this!

You can also use our website
www.medmicro.info

Mikrobiologický ústav LF MU a FN u svaté Anny v Brně



Fórum

Výuka - Atlas

Education - Atlas

Výzkum

INSTITUT PRO MIKROBIOLOGII, FAKULTA LÉKAŘSKÉHO ÚSTAVU A FAKULTNÍ HOSPICÁL SVATÉ ANNY V BRNĚ

Institute for Microbiology, Faculty of Medicine
and St. Ann's Faculty Hospital in Brno, Czech Republic

Photo: archive of IfM

Presence in practicals

- If possible, presence in practicals should be 100 %
- Rare absences (one or two) consulted before or justified afterwards are possible
- Beside presence, you will also have to show your vigilance in practicals at the credit test or another form, that will show us that not only your body, but your soul, too, was present here

More instructions

- You have to draw the pictures (draw cocci, do not write „I can see cocci“)
- Draw the pictures in colours, you have to use coloured pencils
- Draw what you really see (if the cocci are confluent in the microscope, draw them so)
- If you cannot see what you think you have to see, tell to the assistant
- You will show your lab reports to the examiner at the examination and they will definitely influence your final result at the examination!

Here you can see, that lab reports are really important (and card of and Slovak student, partially translated into English)

<u>Semestr:</u> spring	<u>Semestr:</u> autumn
Započteno: 22.5.2006	Započteno: 22.12.2006
Vyučující: [Signature]	Vyučující: [Signature]
Poznámky: 8/8	Poznámky: 12/12
<u>Zkouška dne:</u> 14.2.2007	<u>Termín:</u> řádný

Otázka	Dílčí známka
Praktikum Determine, if they are <i>enterob.</i>	B <i>100%</i>
A: <i>Macrobif. & Enterococci</i>	A
B: <i>Q. difficile</i>	A-B
C: <i>Alfentz. (Klebs.)</i>	A
<i>Propocals: + (very nice!)</i>	
Výsledek: <i>Excellent (A)</i>	Examinátor: [Signature]

The cocci really ARE confluent in the microscope!



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Clinical
microbiology, its
definition and
relations to
other subjects

Classification of our branch

Molecular
biology and
genetics

Plant
microbiology

Infectious
medicine

General
microbiology

Human
medical
microbiology

Epidemiology
of infectious
diseases

Cell
biology

Veterinary
medical
microbiology

Dermato-
venerology

Who are clinical microbiologists

Basic microbiological research

Industrial
microbiology

Medical
microbiology

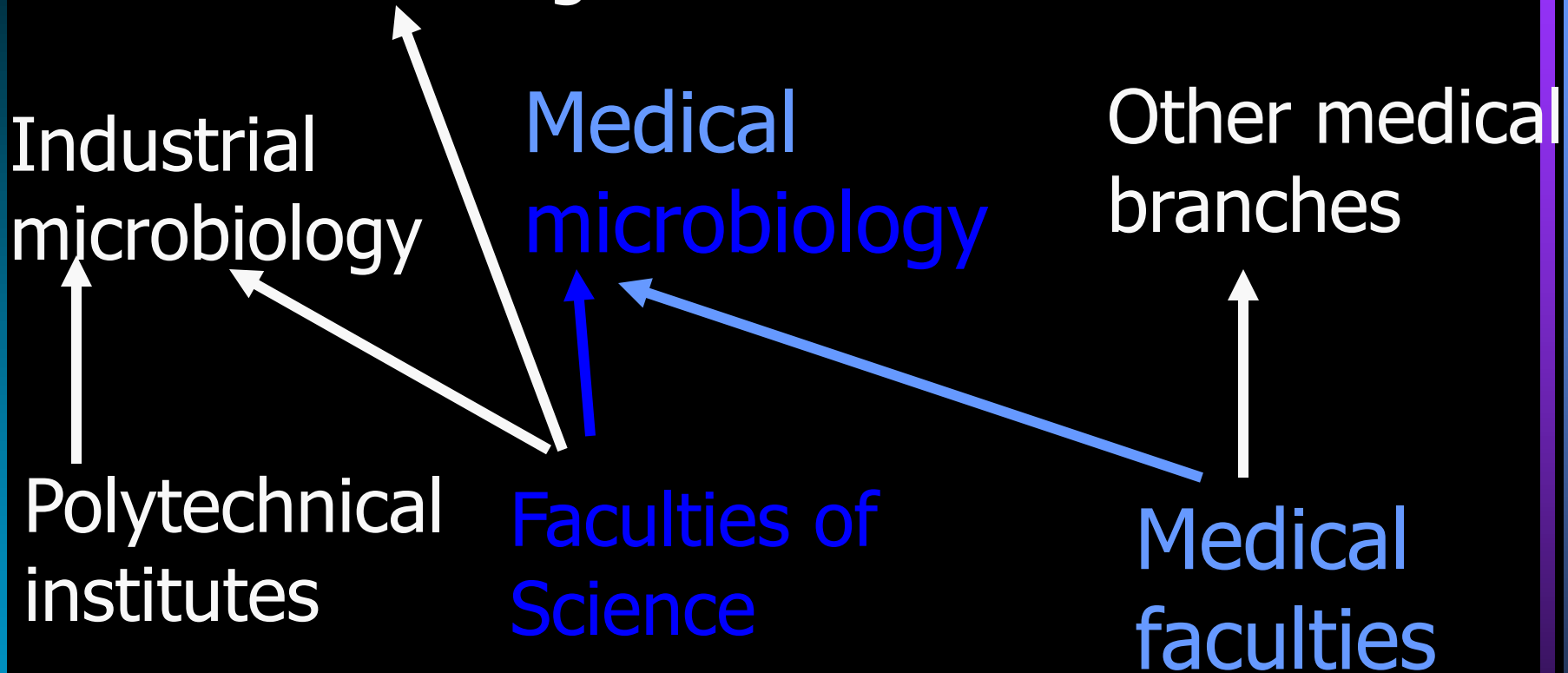
Other medical
branches

Polytechnical
institutes

Faculties of
Science

Medical
faculties

Your teachers are blue 😊



A microbe (microorganism): what does it mean?

- **It should be living.** A grain of dust is not a microbe, although it is microscopical
- **It should be microscopical.** A giraffe is not a microbe, although it is living

The second condition is not absolute. For example, a tapeworm can measure 10 m. But the eggs are microscopical, so it belongs to the microbiology.

