

Institute for microbiology shows



# TRACING THE CRIMINAL

Part six: More gram-negative  
cocci and bacilli

# Survey of topics

Clinical characteristics – G– cocci

Clinical characteristics – „other G– bacilli

Diagnostics of G– cocci

Diagnostics of „other G– bacilli“

# Clinical characteristics – G– cocci



# Story One



- **Johnny was** very childish, he had no experience with women even in 20 years of age. His friends made fun of him. Once they made a plan: they gave him lots of spirits and paid a prostitute for him. Johnny had a feeling, that he is finally a man... only before **pus** started to drop from his urethra...

# Certainly, you know, what is the criminal

- Of course, it is *Neisseria gonorrhoeae*, (gonococcus) causing gonorrhoea.
- Gonorrhoea is urethritis, in females also cervicitis; asymptotically or symptomatically gonococci are found in pharynx and rectum, too.
- In females, it is not a colpitis (vaginal inflammation), so it is not recommended to perform vaginal swabs in gonorrhoea.

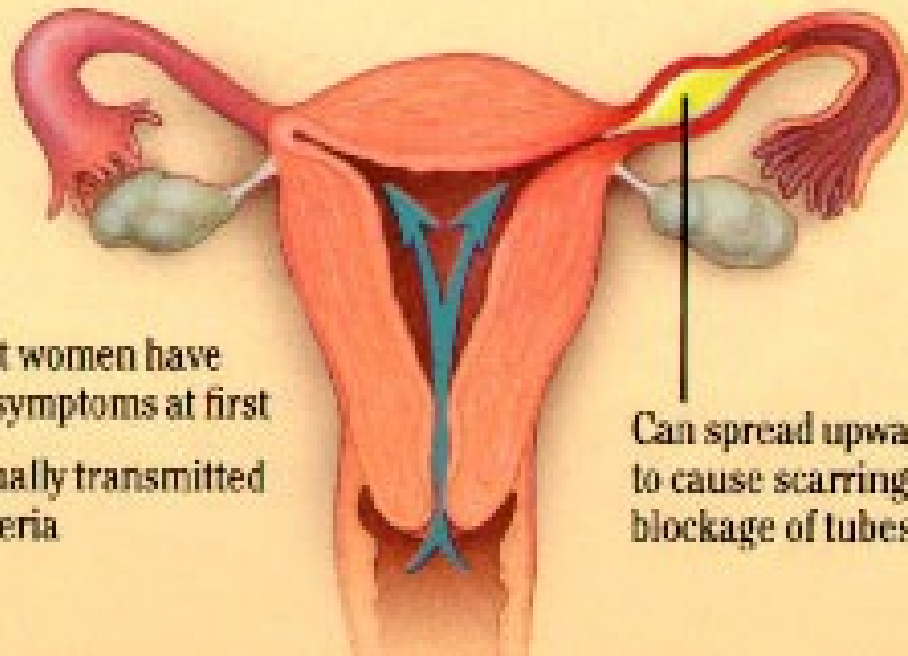


# Partner of Johnny of that night...

nova.medicina.cz

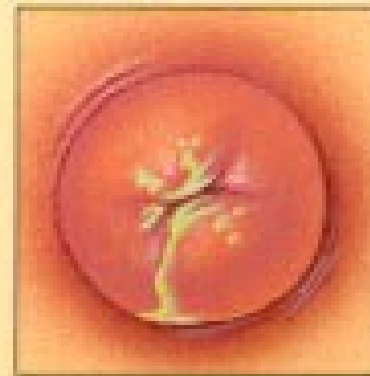


www.lcusd.net

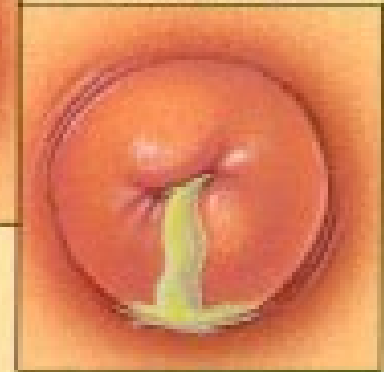


Can spread upwards to cause scarring or blockage of tubes

Chlamydia



Gonorrhoea



Most women have NO symptoms at first

Sexually transmitted bacteria

# Story two

- Lucy learned four weeks for the examination of physiology. **She did not leave the house at all** and only sat at textbooks. At the exam she had intention, that she is not able to say a word, but finally she passed with „E“.
- In the evening, **she visited a dancing party with friends**. The party was full of smoke and they danced during all night. Next day, **Lucy was not well**, she started to **have fever** and rash.

## (continuing)



- In this moment, Lucy was hospitalized at **infection clinic**. In ambulance she failed unconscious and doctors constated **metabolic failure**. After ten hours of attempts to keep Lucy's vital functions, that had no effect, **Lucy died**.
- Such a course of infection may be caused by a **dangerous criminal**. Some of his strains are present in **throat of healthy persons...**



# And the criminal is...

- ... ***Neisseria meningitidis*** or **meningococcus**
- Meningococcus causes meningitis, but also sepsis and other serious problems; all this is product of **clonal strains**.
- Other strains are completely innocents and studies say that some ten percent of population are throat carriers of meningococcus.
- Virulence is related mostly with **protein antigens**
- **Polysacharidic antigens** determine preventability by vaccination

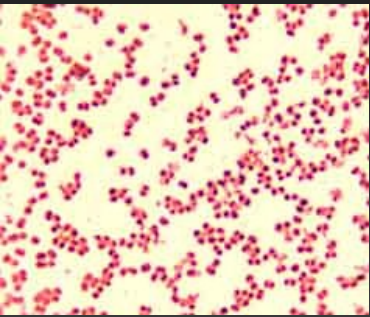


# Why the infection comes sometimes, and sometimes does not

- The invasive infection is only present, when the strain is highly virulent (specific clones of the microorganism) and the host organism is ready to get infected
- Meningococcus is transmitted by a **narrow contact**. Invasive **infection is more likely when mucous membranes are damaged**, e. g. by smoking or previous viral infection.
- Infection is often present after **too big physical activity after long inactivity period**

***Meningococcal infection is serious, but quite rare in Europe. In some other parts of the world, the situation is different – see next slide.***

# Meningococcal meningitis is worldwide very important infection



„Meningitis belt“,  
area of extended  
presence of  
meningococcal  
meningitis



# Treatment

- It is necessary to **ensure patient's survival** (to follow haemorrhagia and acidobasic equilibrium)
- In the same time, antibiotics are administered
- Drug of choice in meningococcal diseases is even now **classical penicillin**. Among other drugs it is also common to use one of 3<sup>rd</sup> generation cephalosporins (**ceftriaxon** – good access to the CSF), or other antibiotics

# Prevention by vaccination

- As the incidence is not high in Europe (although the lethality is), usually not the whole population, but just risk groups are vaccinated (soldiers, people in contact with a risky strain)
- The problem exists with **serogroup B**, as its antigenic determinant is not a sufficiently strong antigen and so it is not possible to get a vaccine that would be sufficiently protective. Nevertheless, it is possible to protect people against some individual serotypes inside B serogroup (but not in Europe, here they are too many; the vaccines are used in Cuba and New Zealand)

# Vaccines

- There are differences between them. **Old polysaccharide** vaccines give less protection than **new conjugated** vaccines
- There exist also difference in **serogroups** (C only, A + C or tetravaccine A + C + W135 + Y)
- B and C are the most common types in Czechia, but e. g. Mecca hajj ( ) pilgrims need get vaccinated against W135



# Different serotypes in the world

<http://www.phac-aspc.gc.ca/publicat/ccdr-rmtc/09vol35/acs-dcc-4/index-eng.php>



# Let's compare neisseriae:



|                                    | In vivo                                     | In vitro  |
|------------------------------------|---|---|
| <b>Gonococcus</b>                  | The most delicate, sexual transmission only | The most delicate, grows on chocolate agar only             |
| <b>Meningococcus</b>               | Less delicate, short distance air transport | Less delicate, if blood agar is enriched, growth is enabled |
| <b>So called „oral“ neisseriae</b> | The least delicate, air transport possible  | The least delicate, grows even on poor blood agar           |



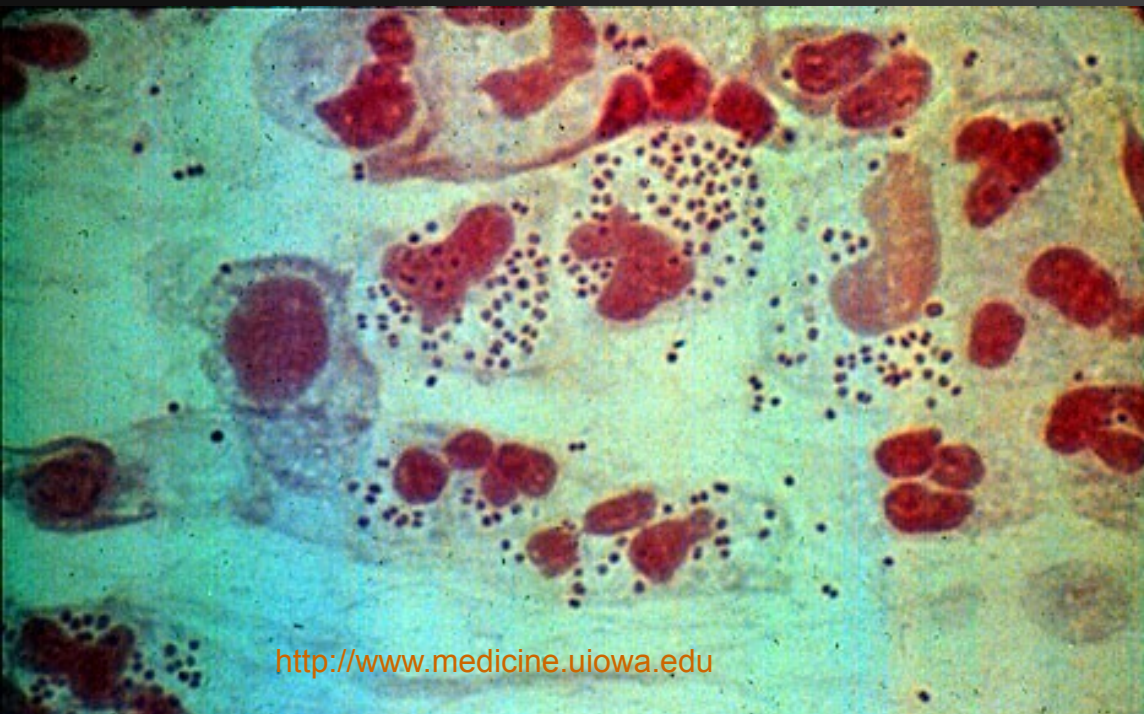
# Story three

- Annie was **crying and touching her ear**. Her mother measured her temperature, and it was elevated.
- At general practitioner's, Annie was examined and diagnosis of **otitis media** was set
- As her **tympanon was already broken**, the pus was taken for examination
- AMOCLLEN (**amoxicilin**) was used for threatment imediately. Later, a **susceptible pathogen** was found.

# And this pathogen was

- ***Moraxella* (sub-genus *Branhamella*) *catarrhalis* – another Gram-negative coccus**
- This organism is often present in small amounts even in healthy persons.
- In pure culture causes **sinusitis, otitis media, pharyngitis** etc.
- Her sub-genus (originally genus) name *Branhamella* was derived from Sarah Branham, one of first women scientists. She was one of brave women to show man that not only men can be good in science

# *Moraxella (Branhamella) catarrhalis*



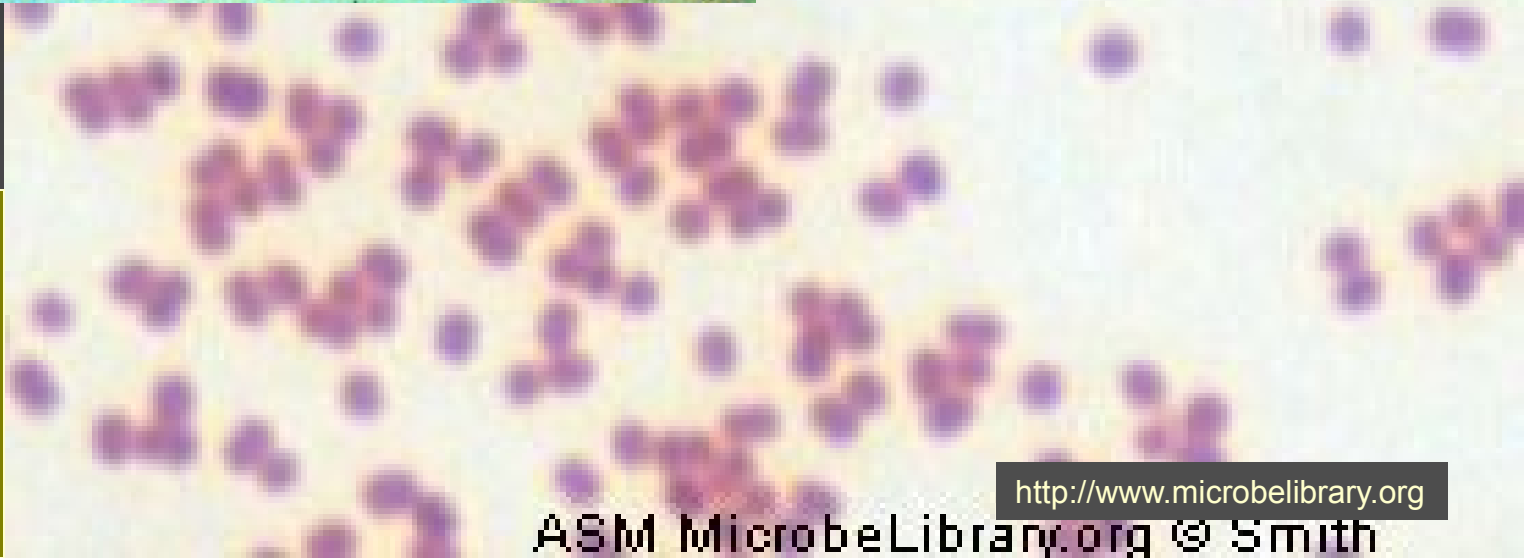
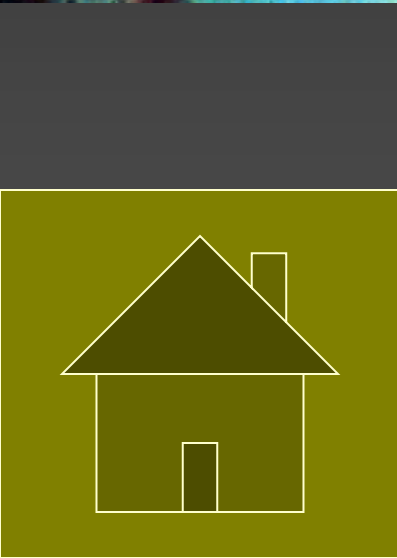
<http://www.medicine.uiowa.edu>



©Dr. med. T. Pietzcker, Ulm

<http://www.uni-ulm.de>

medianovo.de



<http://www.microbelibrary.org>

ASM MicrobeLibrary.org © Smith

Clinical  
characteristics –  
„other G– bacilli“

# Story four

- There was a big movement in the hospital that day: three more patients, all of them **seniors**, became ill, and again it was the same – **breathing problems and fever**
- After a thoroughful examination, the laboratory found the pathogen not only in patient secretions, but also in hot **water pipes** of the hospital. The pipes had to be rebuilt and only after that the infections were stopped finally.