Various microbes

- Among microbes we can find **microscopical algae and cyanobacteria, archea** (formerly archeobacteria), various organisms able to live long time **deep in the sea** or in extreme conditions of **hot springs**
- For us, clinical microbiologists, these microbes are not target of our interest. Nevertheless, they are very interesting.

What these microbes can

- They live **in depth** of 10 km
- They survive **temperatures** around 110 °C
- They stand strong **radioactivity**
- Instead of oxygen, they are able to „breathe“ sulphur or nitrogen (or: they have another electron acceptor than oxygen)
- Nevertheless, many interesting things are performed even by medically important microbes, as you'll see later.



Neisseriae

Classification of living organisms

- **Prions** – *no DNA, usually not counted to be living organisms at all*
- **Viruses and bacteriophages**
- **Cellular organisms**
 - **Archaea** (archeobacteria)
 - **Eubacteria** (eubacteria)
 - **Eucarya** (eukaryotic organisms)
 - monocellular
 - polycellular

Medically important microbes 1

- Medically important microbes are such, that are **important for human body** (so not for human = creator, but for human = object)
- „Important for body“ is not at all the same as „harmful for body“. On the contrary, **many of them are harmless, or even helping us!**

Medically important microbes 2

- **Each organism has its medically important microbes:** human, each species of animal or plant
- Even some microbes (e. g. bacteria) have their own microbes (bacteriophagi).

Neisseria gonorrhoeae



http://medicine.plosjournals.org/archive/1549-1676/2/1/figure/10.1371_journal.pmed.0020024.g001-M.jpg

Main medically important microbes

- **Viruses** (and prions)
- **Bacteria** (e. g. and *Streptococcus* or an *Escherichia*)
- **Fungi** (yeasts and molds)
- **Parasites** – not all of them are microbes:
 - **Inner parasites**
 - **Protozoa** (e. g. *Plasmodium malariae*)
 - **Flukes** (e. g. *Schistosoma haematobium*)
 - **Roundworms** (e. g. *Ascaris lumbricoides*)
 - **Tapeworms** (e. g. *Taenia saginata*)
 - **Outer parasites** (lice, fleas, bugs)



*Corynebacterium
diphtheriae*

Picture is made by
as. MUDr. Petr Ondrovčík, CSc.,
former lecturer of the Institute,
deceased young in autumn 2007

**Bacteria are
interesting!**



Morphology of bacteria

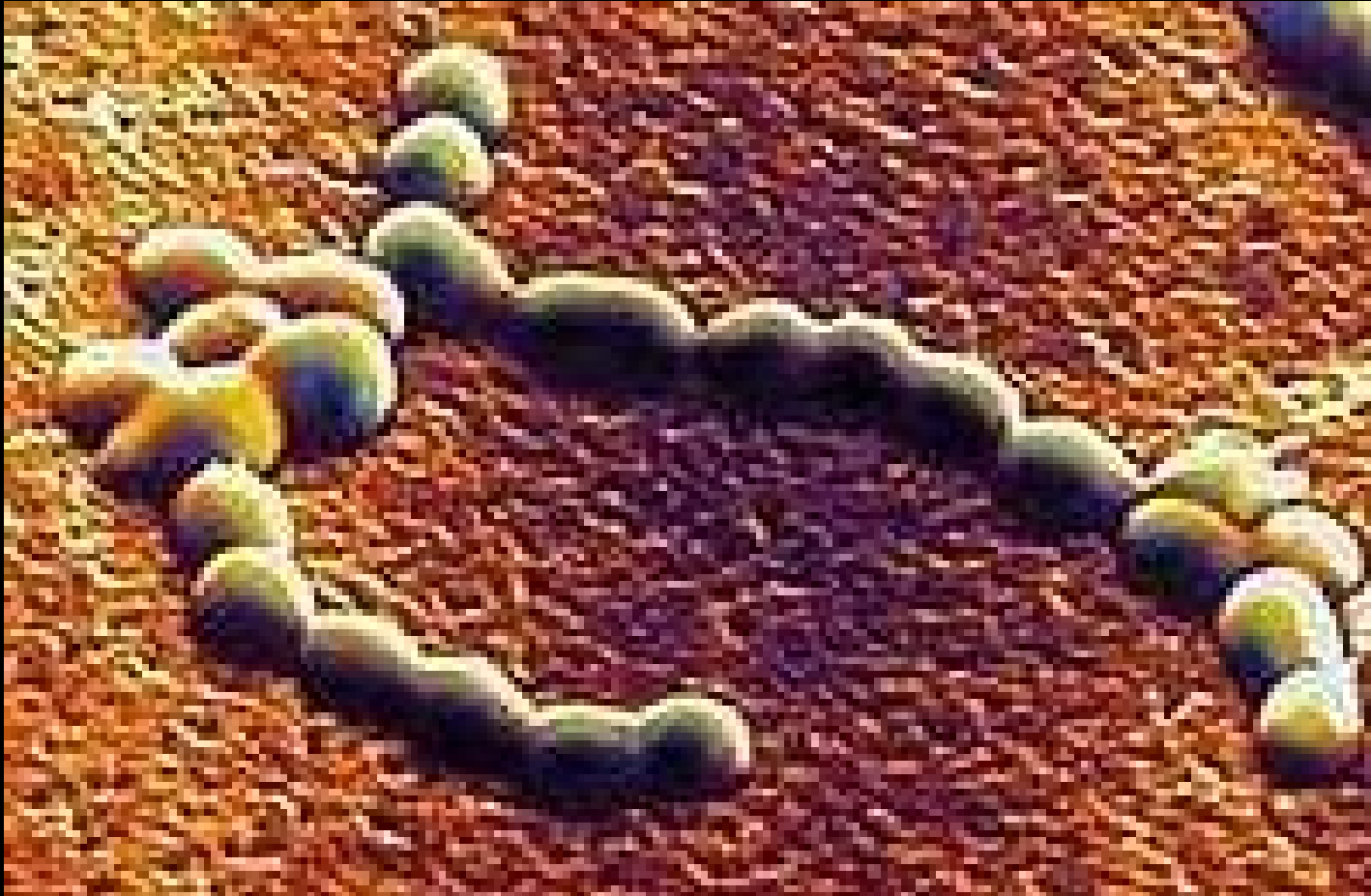
Morphology of medically important microorganisms

- **Viruses** are composed of **DNA** or **RNA** and **proteins**; some viruses possess an envelope „stolen“ to a host cell
- **Viruses** have **cubic** or **helicoidal symmetry**. Several of them are able to form „pseudocrystals“
- **Yeasts** are egg shaped, they can form buds and so named pseudomycelia. On the surface they have a **cell wall**
- **Filamentous fungi** and **parasites** are very variable in their shapes and they have various **development stages**

Morphology of bacteria

- **Cocci** in couples (diplococci), in chains and clusters (do not say „streptococci“ and „staphylococci“, it would be confusing)
- **Rods** straight or curved (vibria), eventually several times curved (spirillae), short or long, forming filaments or branched filaments; their ends may be round or edged and also rods may be arranged in various way
- **Spirochets** – thin spiral bacteria
- **Amorph bacteria**, e. g. mycoplasmas (they do not have any wall, so do not have shape)

Cocci in chains (electronoptic microphotograph of *Enterococcus* sp.)



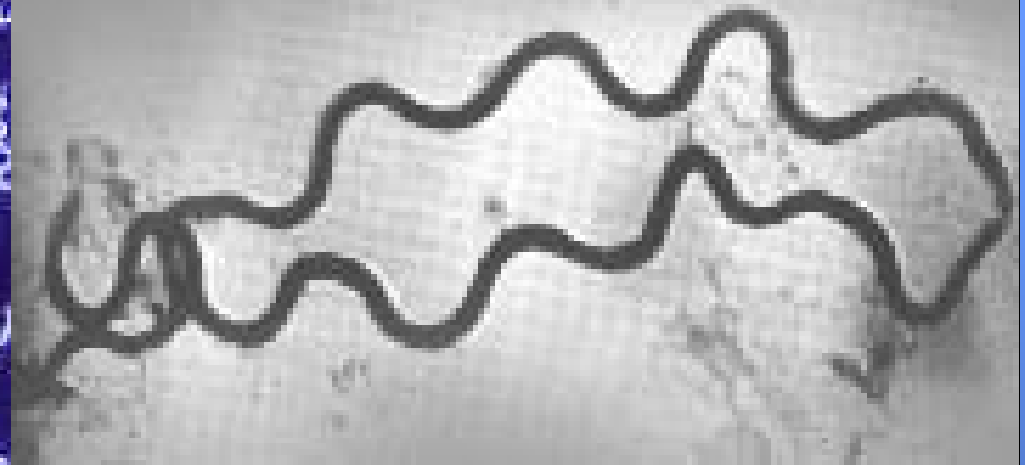
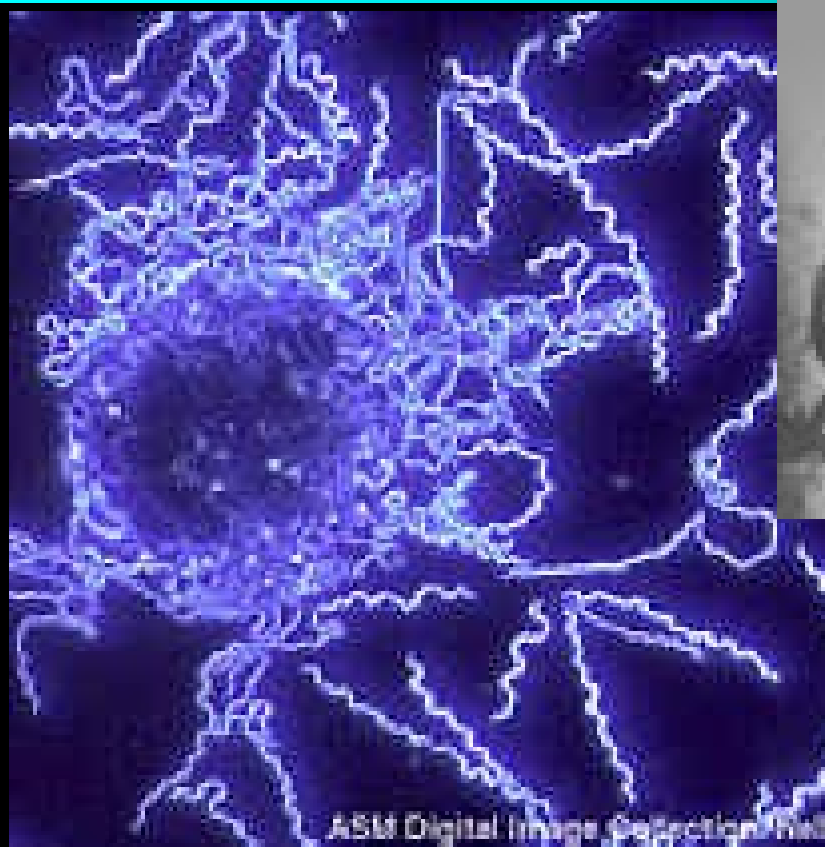
<http://www.morgenwelt.de/typo3temp/5ce14d39b5.jpg>