# Legionaire's disease

It is a disease caused by *Legionella pneumophila*

Legionaire's disease is a variant of disease that is quite severe; another variant, **Pontiac fever**, is rather mild

The bacterium of other have reservoir in **water instalation, air condition**, etc.

When building new hospital departments (but also senior houses, hotels, spa...) measures to prevent legionellosis should be taken, mostly at planing water pipe system (namely it is necessary to avoid blind branches, they cannot be run through by hot water or a disinfectant when necessary).



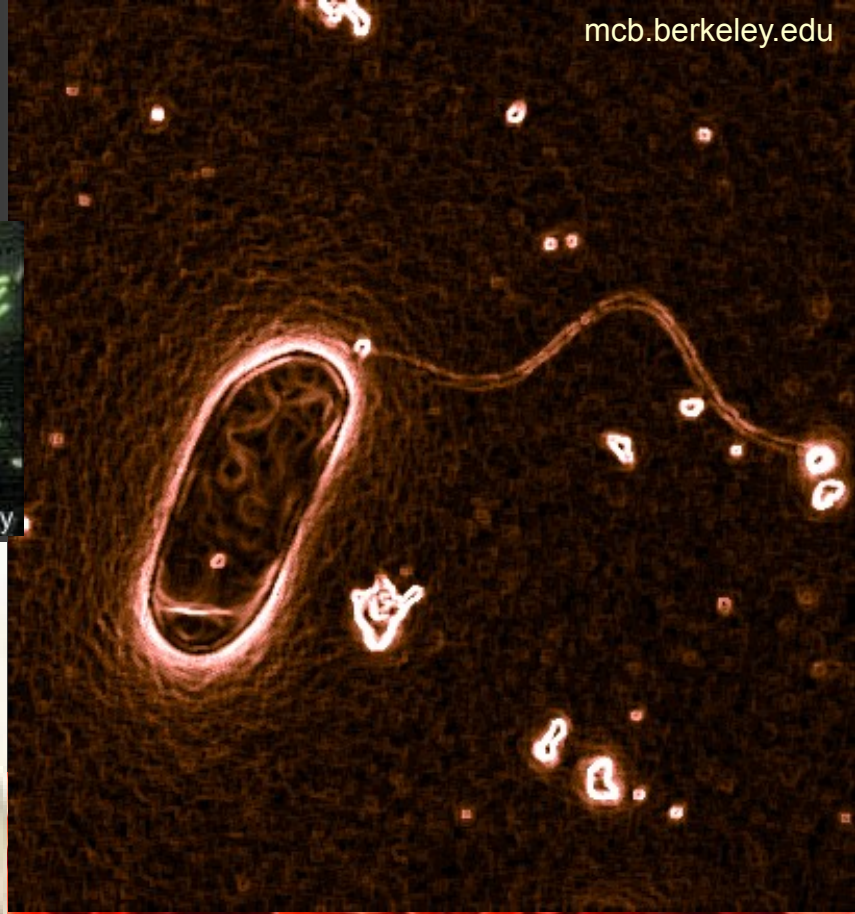
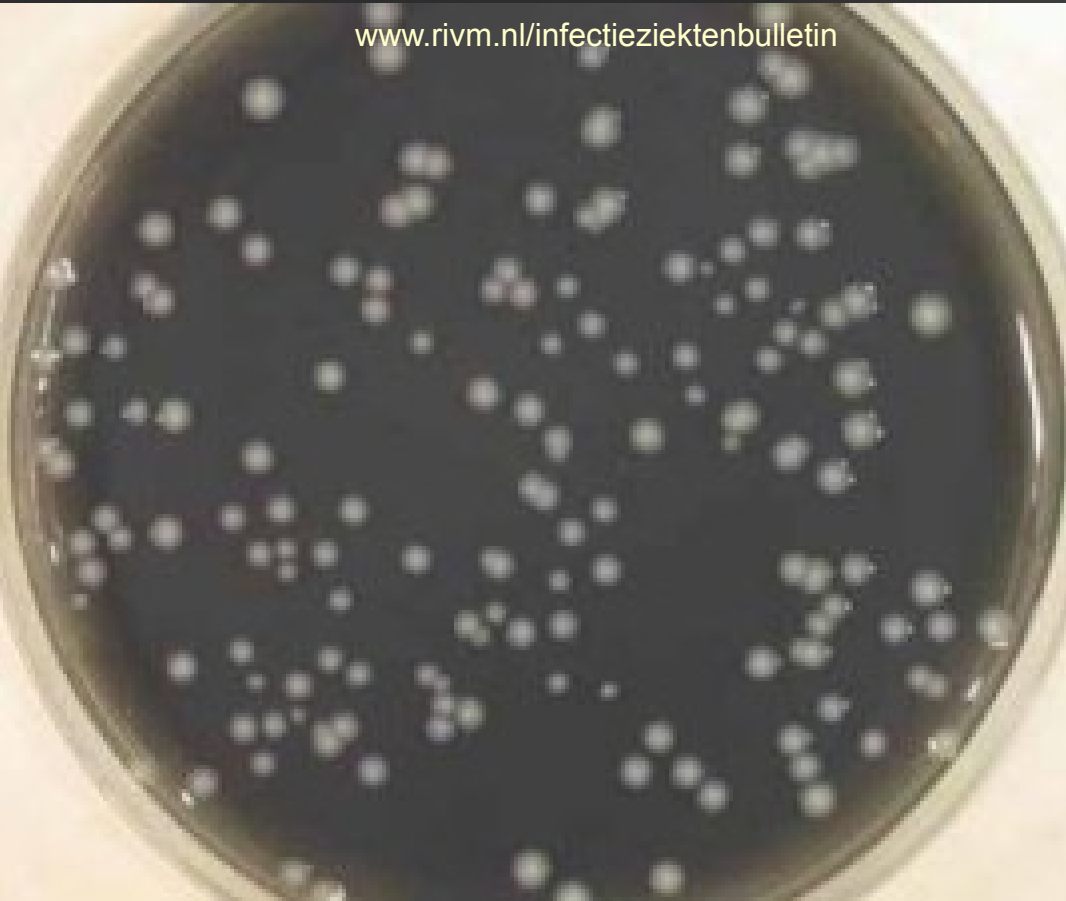
# Legionella

<http://www.eldersllp.com>



ASM MicrobeLibrary.org © Delisle and Tomalty

[www.rivm.nl/infectieziektenbulletin](http://www.rivm.nl/infectieziektenbulletin)



[www.chemistryquestion.com](http://www.chemistryquestion.com)

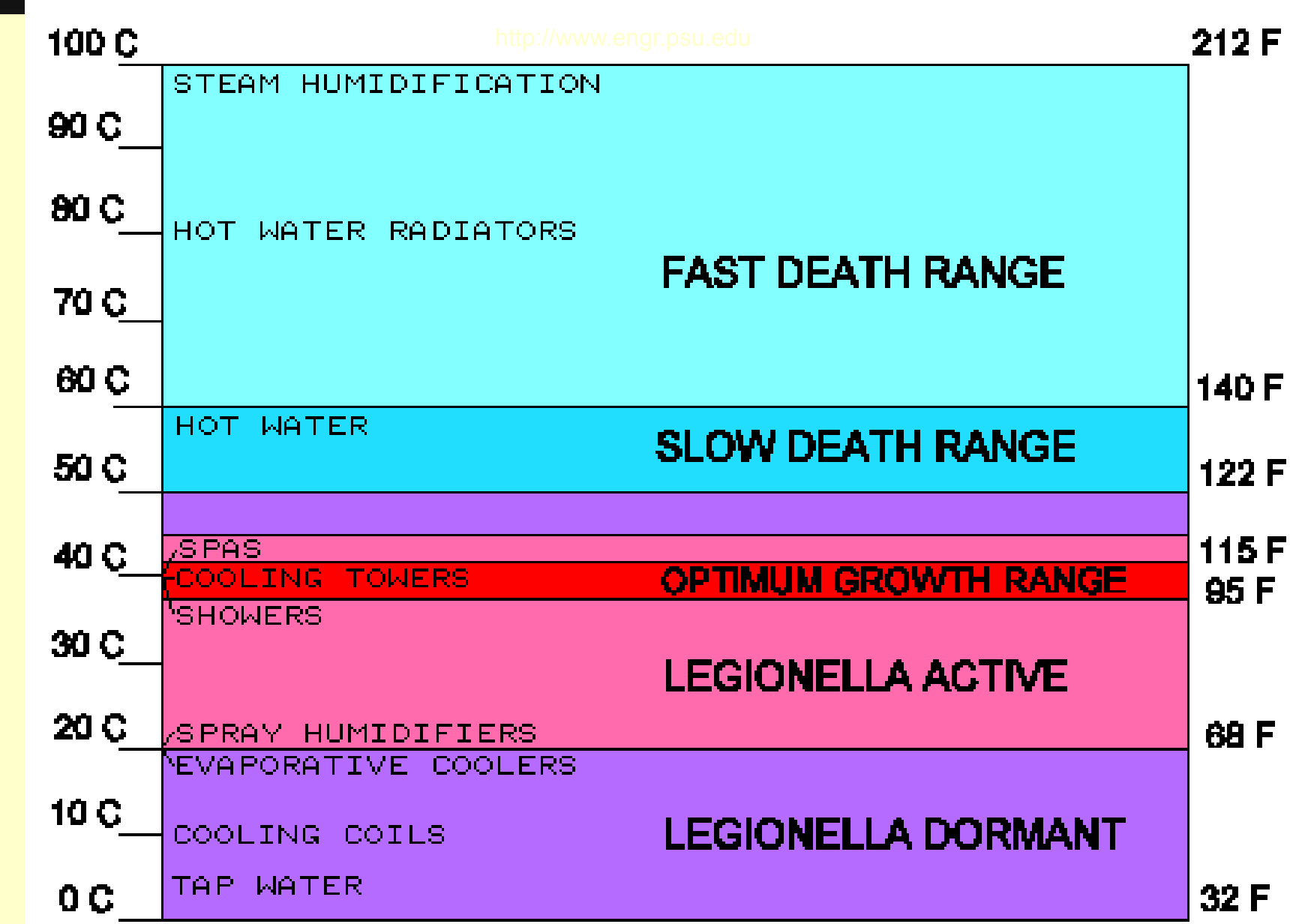
# Legionnaire's disease





# Legionella and temperature

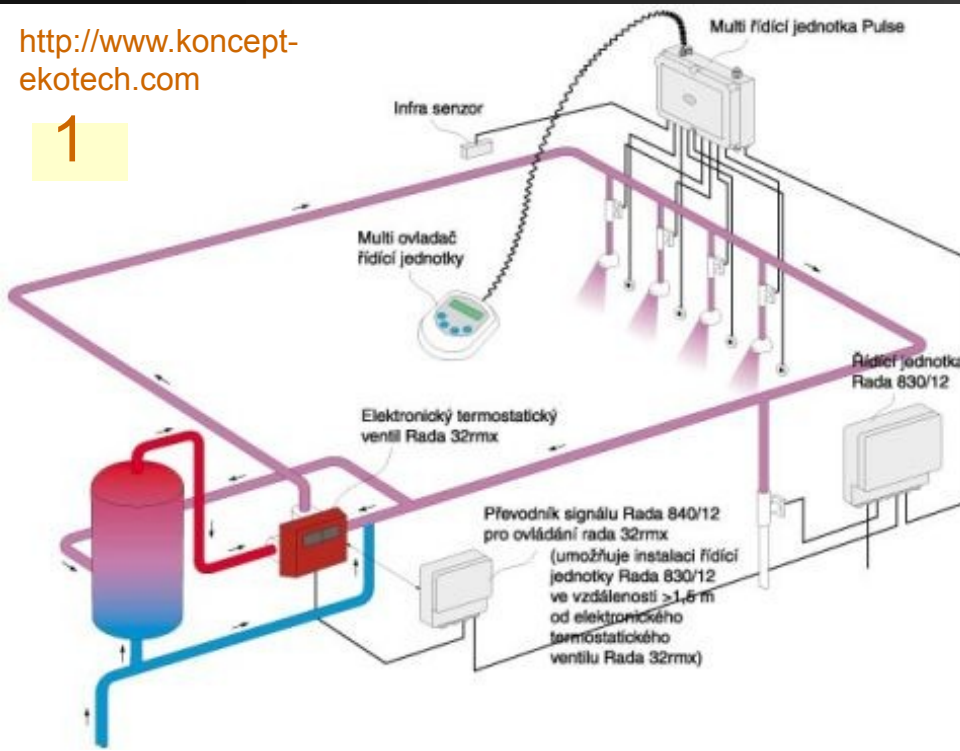
<http://www.engr.psu.edu>



# Some ways of water disinfection

<http://www.koncept-ekotech.com>

1



2



- 1 hot temperature
- 2 UV rays
- 3 filtration
- 4 chloration

3



<http://www.awtwater.co.uk>

4

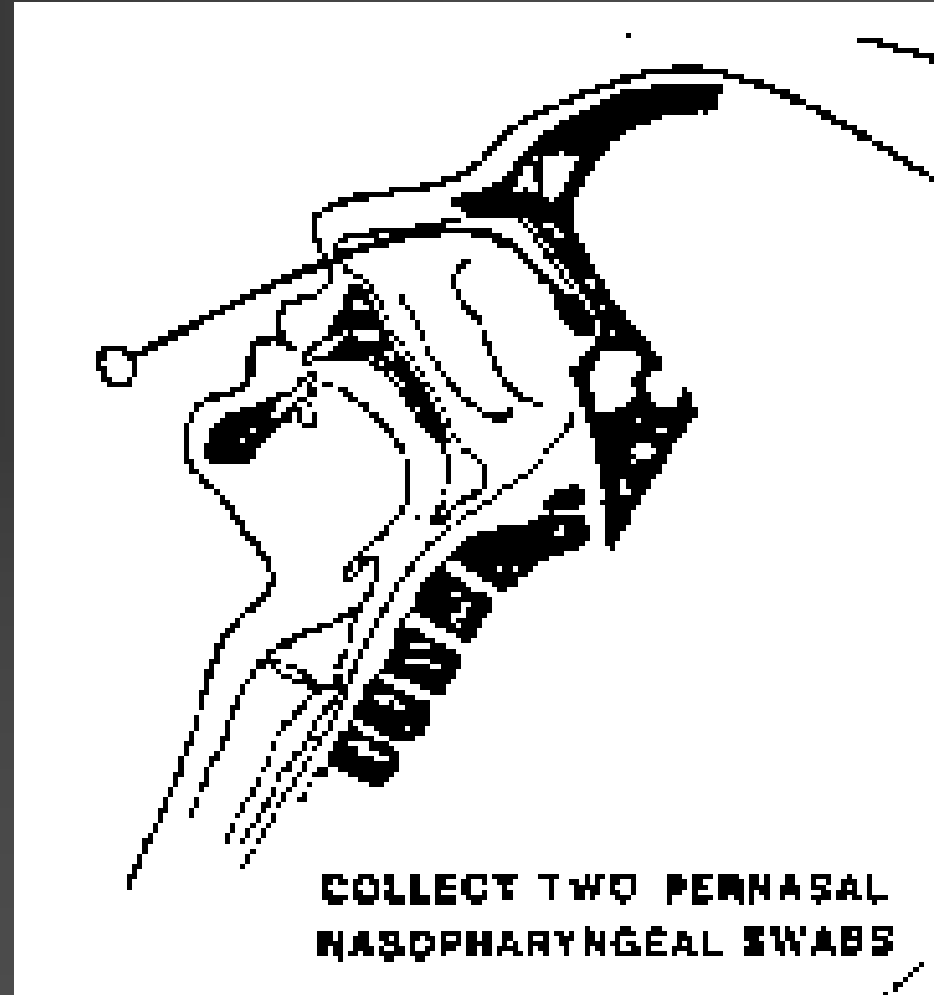


# „Other Gram-negative bacteria“

- It is not a real „group“ or „family“. Nevertheless, these are quite rare bacteria, usually not growing on Endo agar, some of them growing on blood agar, and causing various diseases
- Besides ***Legionella***, we should mention at least three genera: ***Bordetella***, ***Brucella*** and ***Francisella***

# Genus *Bordetella*

- *B. pertussis* and *B. parapertussis* cause whooping cough
- *B. bronchiseptica* causes various pathologies in humans and animals.
- Whooping cough is very rare due to vaccination
- **Pernasal swab** is used when necessary (swab from nasopharynx, taken through nasal cavity)



# Bordetella

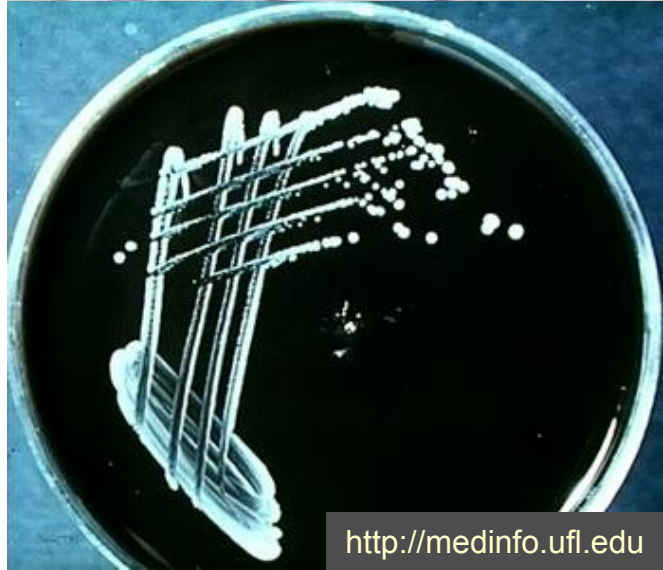
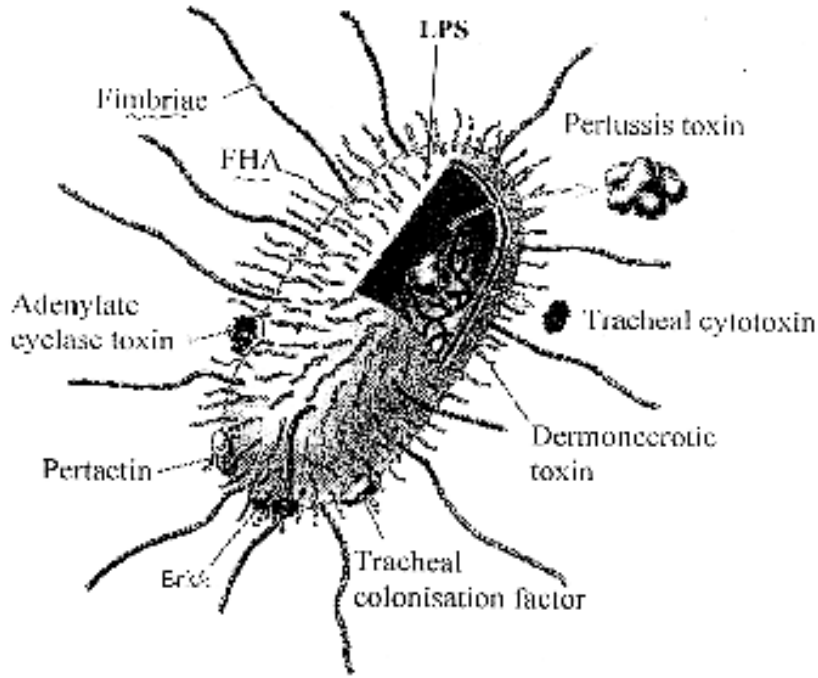


## *Bordetella pertussis*

### Causitive agent of Whooping cough

- Small gram negative bacterium
- Many virulence factors

- Adhesins: **Pertactin**  
 FHA  
 Fimbriae
- Toxins: **Pertussis toxin**  
 ACT  
 TCT  
 LPS



*Bordetella*  
was  
isolated in  
1906 by  
Jules  
Bordet et  
Octave  
Gengou



# *Bordetella bronchiseptica:* cat infection



# Genus *Brucella*



- It is a causative agent of zoonoses
- ***Brucella abortus*** is a bovine pathogen. It infects commonly bovine placenta, causing abortions in cattle. In human it causes so named **Bang disease** (fever, problems with various organs etc.)
- Other brucellae are ***Brucella suis*** from hogs, ***Brucella melitensis*** from sheep and goats and ***Brucella canis*** from dogs



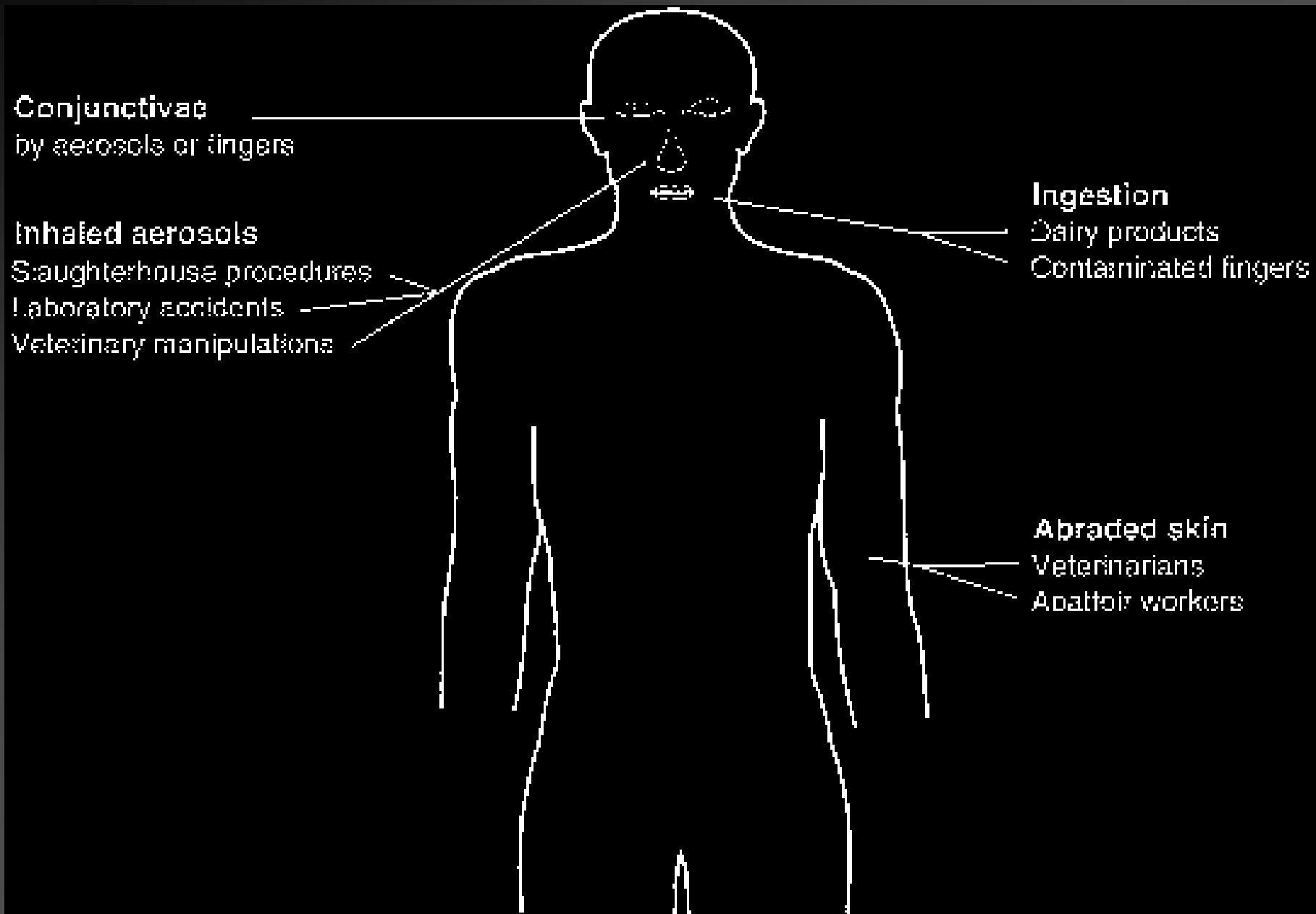
# Brucella



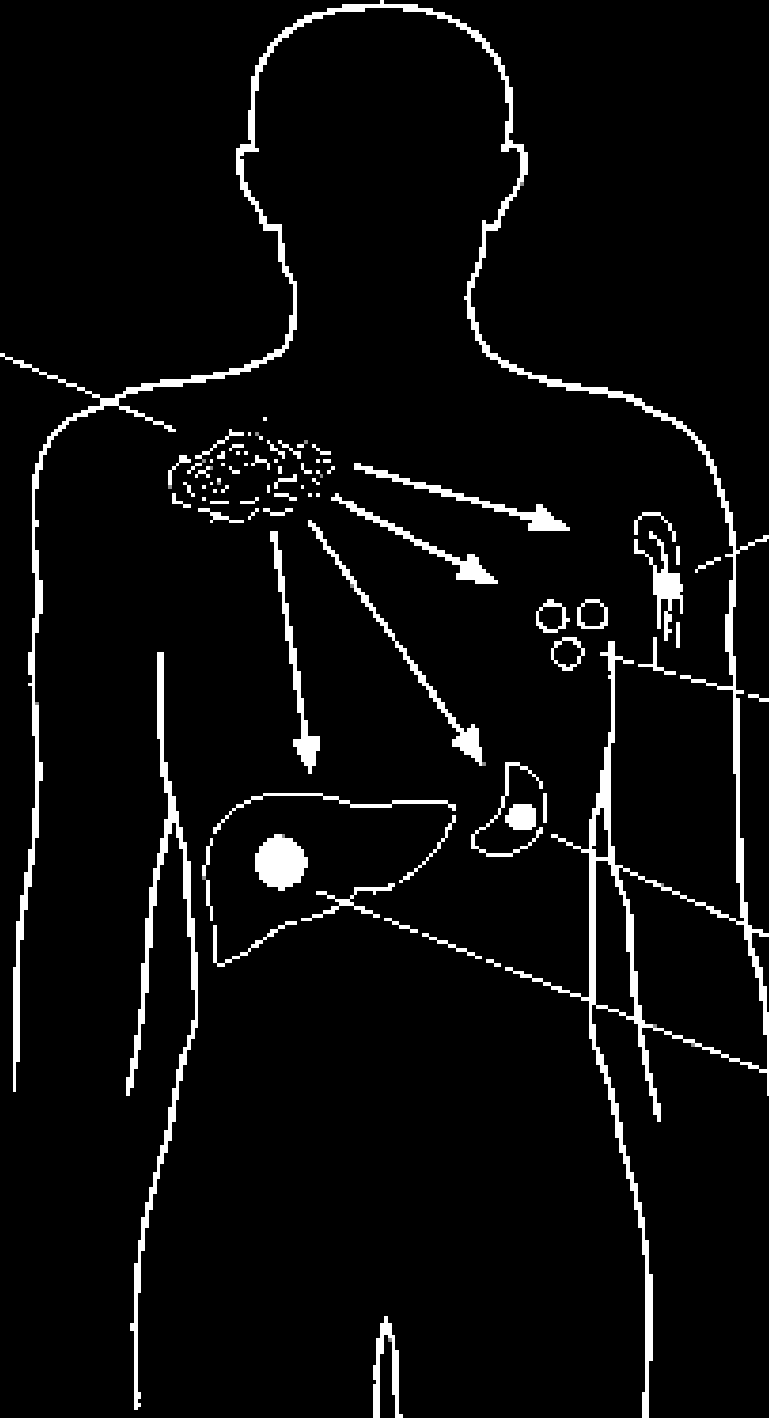
<http://pathport.vbi.vt.edu>

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<http://upload.wikimedia.org>