Several times curved rods – Helicobacter

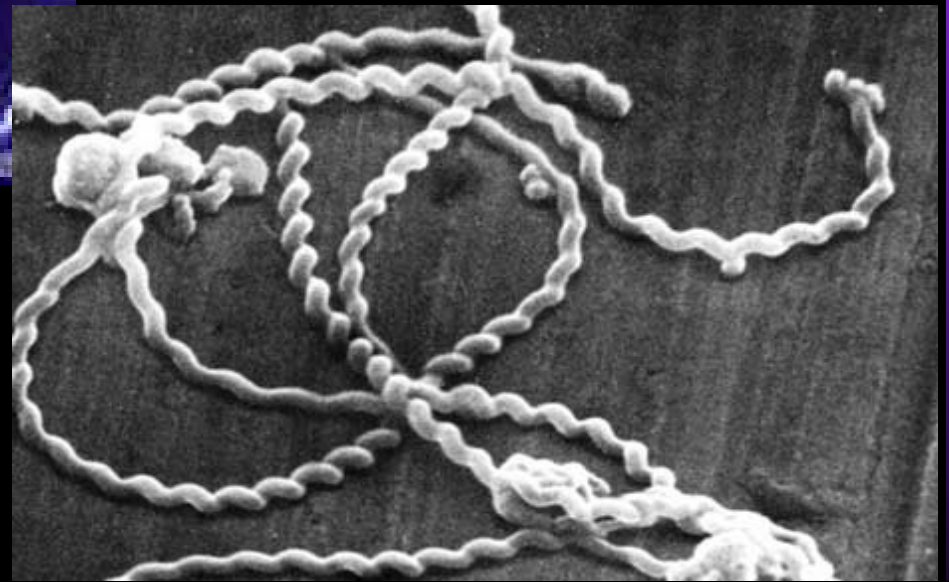


<http://vietsciences.free.fr/nobel/medecine/images/helicobacter%2520pylori.JPG>

www.primer.ru/std/gallery_std/treponema.htm



Spirochetes



<http://nl.wikipedia.org/wiki/Afbeelding:TreponemaPallidum.jpg>

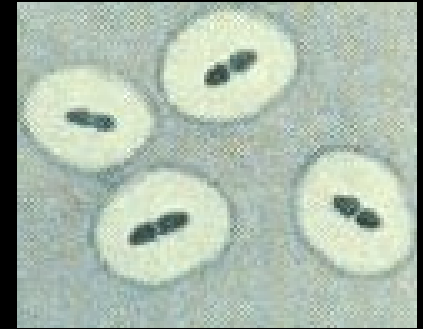
Fimbriae and a flagella

- Many bacteria are able to **move**
- They move mostly by **a flagella**
- **Fimbriae** are used to movement, adhesion and to **exchange of genetical information**
- Bacterial flagellae are different from flagellae of eukaryotic organisms
- Fimbriae and flagellae are invisible in optical, but often in electronoptical microscopy

Bacteria with flagellae (*Escherichia coli*)



http://www.biotox.cz/toxikon/bacteria/bacteria/obr/escherichia_coli_1.htm



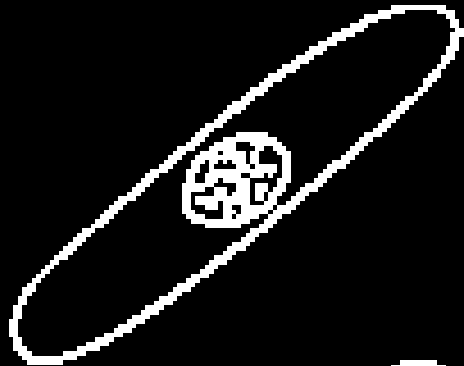
Capsulla and biofilm

- **Capsulla** surrounds an individual bacterium or a couple of bacteria. It is not an integral part of bacterial cell, rather complex of molecules (mostly polysacharids), protecting the cell
- **Biofilm** is an integral layer formed by bacteria, their capsullae and other material. It is much more resistant than an individual bacterium living in so named planctonic form

Sporulation

- Sporulation is **something like winter sleep**, but much stronger
- Spores can survive **high temperatures, drying, disinfection** and so on
- A spore is formed as an **endospore**: cell is divided, but not entirely: one part is transformed into an endospore, that comes inside the second part
- *Bacterial spores × fungal spores!*

Endopores of various species of genus *Bacillus*

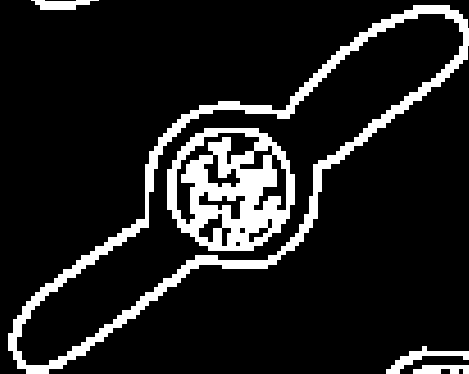


ex : *B. Subtilis*

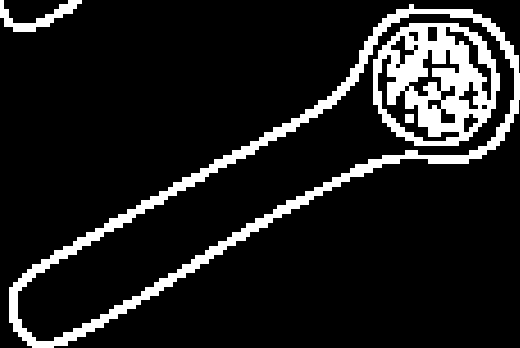
B. Cereus

B. Thuringiensis

B. Anthracis



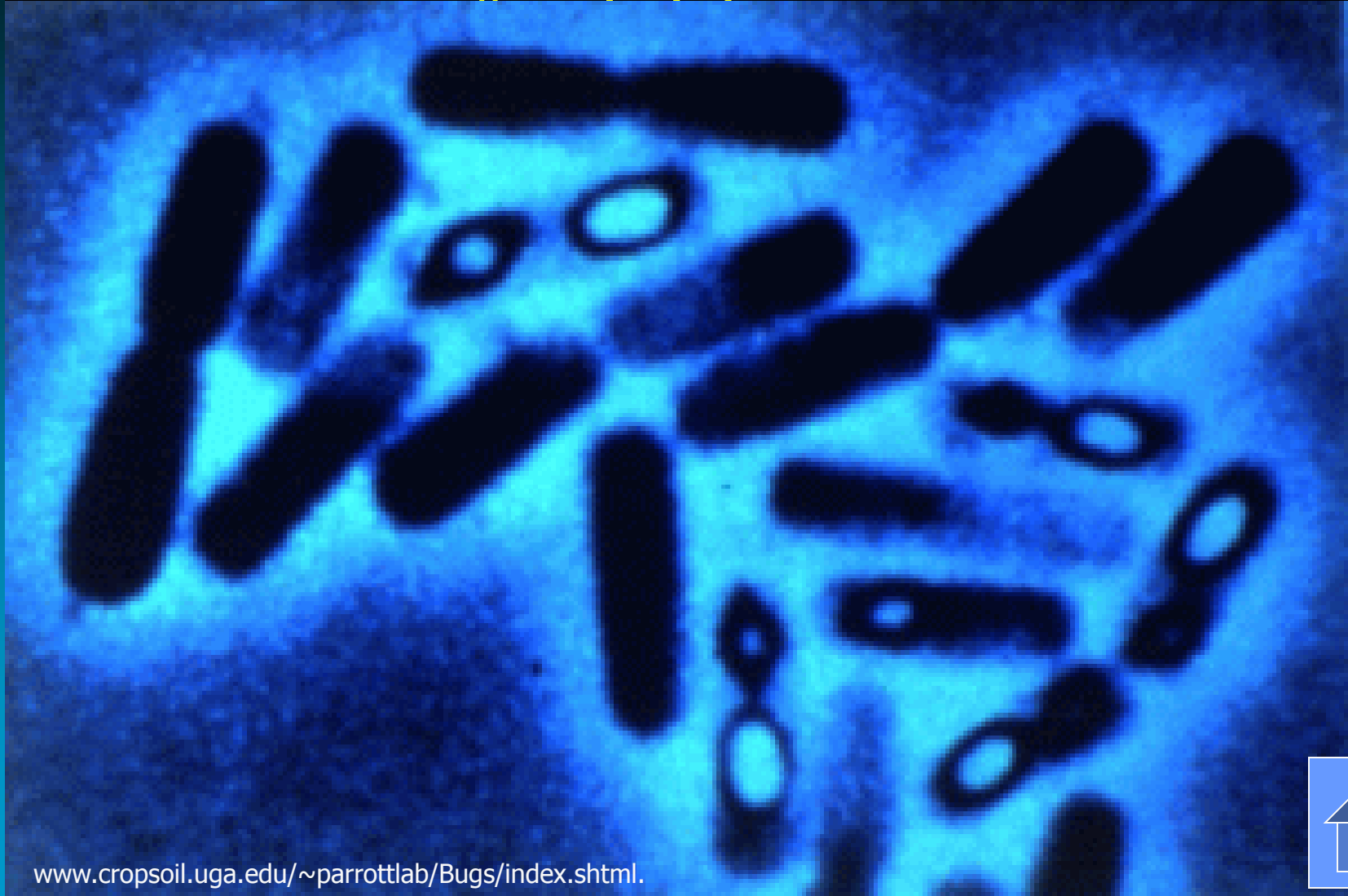
ex : *B. Polymyxa* (fixe le N₂)