*Brucella*-infected  
phagocytic cell



Bone marrow

Lymph nodes

Spleen

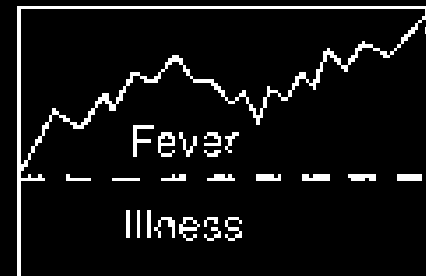
Liver

# History and Physical Exam

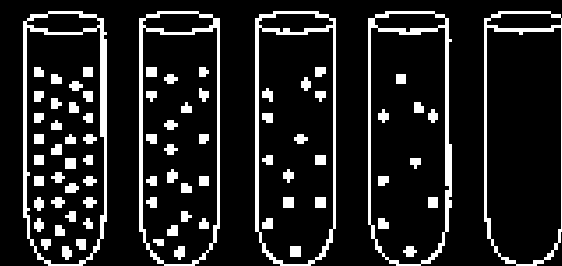
## Clinical

Occupation  
Raw milk  
Fresh cheese  
Travel  
Symptoms  
Signs

## Course of disease



## Quantitative serology

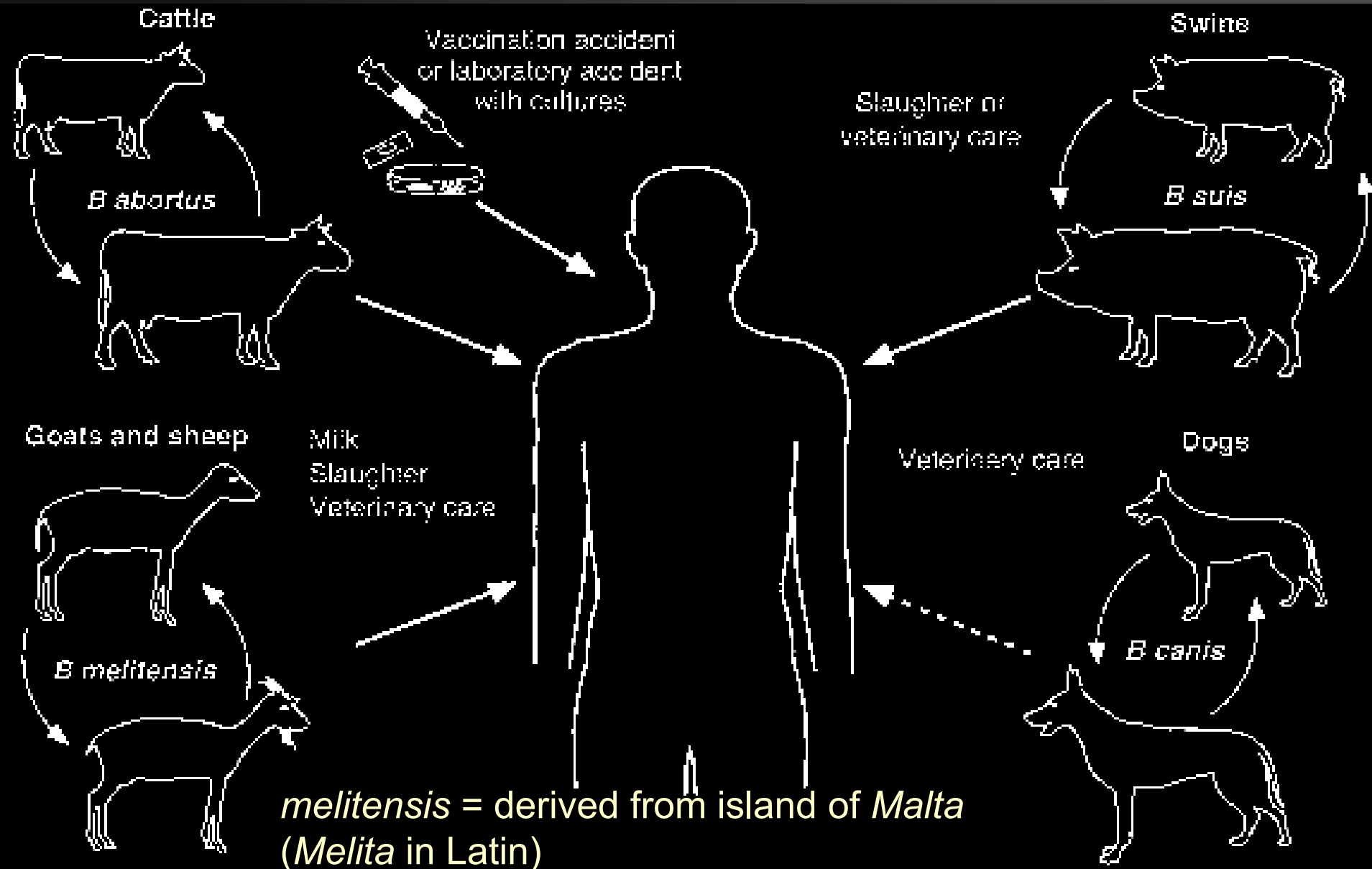


## Laboratory

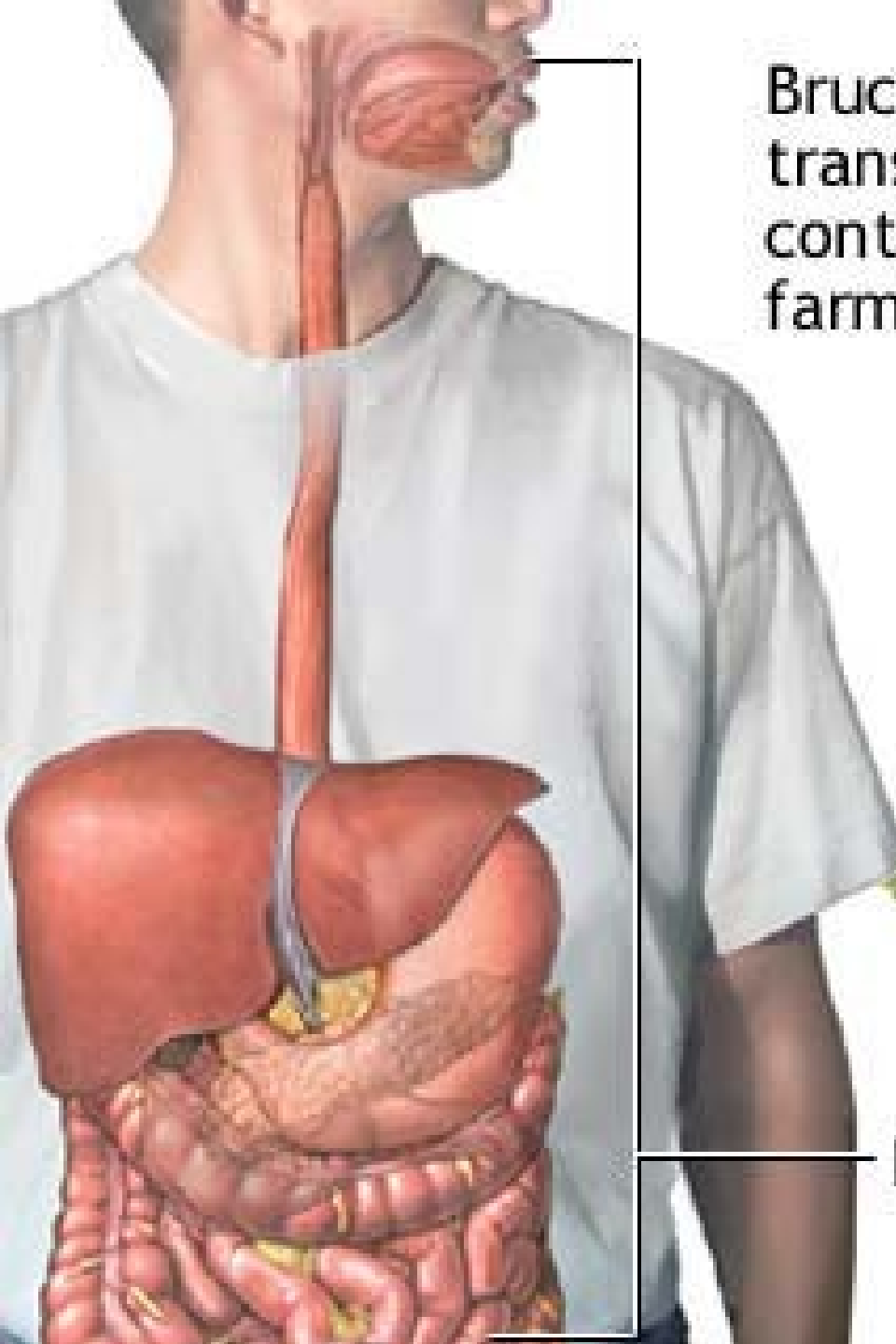
### Blood cultures



# Remembering Brucella



Brucella bacteria is usually transmitted to humans by contact with infected farm animals.



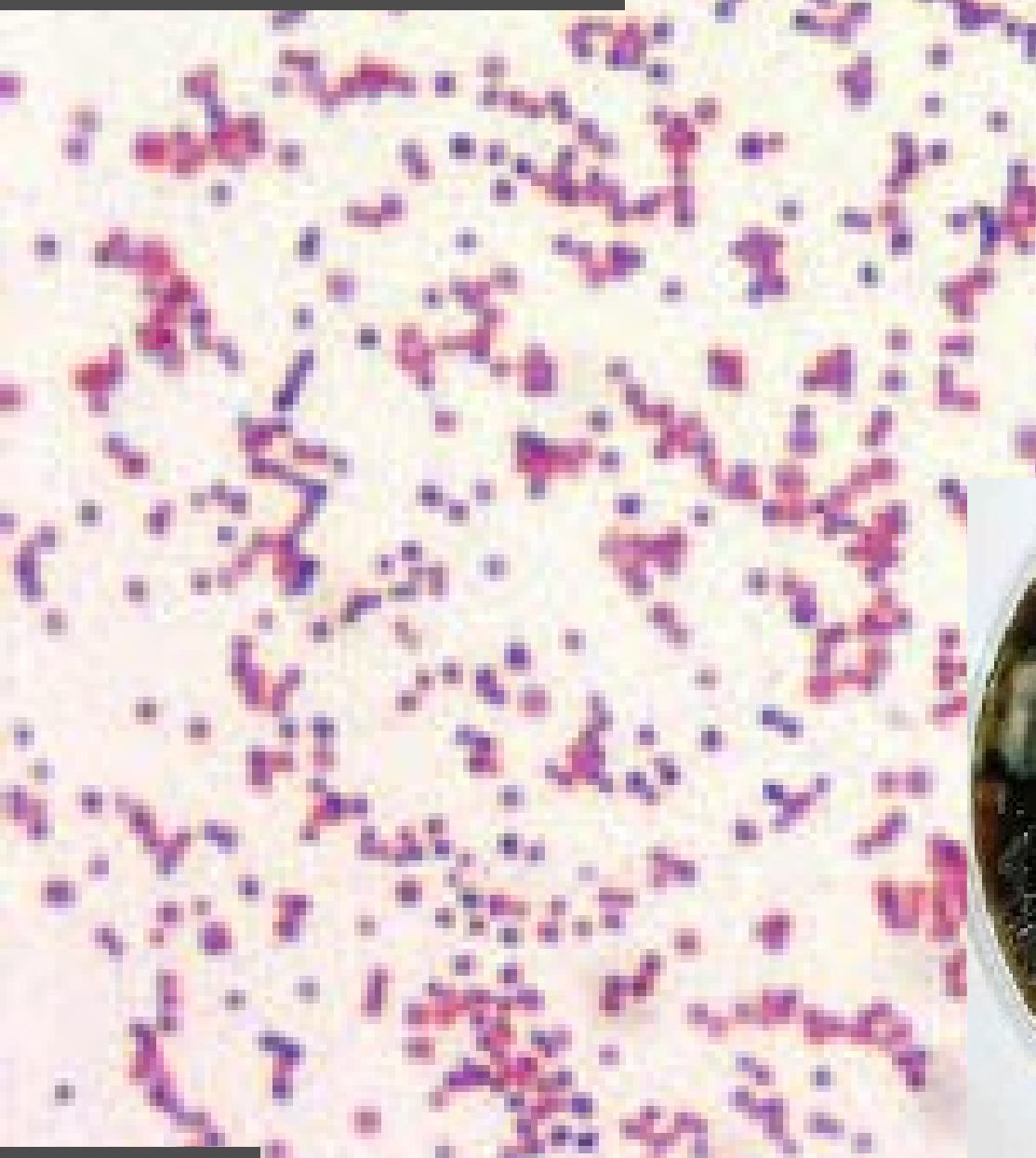
Digestive system

# Genus *Francisella*

- Most important species – *F. tularensis*
- Causes **tularemia** – „hare plague“
- **Gamekeepers**, but even more **cooks preparing game** are in risk of infections
- The organism may infect **wounds**, but also it is possible to inhale it, thus leading to **pneumoniae**

# Francisella

[www.infektionsnetz.at](http://www.infektionsnetz.at)



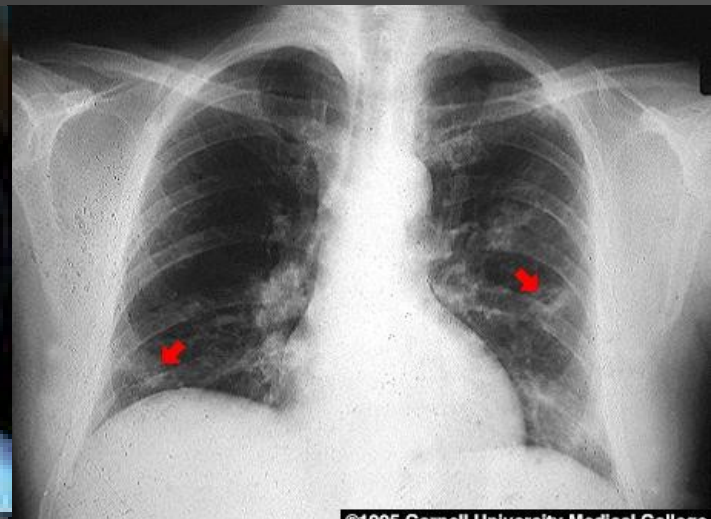
[www.wnysmart.org/tularemia](http://www.wnysmart.org/tularemia)

[www.stedim.com](http://www.stedim.com)





<http://www.antropozoonosi.it> (4x)



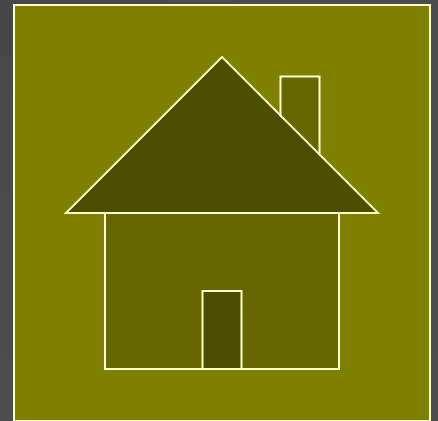
# From report about epidemiological situation (IX/2008) – I (abbreviated)

- **Tularemia – A21:** Woman, born 1970 from Valtice, gardener. 20<sup>th</sup> June GP visit for T 38°C, sore throat, enlarged lymphonodes next to right clavícula. The lymphonode was extirpated. First serological examination negative, second (2<sup>nd</sup> July) positive
- **Brucellosis – A23:** Man, born 1972, Brno. 10<sup>th</sup> May intestinal problems, febrilia, hepatopatia, dg. proven serologically. *Brucella abortus* positive, CFT 1:8. In anamnesis a travel to Borneo (Kalimantan) in april, trek in a tropical forest. Delayed report.