ex : *B. Pasteurii* (dégrade l'urée)

[http://membres.lycos.fr/neb5000/BacteriologieI/Groupe s%20Bacteriens/Battonnets%20et%20coque%20Gram-positifs%20formant%20des%20endospores.htm](http://membres.lycos.fr/neb5000/BacteriologieI/Groupe%20Bacteriens/Battonnets%20et%20coque%20Gram-positifs%20formant%20des%20endospores.htm)

Endospores do not stain themselves. They are visible as „empty place“ inside bacteria



Diagnositics of bacteria

Diagnostics: detection of bacteria and their determination

- **Practical medical microbiology** means that a **doctor** (general practitioner, specialist, doctor from hospital) **sends a specimen to the lab**
- **A laboratory of medical microbiology has to prove eventual presence of bacteria** in such a specimen, and eventually **to determine them.**
- The determination does not need to be perfect, but it has to give **enough information for treatment**

Specimen versus strain I: specimen

Specimen is what is taken from the patient and comes for laboratory examination

- **liquid or solid material in a test tube** or other test tube (blood, serum, urine...)
- **cotton swab**, usually in transport medium.

Specimen versus strain II: strain

Strain is pure culture („cultivate“) of one species of a microbe

Strain can be gained only by cultivation of a microbe on a solid medium.

Koch`s discovery, that bacteria can be cultured like that, was essential for modern microbiology.

Survey of methods

- **Direct methods: We search for a microbe, its part or its product** (e. g. a bacterial toxin)
 - **Direct detection in specimen** – we use the whole specimen (blood, urine, CSF etc.)
 - **Strain identification** – isolate determination
- **Indirect methods: We search for antibodies.** An antibody is neither a part nor a product of a microbe – it is a macroorganism product, after being challenged by a microbe

Survey of direct methods



Method	Specimen examination	Identification
Microscopy	yes	yes
Cultivation	yes	yes
Biochemical identificat.	no	yes
Antigen detection	yes	yes
Animal experiment	yes	usually not
Molecular methods	yes	usually not*

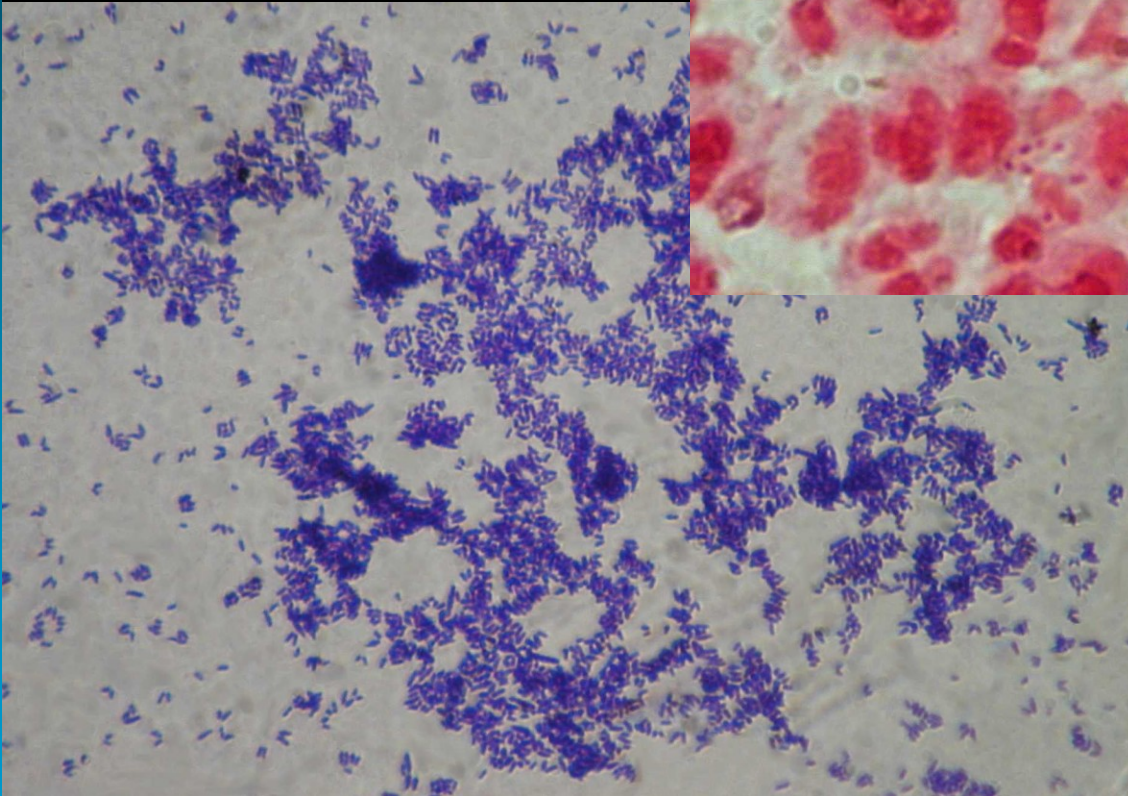
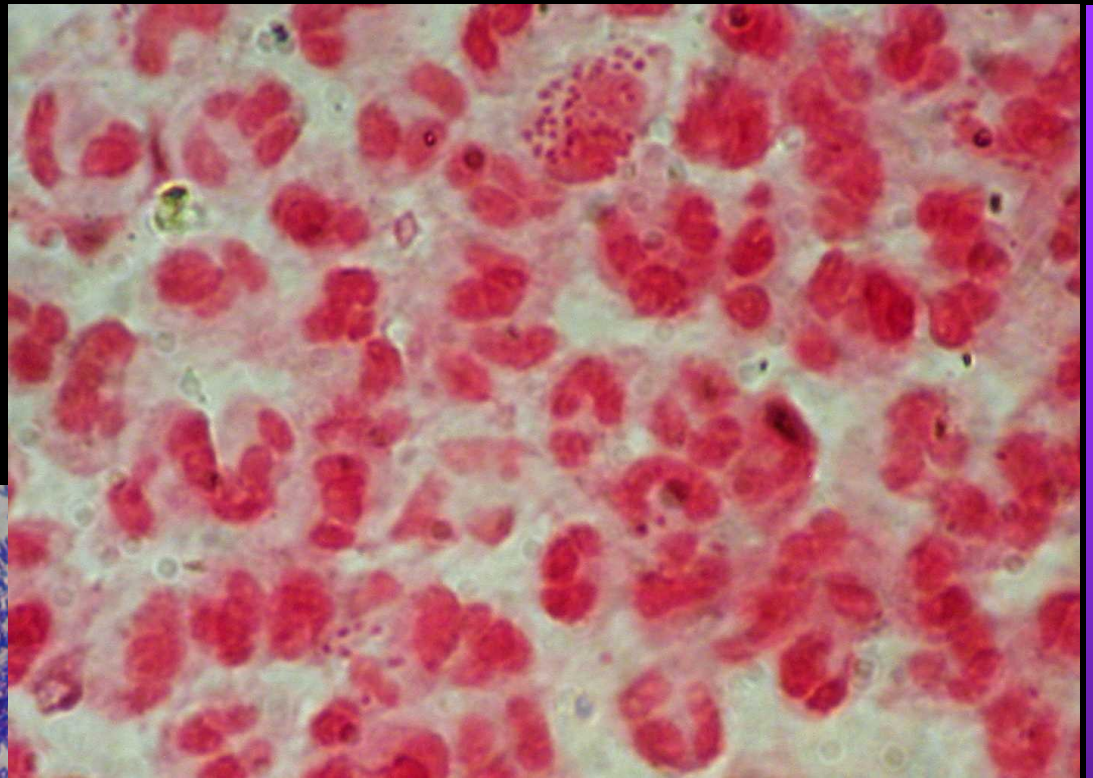
*but in molecular epidemiology – detection of simillarity of strains - yes

Microscopy

Microscopy

- **We observe microbes, in specimen also cells of host organism** (epitheliae, WBCs etc.)
- **Wet mount** – for large and/or motile microbes (parasites, fungi, motile bacteria)
- **Dark field wet mount** (mainly spirochets)
- **Fixated and stained preparations** – Gram staining, Giemsa staining, Ziehl Neelsen staining (use for various groups of bacteria, fungi, parasites)
- **Electron microscopy** – in viruses; rather for research than for common virological diagnostics

Microscopy of a specimen



Microscopy of a strain

Main microscipical methods in medical microbiology

	Drying and fixation	Coverslip	Imersion system
Wet mount	no	yes	no
Darkfield wet mount	no	yes	yes
Stained preparat.	yes	no	yes

Preparing a microscopical preparation

- We make a **smear of a swab** made by a cotton swab (in stained preparations only)
- **Liquid specimen** are **dropped on a slide**
- If we have a strain, **we make a drop of physiological saline onto the slide**. We sterilize a microbiological loop in flame and after drying we take **a little of bacterial mass**. We mix it in a drop of **saline**.

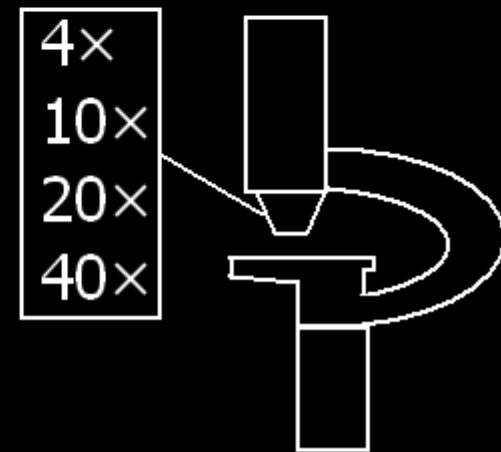
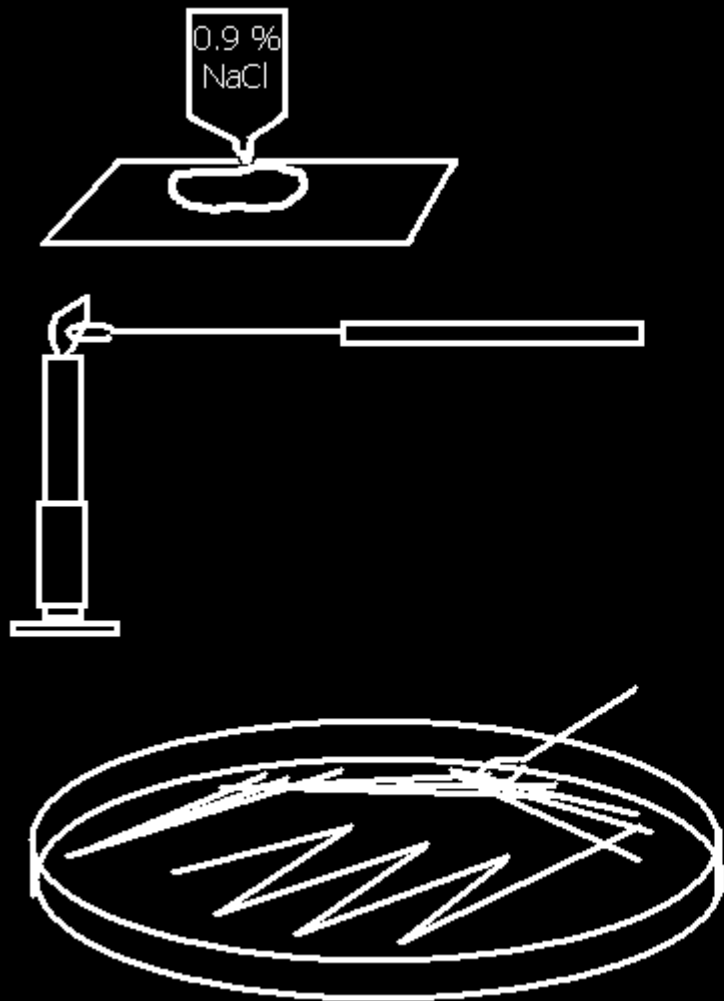