*(Reported by Public Health Office for South Moravia)*

# From report about epidemiological situation (IX/2008) – II

- **Pertussis (A37.0):** 4 cases reported (Brno-environment, Hodonín), people aged 14 to 17, all vaccinated, one with missing re-vaccination
- **Parapertussis (A37.1):** 3 cases of disease, coinfection, Brno environment, Hodonín



# Diagnosatics of G- cocci

# *Neisseria gonorrhoeae* – sampling

In gonorrhoea suspicion it is very important to perform sampling properly. Despite all care it is likely that the pathogen would not be able to survive. That is why it is recommended to send also smear on a slide from cervix and urethra (but not rectum and pharynx)

So „complete gonorrhoea examination“ consists of following parts:

- **urethral swab** in Amies + **smear**
- **cervical swab** in Amies + **smear** ( ♀ )
- **rectal swab** in Amies (no smear)
- **pharyngeal swab** in Amies (no smear)

# Sampling and acute diagnostics in purulent meningitis

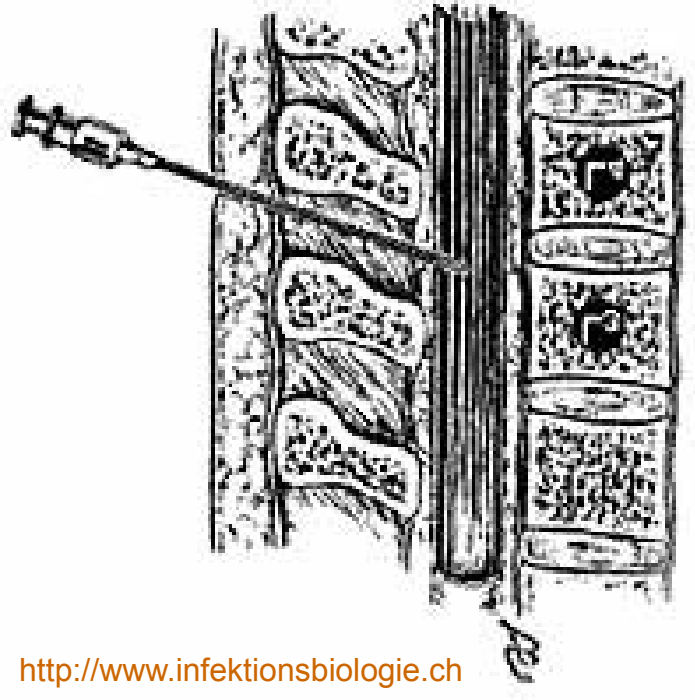
In case of suspicion for purulent meningitis, usually **cerebrospinal fluid** is taken, eventually also blood for blood culture. CSF can be examined biochemically, cytologically and microbiologically.

The person taking CSF can mention, that **CSF is turbid and flows out under the pressure**

In laboratory, two **quick methods** are available

- microscopy
- direct antigen detection

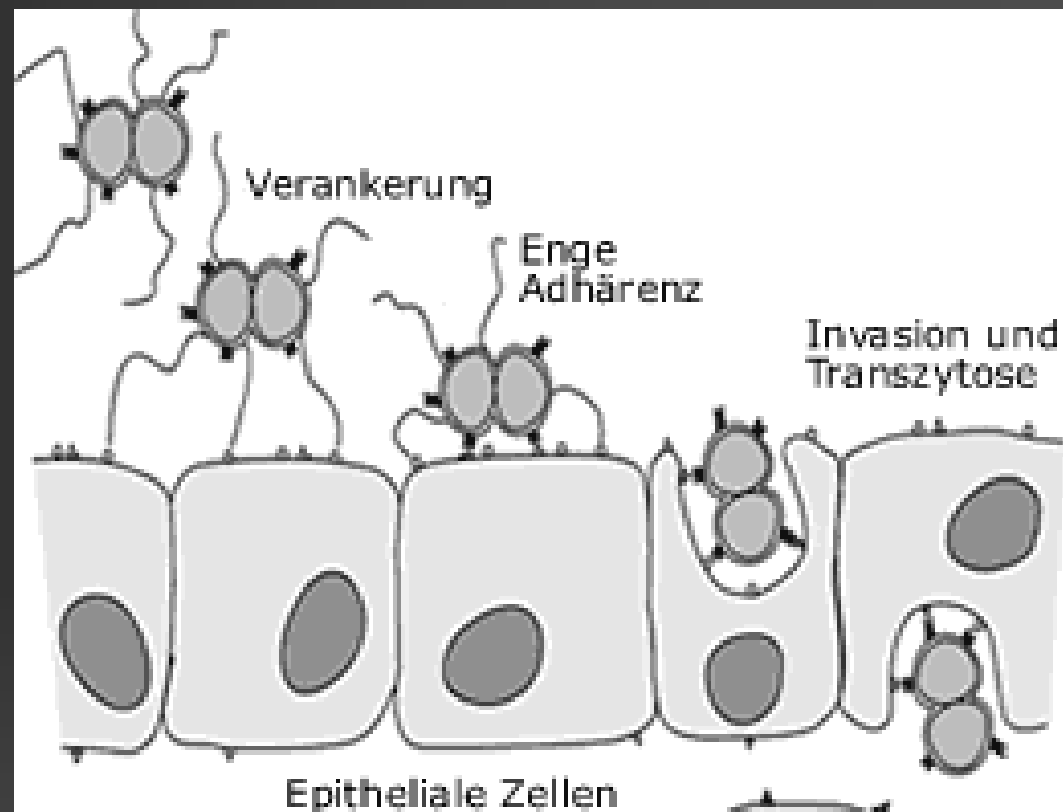
*Not regarding the diagnostics and its results, the most important is instant start of patient treatment!*



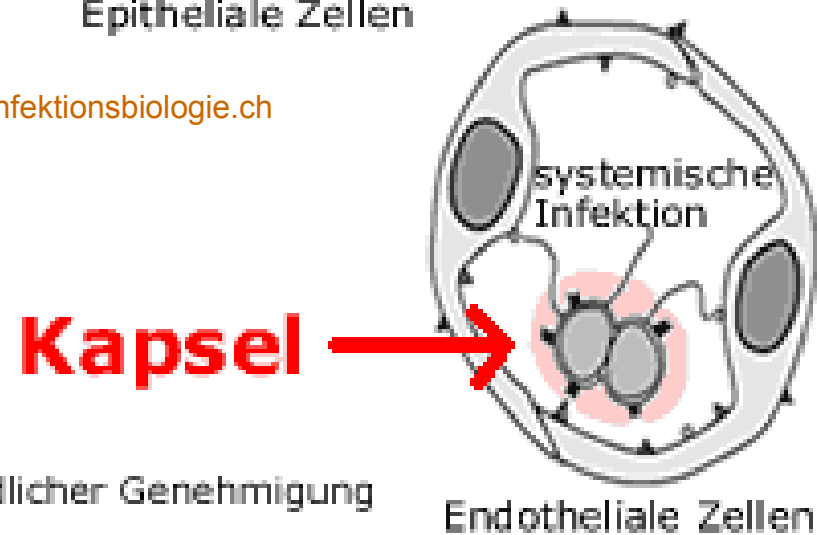
<http://www.infektionsbiologie.ch>

# CSF sampling

How they  
come into  
the tissue



<http://www.infektionsbiologie.ch>



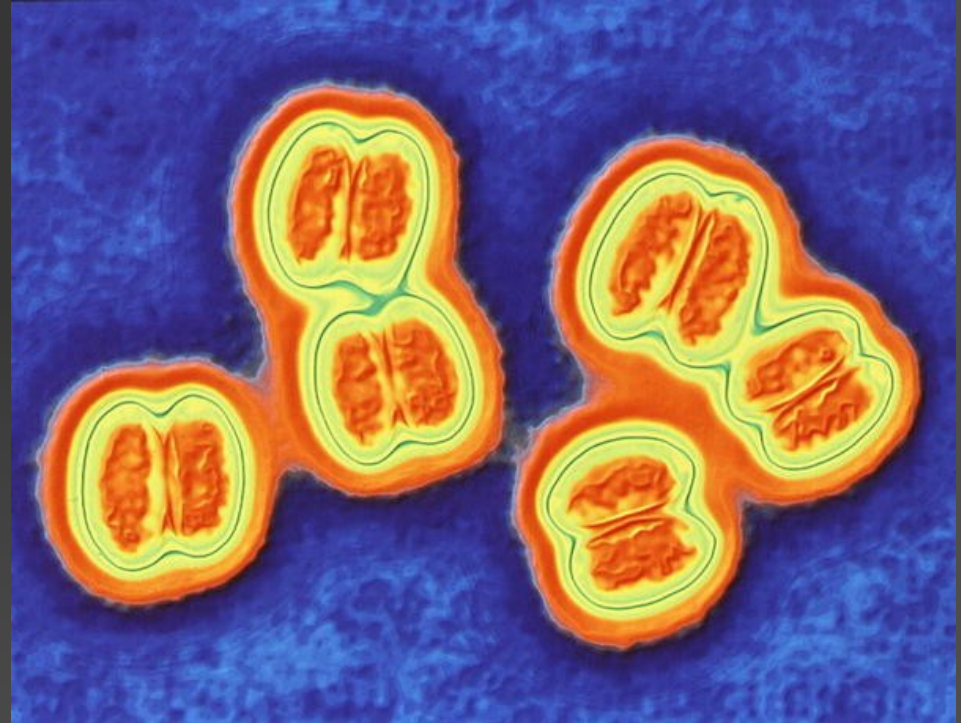
Mit freundlicher Genehmigung  
© Dehio

Endotheliale Zellen

# Neisseriae and Moraxellae – characteristics I

<http://medicine.plosjournals.org>

- **Microscopy:** G – diplococci, coffee bean shaped, often intraleucocytar (see next slide)

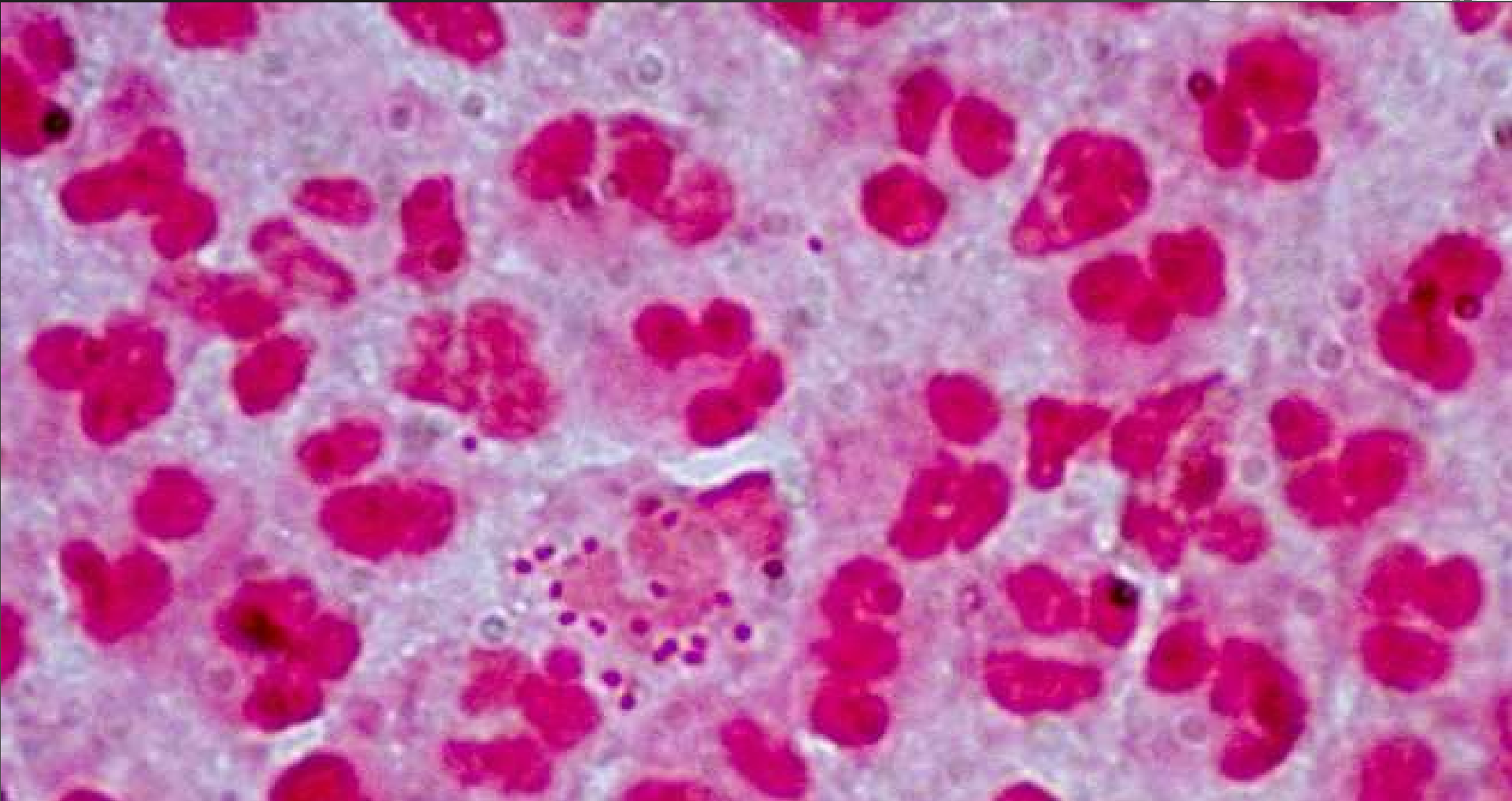
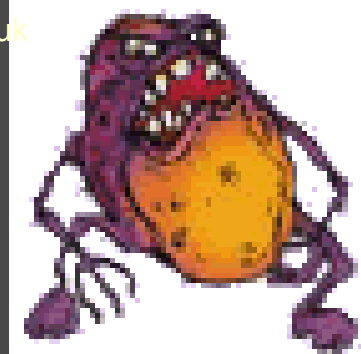


*Some neisseriae a moraxellae may be prolonged and so they might be coccobacilli or bacilli (e. g. Neisseria elongata)*

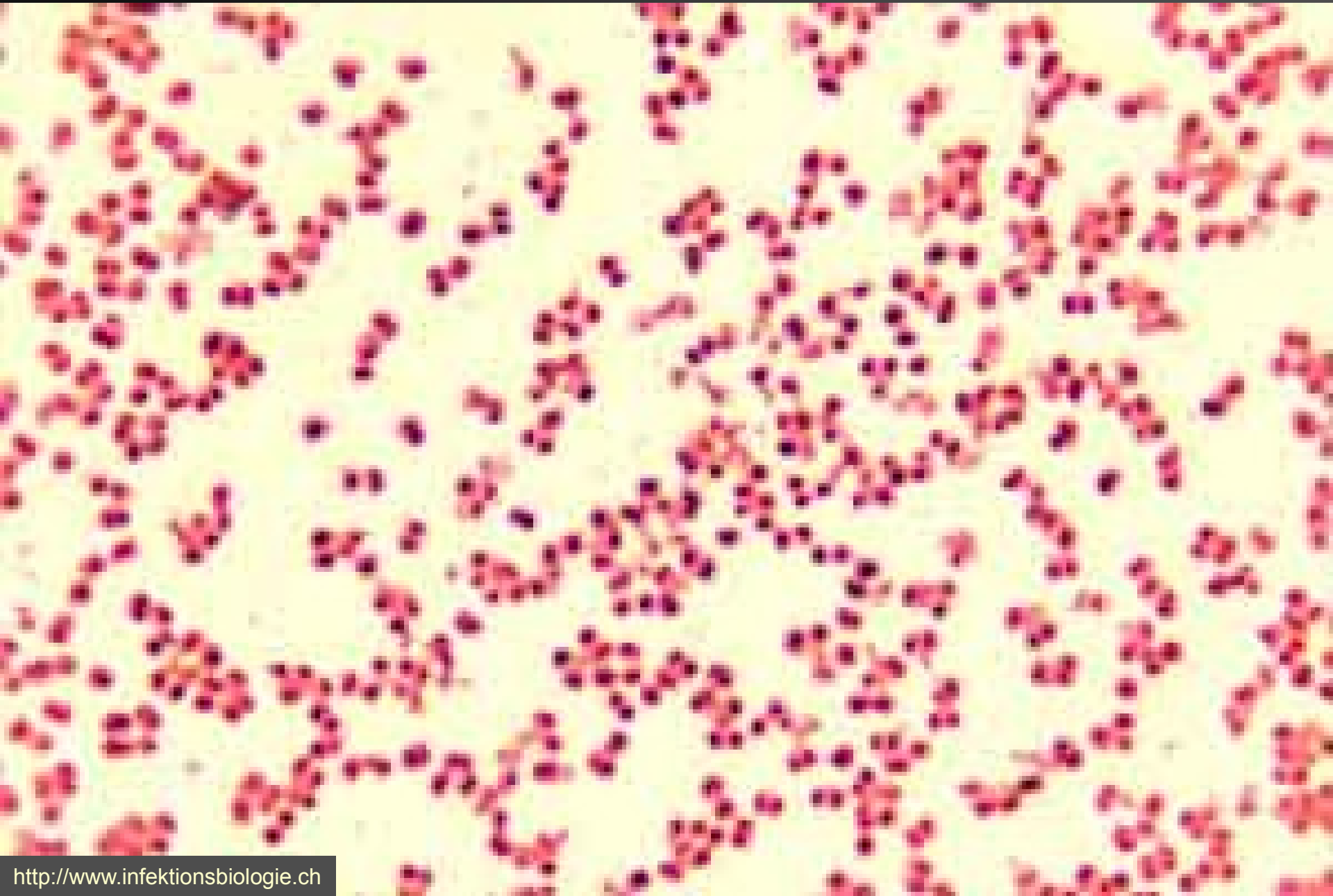


# Gonococci

<http://www.warwickshire.gov.uk>



# Meningococcus



## Microscopic Pictures Of Neisseria (Gram-negative Diplococci)

Neisseria Gonorrhoeae



Neisseria Meningitidis  
intracellularis in spinal fluid



■  $\beta$ -lactam drugs

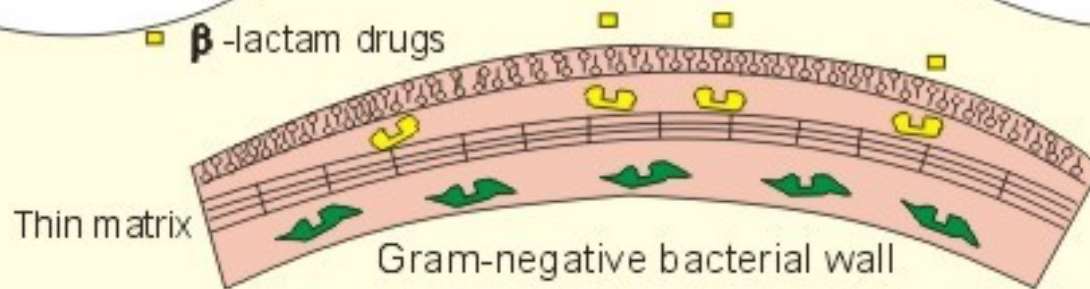
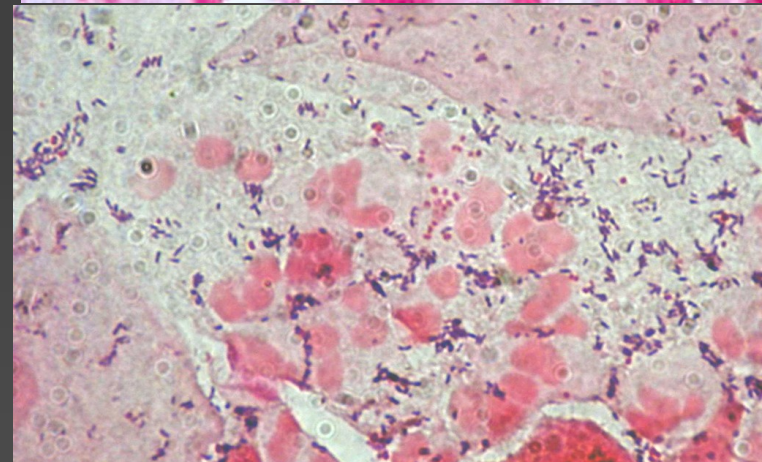
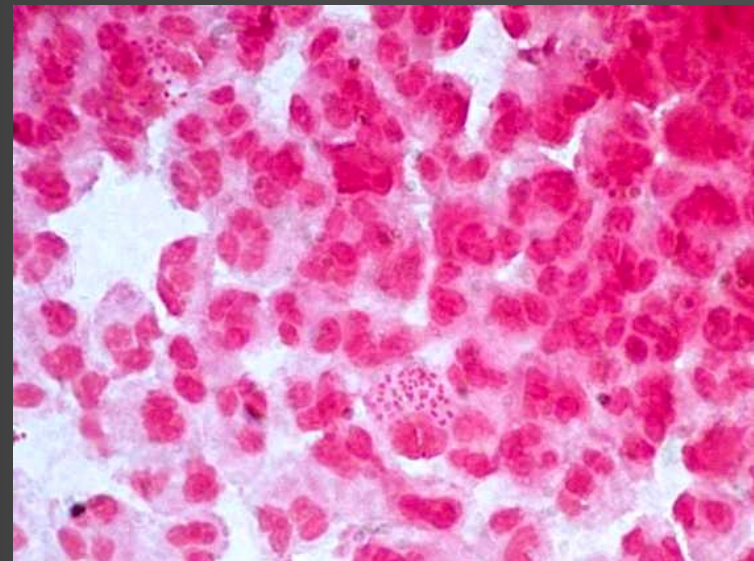


Fig. 33-3

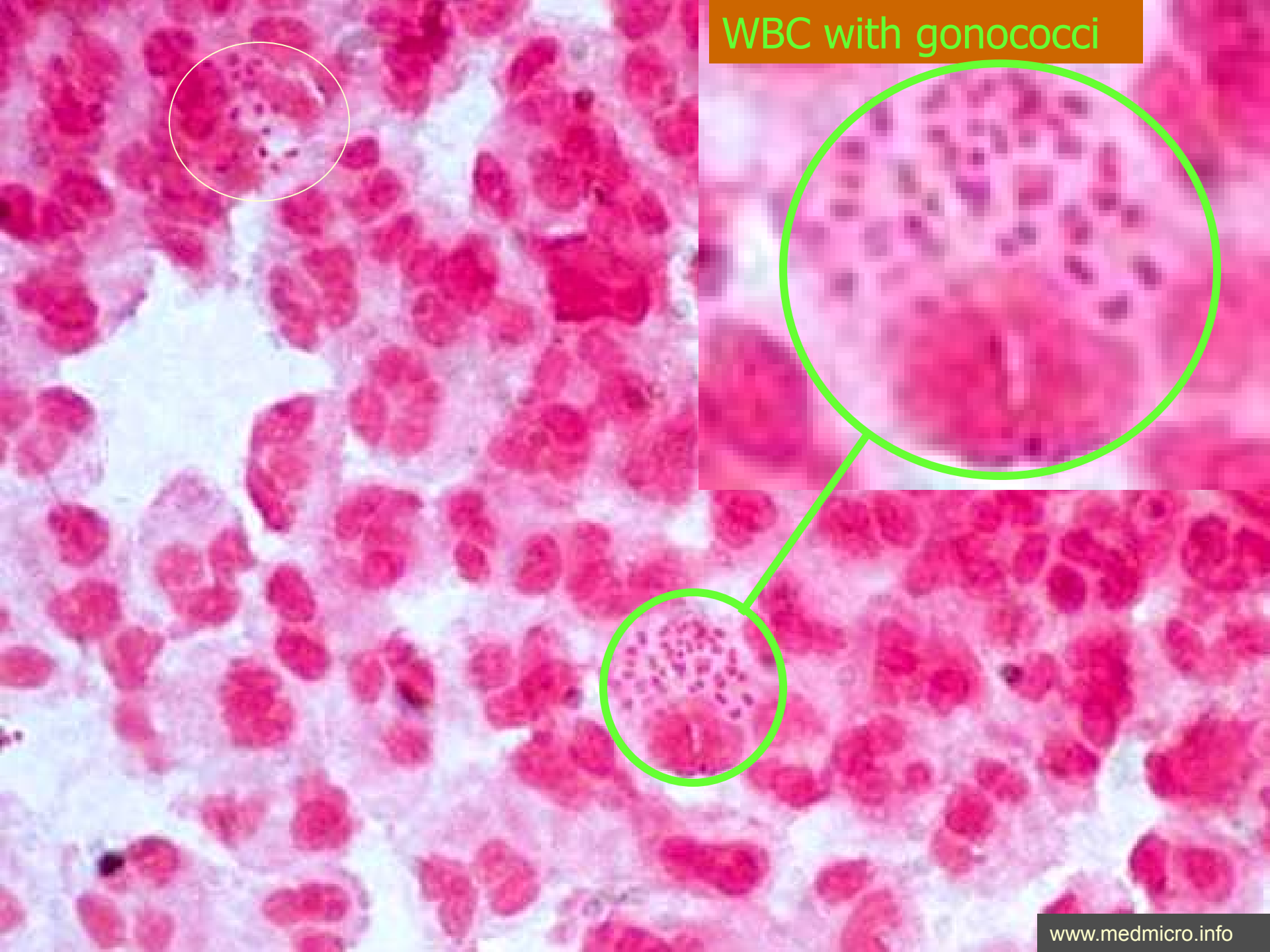
# Observation of gonorrhoea smear

- Gonococci (but also meningococci) are Gram-negative diplococci, coffee-bean shaped, mostly intracellular. Presence of cocci inside leucocytes is their typical property.



*In females, the microscopy is slightly different from males.*

WBC with gonococci



# Neisseriae and Moraxellae – characteristics II

- **Culture:** tiny, colourless or yellowish (according to the species) colony, growing (species specific) on blood or chocolate agar
- Blood agar or chocolate agar necessary also for **diffusion disc test**



Gonococcus →  
← Meningococcus

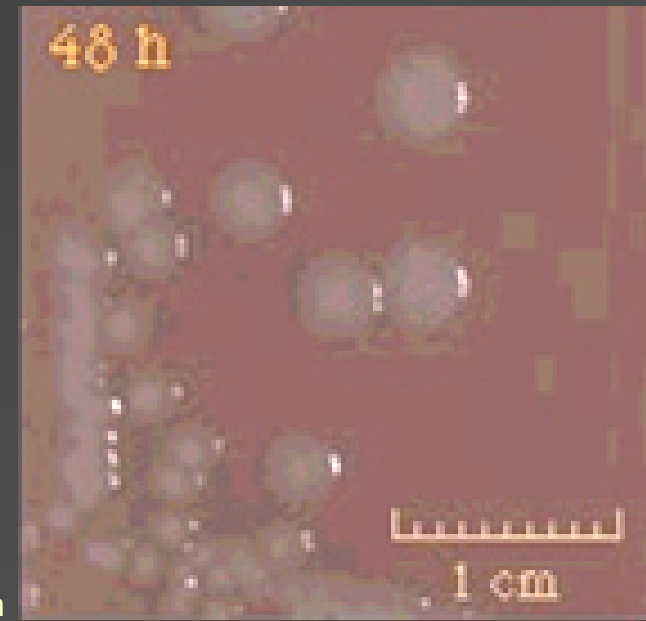


Foto O. Z.



# Culture of *Neisseria* and *Moraxella*

There exist differences between G- cocci in culture properties:

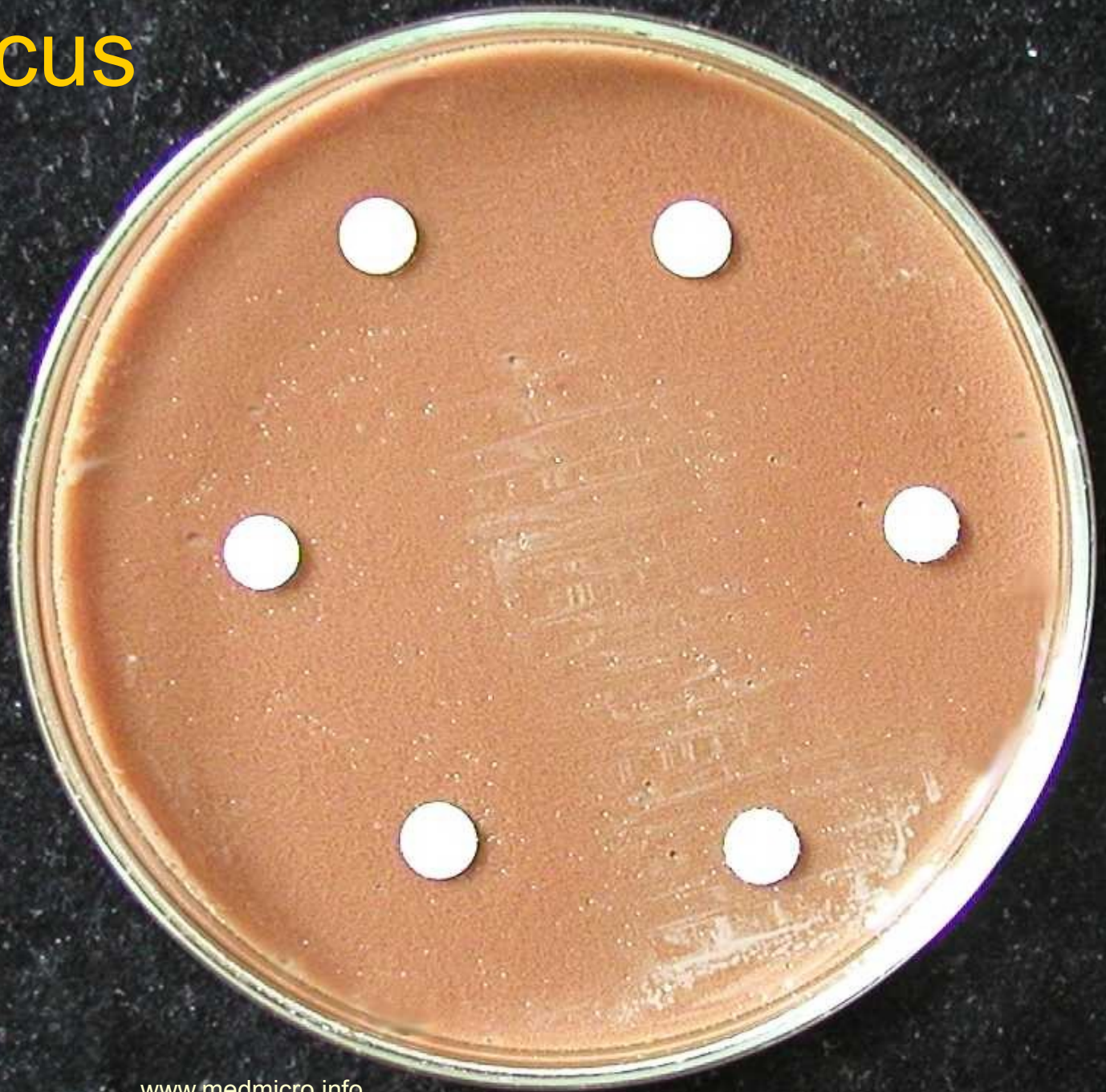
**Oral neisseriae and moraxellae (O+M)** grow on blood agar (BA).