Wet mount

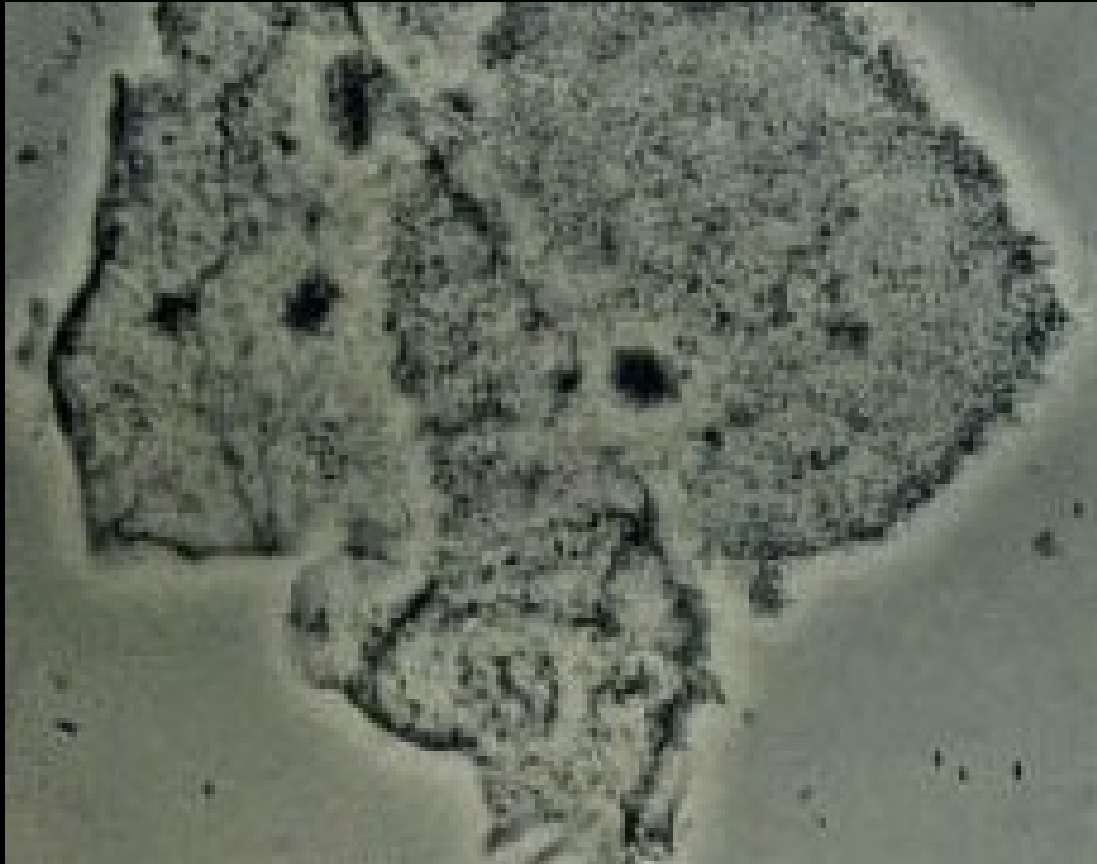
Wet mount (native preparation)

- In case of a wet mount a drop, in which there is a specimen or mixed strain, we do not dry. We only cover the preparation by a coverslip and we observe by objectives, magnifying e. g. 4×, 10×, 20× or 40×.
- We use no immersion oil
- **Task 2b: drop on slide a drop of a C. A. T. medium with patient's swab. Observe microbes, but also epithelial cells, eventually leucocytes.**

Wet mount – procedure



An example of a wet mount C. A. T.



<http://www.kcom.edu/faculty/chamberlain/Website/lectures/lecture/image/clue3.jpg>

Simple staining

Preparing a stained preparation

- We start again by a drop of specimen or of a strain mixed in saline. In this case, **the smaller the drop is, the better.**
- A drop is let to dry. It is allowed to help drying by placing **near to the** burner.
- After drying, the preparation is fixated by drawing the slide **through** the flame of the burner. It is necessary to check the temperature by your hand.