**Meningococci (ME)** grow only on nutrient-rich variants of BA.

**Gonococci (GO)** do not grow on BA at all, they require chocolat agar.

|     | BA  | BA+ | ChA |
|-----|-----|-----|-----|
| GO  | NO  | NO  | YES |
| ME  | NO  | YES | YES |
| O+M | YES | YES | YES |

# Gonococcus atb testing





# Neisseriae and Moraxellae – characteristics III

- **Biochemical diagnostic:** all of them are catalase positive, oxidase positive; *Moraxella catarrhalis* also positive in a specific test, called INAC (indoxyl-acetate test)
- **Antigen analysis:** performed usually by means of by latex agglutination, very important in meningococci for differentiation between serogroup B (not preventable by vaccination) and other serogroups (preventable)

# Basic biochemical tests

- **Quick tests** with diagnostic strips simplify the diagnostics
- Neisseriae are **oxidase positive**, moraxellae too, but their reaction might be late.
- Moraxella is typically **positive in INAC test**
- INAC test is similar to oxidase test, but **the strip should be moistened and one has to wait 5 minutes. The colour is blue-green.**

# Species determination of neisseriae and moraxellae

- For detailed identification of neisseriae and moraxellae, biochemical tests are used, in Czechia mostly NEISSERIAtest, in other countries other tests (at those below or on the next slide)
- Both pathogenous neisseriae have little biochemical activity: **Gonococcus** splits **glucose** only, **Meningococcus** glucose and **maltose**.





## NEISSERIA 4H

|               |                      |                        |                          |          |           |          |           |
|---------------|----------------------|------------------------|--------------------------|----------|-----------|----------|-----------|
| CONT.<br>NEG. | GLU<br>+             | MAL<br>+               | FRU<br>-                 | SAC<br>- | ONPG<br>- | TRI<br>- | PS<br>24h |
| GGT<br>+      | NO <sub>3</sub><br>- | NO <sub>2</sub><br>(1) | NO <sub>2</sub><br>+ (2) | 1395     |           |          |           |

# Neisseriae and Moraxellae – differential diagnostics 1

- **Gram staining:** G – (diplo)cocci
- **Oxidase** differentiates some other G– cocci (e. g. *Acinetobacter* – a G– non-fermenter – is also often coccoid)
- **Growth on various media** differentiates
  - **gonococcus** (growth on chocolate agar only),
  - **meningococcus** (growth on rich blood agar and chocolate agar)
  - **oral neisseriae** (growth on both poor and rich blood agar and chocolate agar)
  - ***M. catarrhalis*** (growth like oral neisseriae)

# Neisseriae and Moraxellae – differential diagnostics 2

- **INAC test** (a strip test similar to oxidase test) – positive in *Moraxella catarrhalis*
- **Complex biochemical test** (NEISSERIAtest), is used especially for mutual differentiation of oral neisseriae
- **Antigen analysis** (determination of meningococcal serogroup in invasive infections)

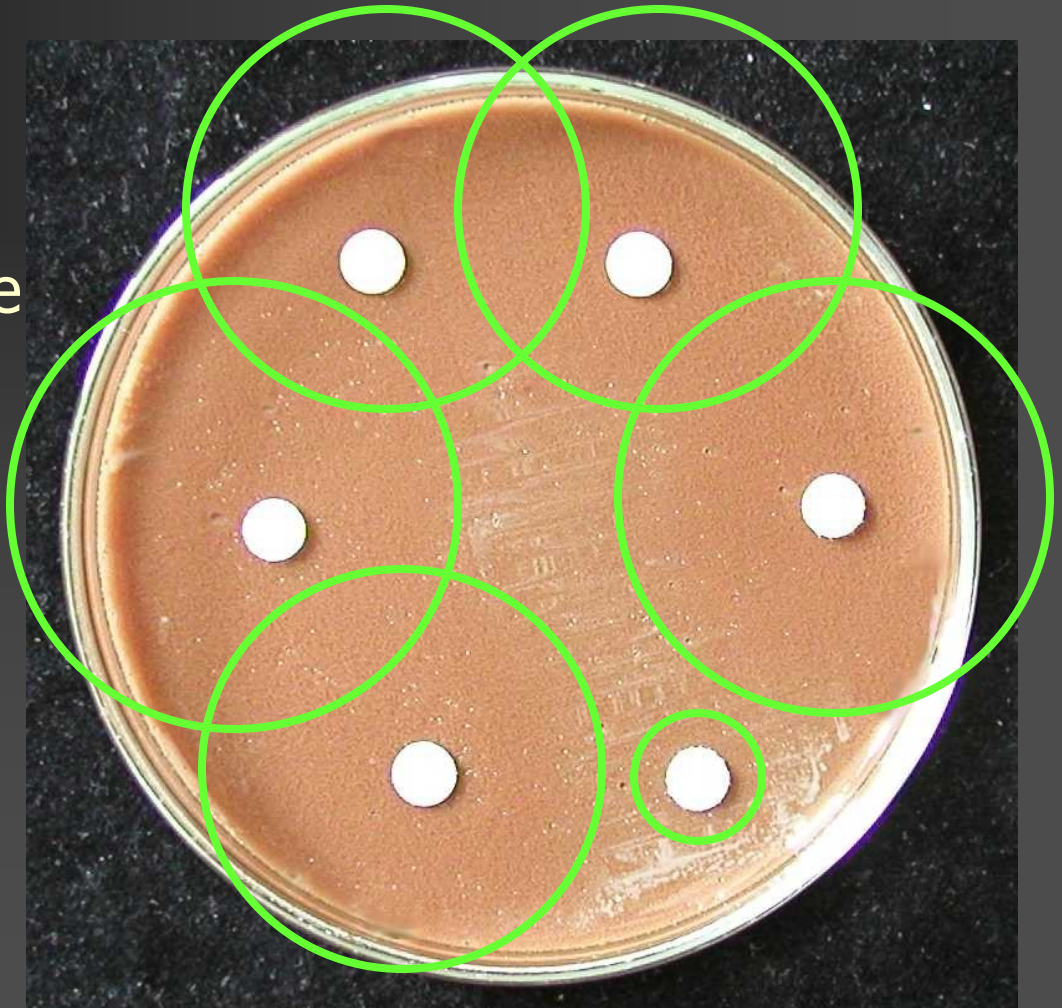
# Antibiotic susceptibility testing in neisseriae and related bacteria

- Antibiotic susceptibility in pathogenous neisseriae is determined on media, on which they are able to grow, i. e. not on MH agar
- First election drug for meningococcus is even today classical penicillin. It is used also for Gonococcus. Other drugs are macrolids, quinolones or ceftriaxone.

# In neisseriae, zones are often large and confluent.

When the zones are so large that it is not possible to measure them, we do not measure them and we write down simply „susceptible“.

*Hypothetical margins of zones are in green: mention, that they are mostly either confluent, or behind the margin of the Petri dish!*



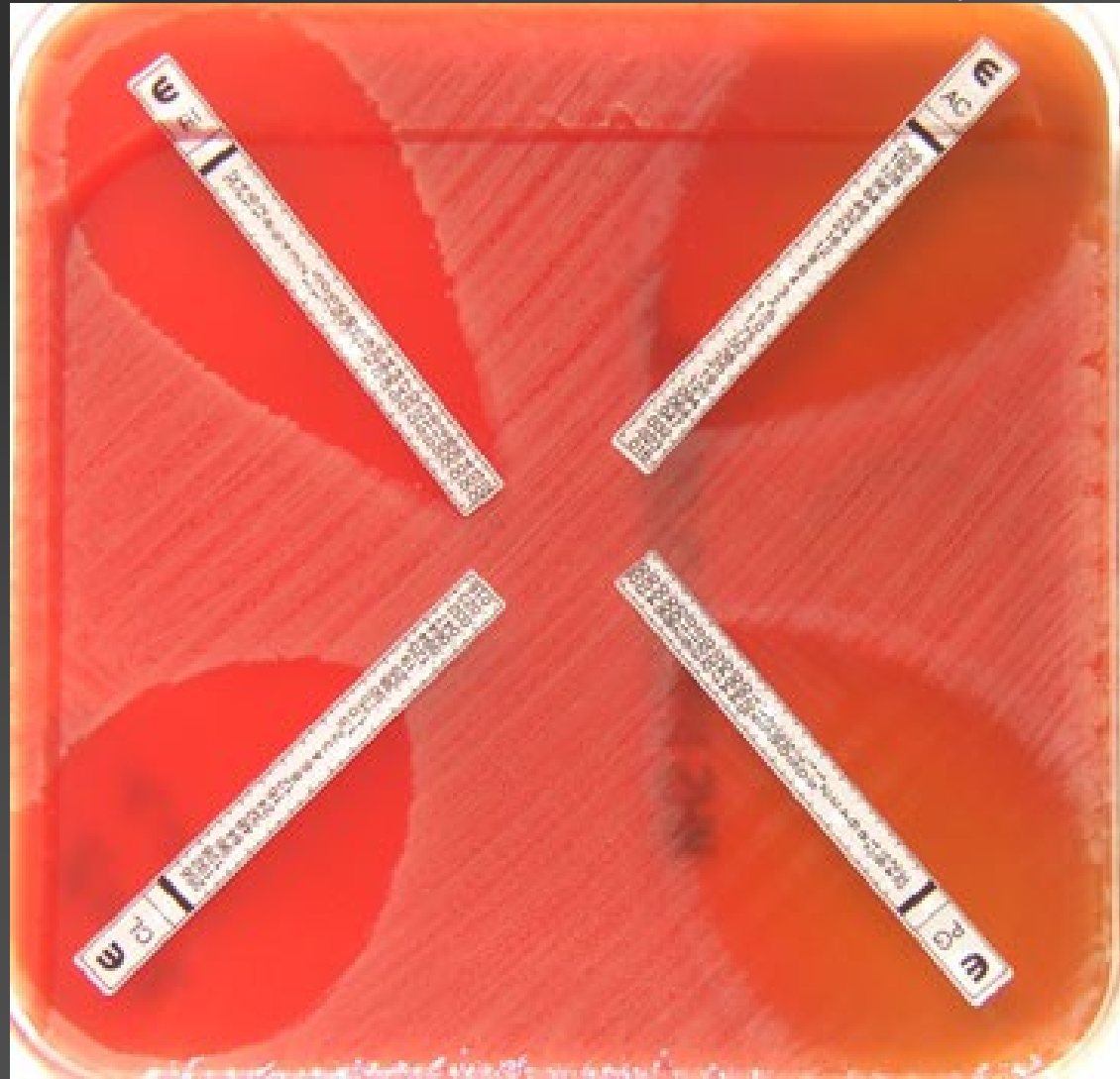


# A set of antibiotics against pathogenic neisseriae

| Antibiotic                    | Abbr. | Reference zone |
|-------------------------------|-------|----------------|
| Penicillin (basic penicillin) | AMP   | 28 mm          |
| Chloramphenicol               | C     | 18 mm          |
| Azithromycin (macrolide)      | AZM   | 12 mm          |
| Doxycycline (tetracycline)    | DO    | 19 mm          |
| Ampicillin (aminopenicillin)  | AMP   | 29 mm          |
| Co-trimoxazol (mixture)       | SXT   | 16 mm          |
|                               |       |                |

# Somewhere, E-test is used

[www.actu-pharo.com](http://www.actu-pharo.com)



# Antigen detection / antigen analysis

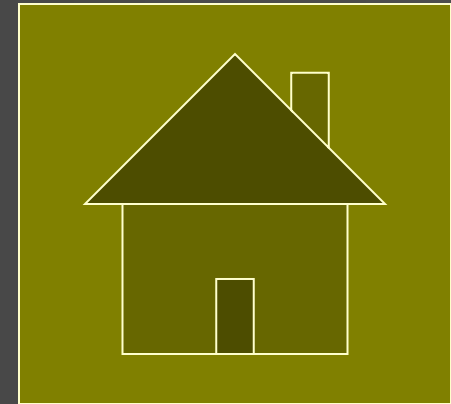
- Agglutination set for CSF agglutination is used for identification of pathogens. In case of a may be identified.
- In *Meningococcus* also the serogroups may be assessed. Therefore we can use it also for antigen analysis of an already cultured strain.

Photo O. Z.