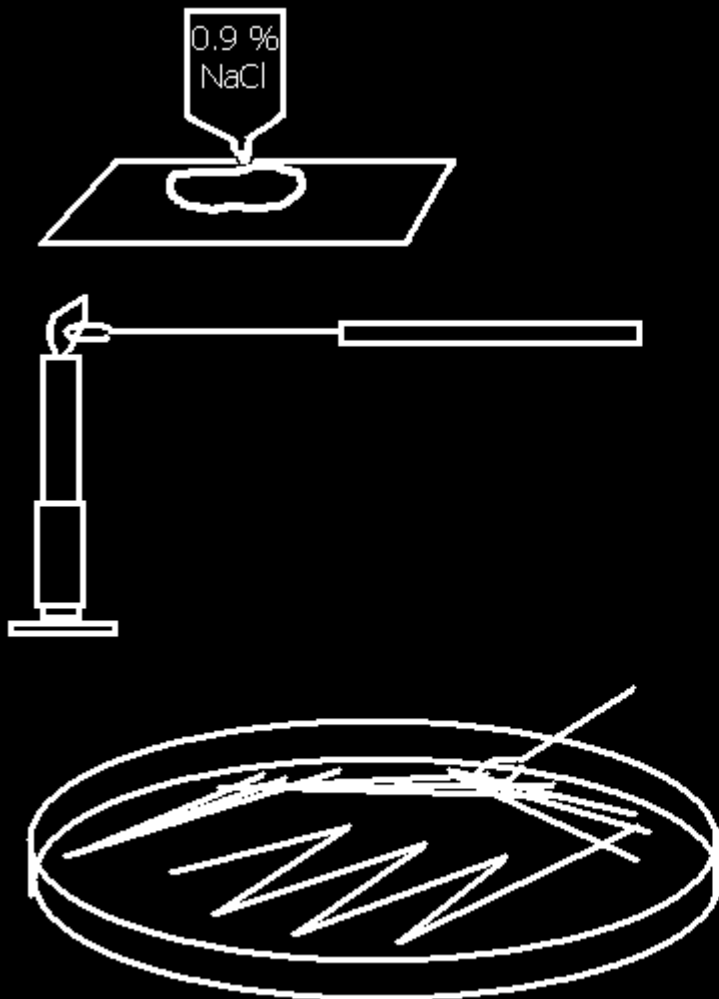
Simple staining

- May be used **if necessary directly by a general practitioner**, that is far from the nearest microbiological laboratory and has methylene blue and a microscope.
- **Not commonly used in practice**
- Shows us **size, shape and arrangement of microbes**

Simple staining – how to do it

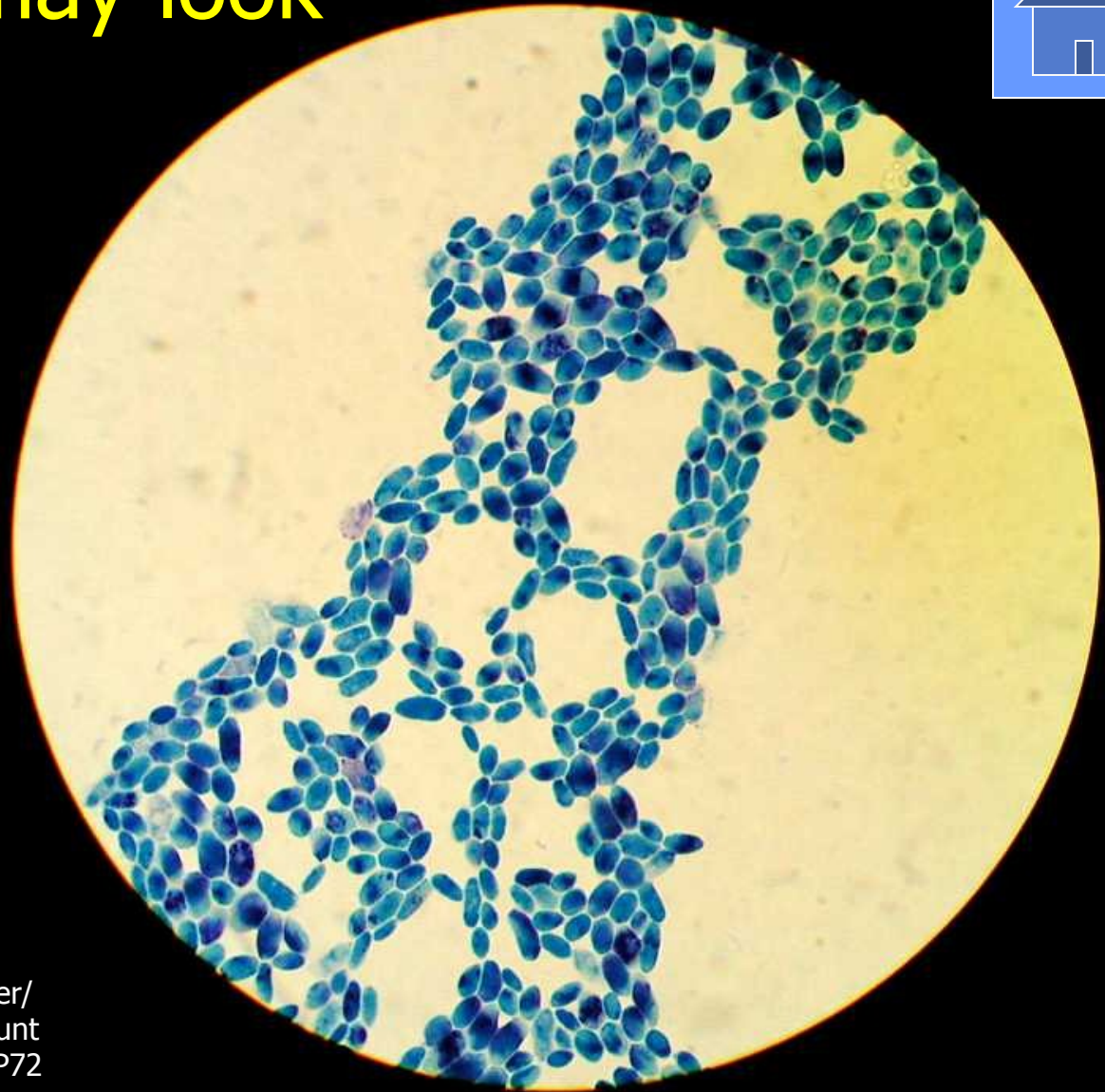
- Fixated preparation **place on a grid in sink** and **pour methylene blue on it**
- Let it be **some two minutes**
- Then **wash by tap water**, and dry by filtration paper
- Add **a small drop of immersion oil** and observe by an objective magnifying 100 ×

Simple staining



DRYING
FIXATION
METHYLENE BLUE
2 minutes
Drying with filtration paper
MICROSCOPY:
IMMERSION OIL
100 × OBJECTIVE

The result may look like this (yeasts):



http://biology.clc.uc.edu/fankhauser/Labs/Microbiology/Yeast_Plate_Count/09_Yeast_Meth_Blue_P7201177.jpP7201179.jpg

Practical notes to microscopy

Practical advice:

- Objectives should be cleaned **by soft tissue only**. Once used tissue is thrown out and not used once more.
- The microscopical table is cleaned by **paper square**. Also this is thrown out after use

After having finished the microscopy, it is necessary:

- When using immersion, clean the immersion objective by gase and petrol
- Eventually clean like this also other objectives, if they are dirty
- The table of the microscope clean by a square of paper, eventually also with petrol
- Switch off and cover your microscope

The End