# Antigens detected at CSF antigen analysis

- *Neisseria meningitidis* A
  - *Neisseria meningitidis* B
  - *Neisseria meningitidis* C
  - *N. meningitidis* Y/W135
- } **teens, children**
- *Haemophilus influenzae* b **children**
  - *Streptococcus pneumoniae* **seniors**
  - *Streptococcus agalactiae* **newborns**
  - **In green colour there is the age group, where the infection is the most typical**



# Diagnosics of „other G– bacilli“

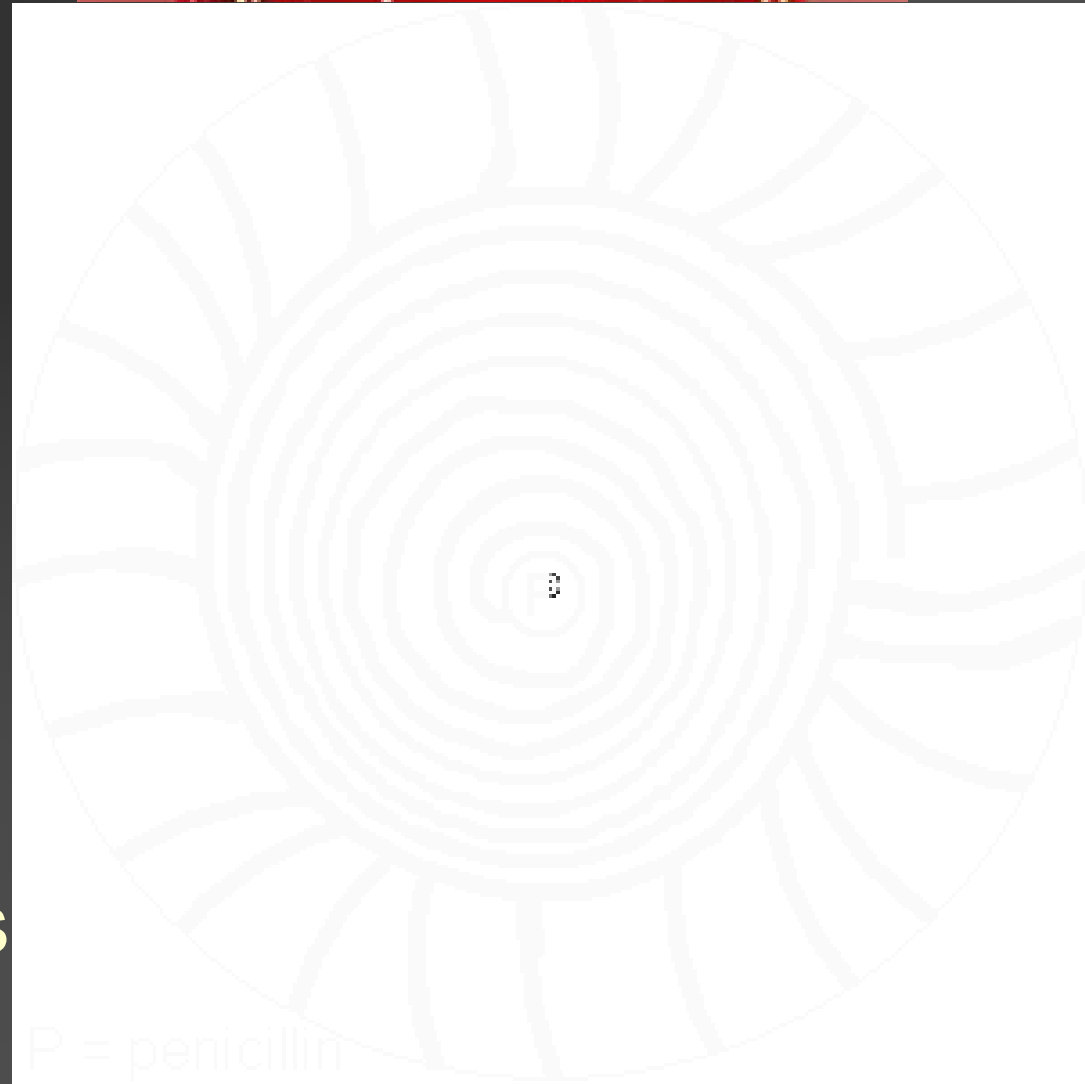
# „Other gram negative“ characteristic

- **Microscopy:** G – bacilli, often short
- **Culture:** we use mostly special media (BG for *Bordetella*, BCYE for *Legionella* etc.)
- **Biochemical diagnostic:** some characteristics might be used
- **Antigen analysis:** sometimes useful
- **Indirect methods** used, mostly for tularemia
- **Differential diagnostics** is not algorithmic here. Usually specimens are sent to the laboratory with suspicion for legionellosis, whooping cough, Bang disease etc.

# *Bordetella*: Inoculation on BG agar

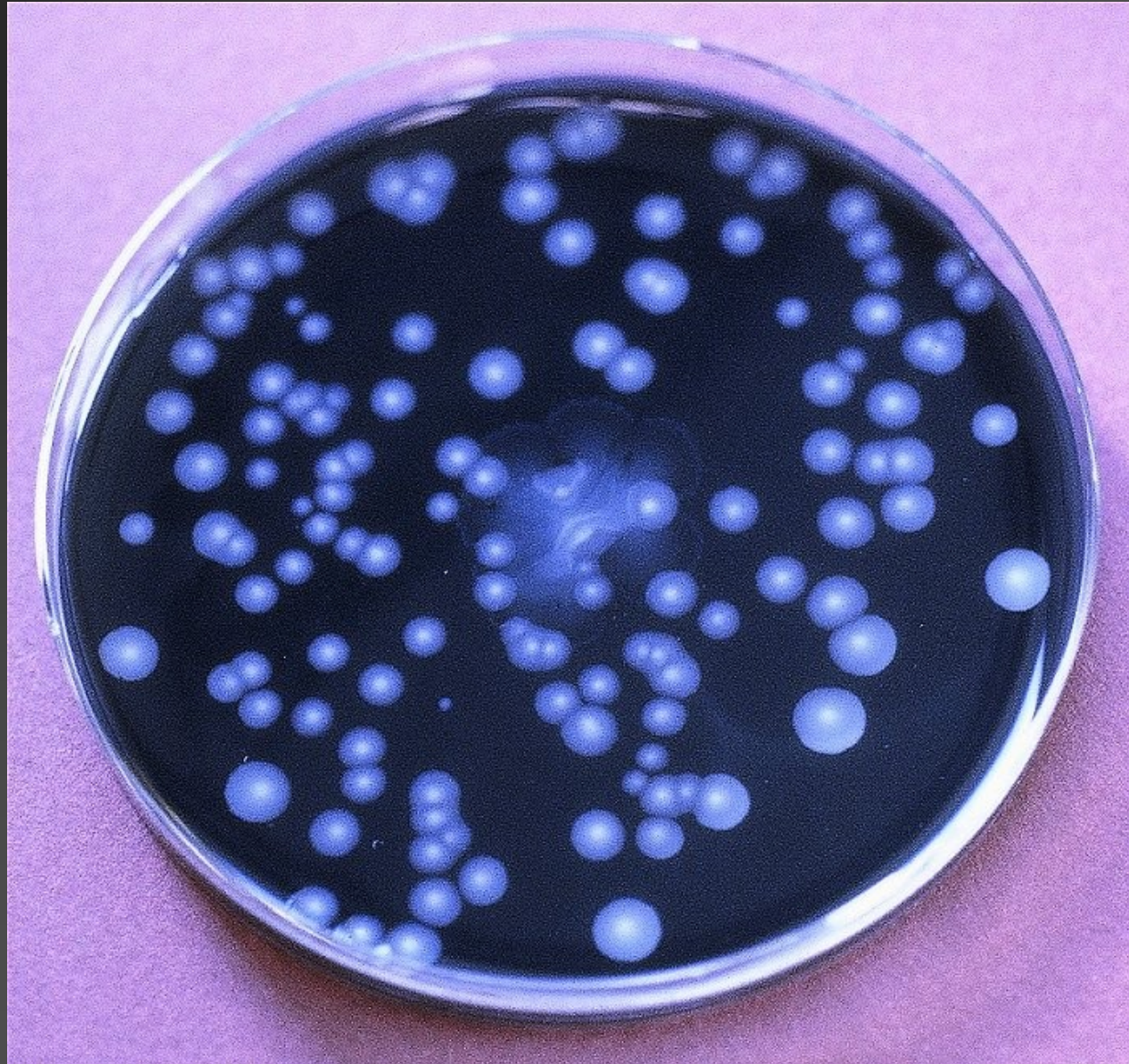
This strange way of inoculation is used, as the experience showed, that it increases successful diagnostics.

- 1) inoculation of central field (to a drop of penicillin)
- 2) Spiral to margins
- 3) Radial rays



# BCYE medium for Legionella

**B**uffered  
**C**harcoal  
**Y**east  
**E**xtract



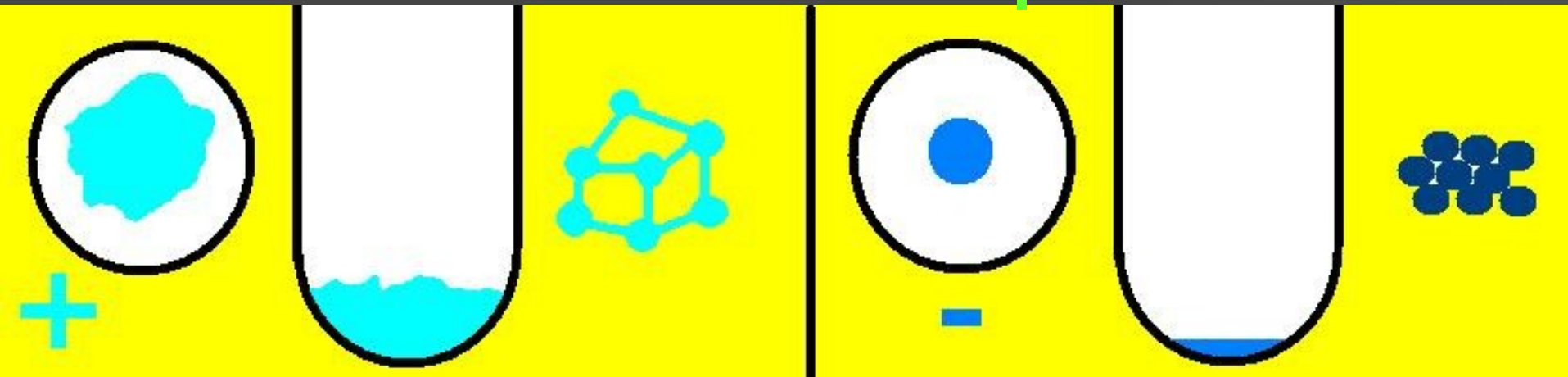
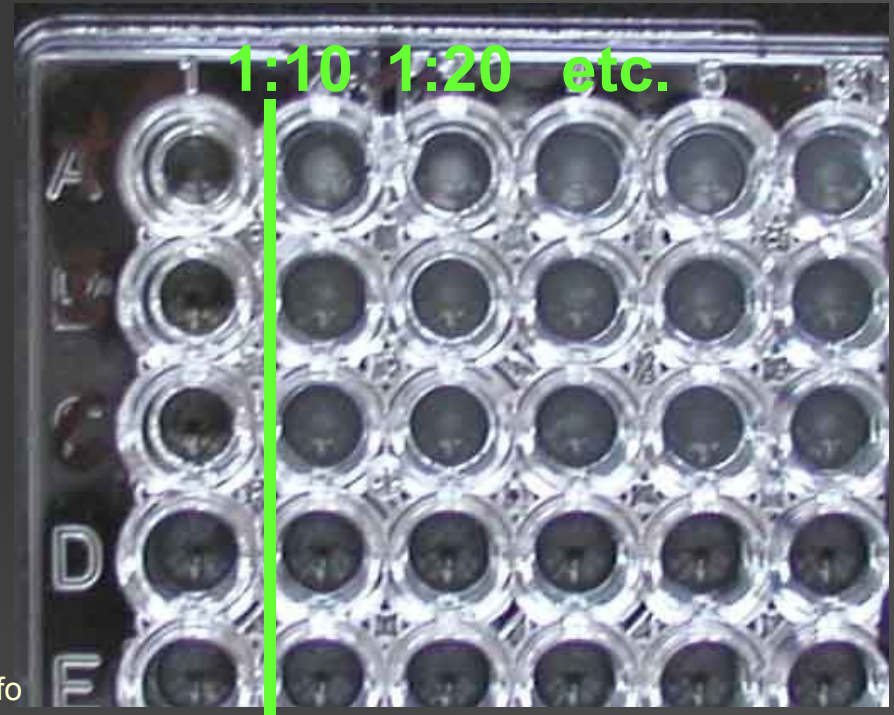


# Francisella diagnostics: Reading the agglutination set

Titre is counted, i. e. the highest dilution giving still a positive reaction

**Any** titer (i. e. everything except negative results) is interpreted as suspicious!

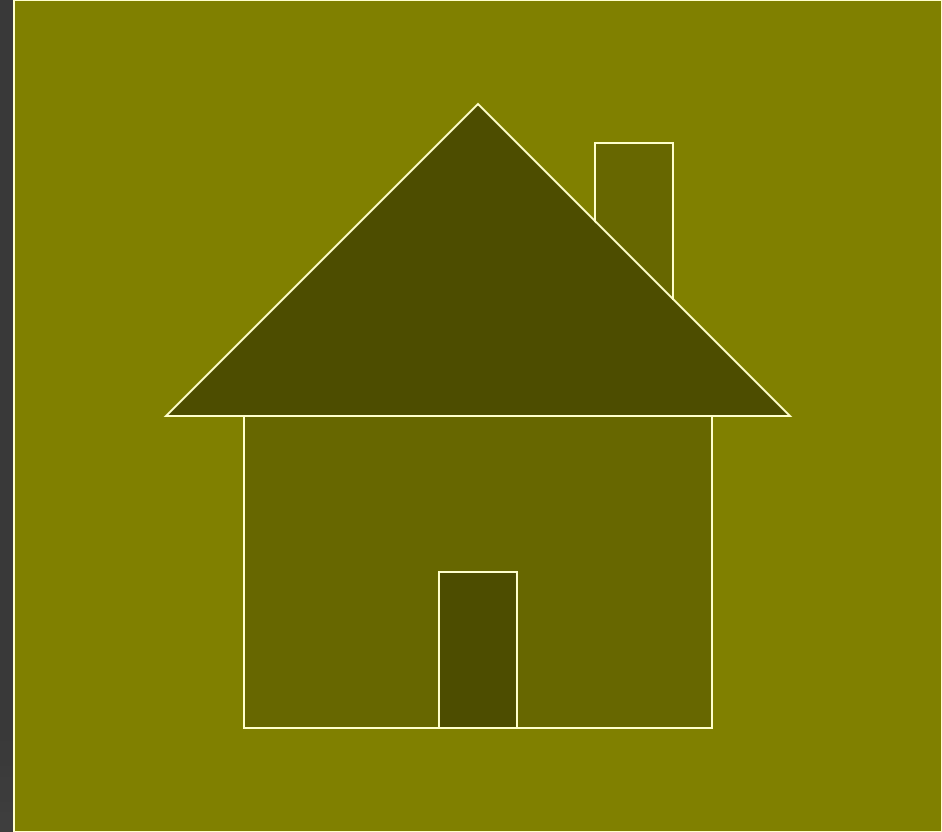
[www.medmicro.info](http://www.medmicro.info)



# The End

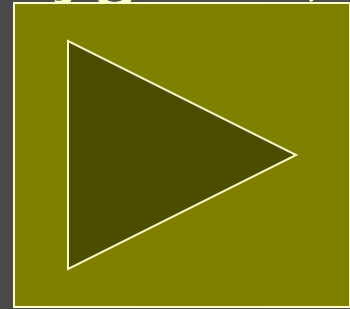


[www.siumed.edu](http://www.siumed.edu)



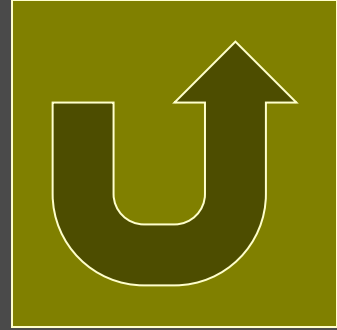
# How to build the buildings for health care to prevent legionellosis

- to ensure **properties of the building** for the healthcare (enough space for personnel, hygiene, storage etc.)
- Protection of **hot and cool water**
- Protection of **sewage and solid waste**
- Protection of **heating, air conditioning** etc.



*Already when searching for an architect it is recommended to ensure that the architect has basic knowledge of healthcare management*

# Especially for legionellosis



- The infection highly related with the status of the building is **legionellosis**.
- In many cases an outbreak of legionellosis is a result of **bad project of water pipes, air conditioning** etc.
- In case of water pipes, especially **blind stream branches**, that cannot be washed through and so they might as a reservoir of legionellosis
- Correction is only possible by **rebuilding all the pipe system**