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

- Johny 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. Johny had a feeling, that he is finally a man… only before pus started to drop from his urethra…

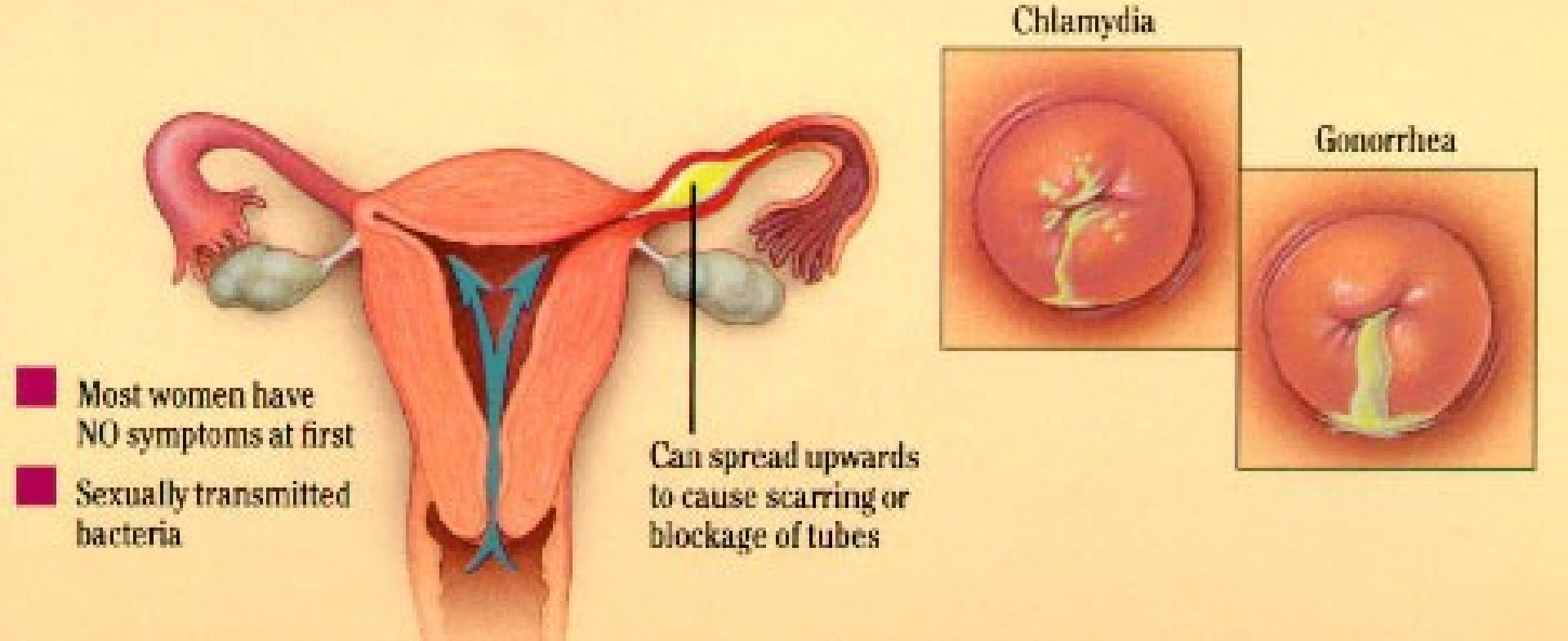
Certainly, you know, what is the criminal

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

Partner of Johny of that night…

nova.medicina.cz

www.lcusd.net



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 unconcious 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…

http://www.waterscan.co.yu

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 completelly inocents 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

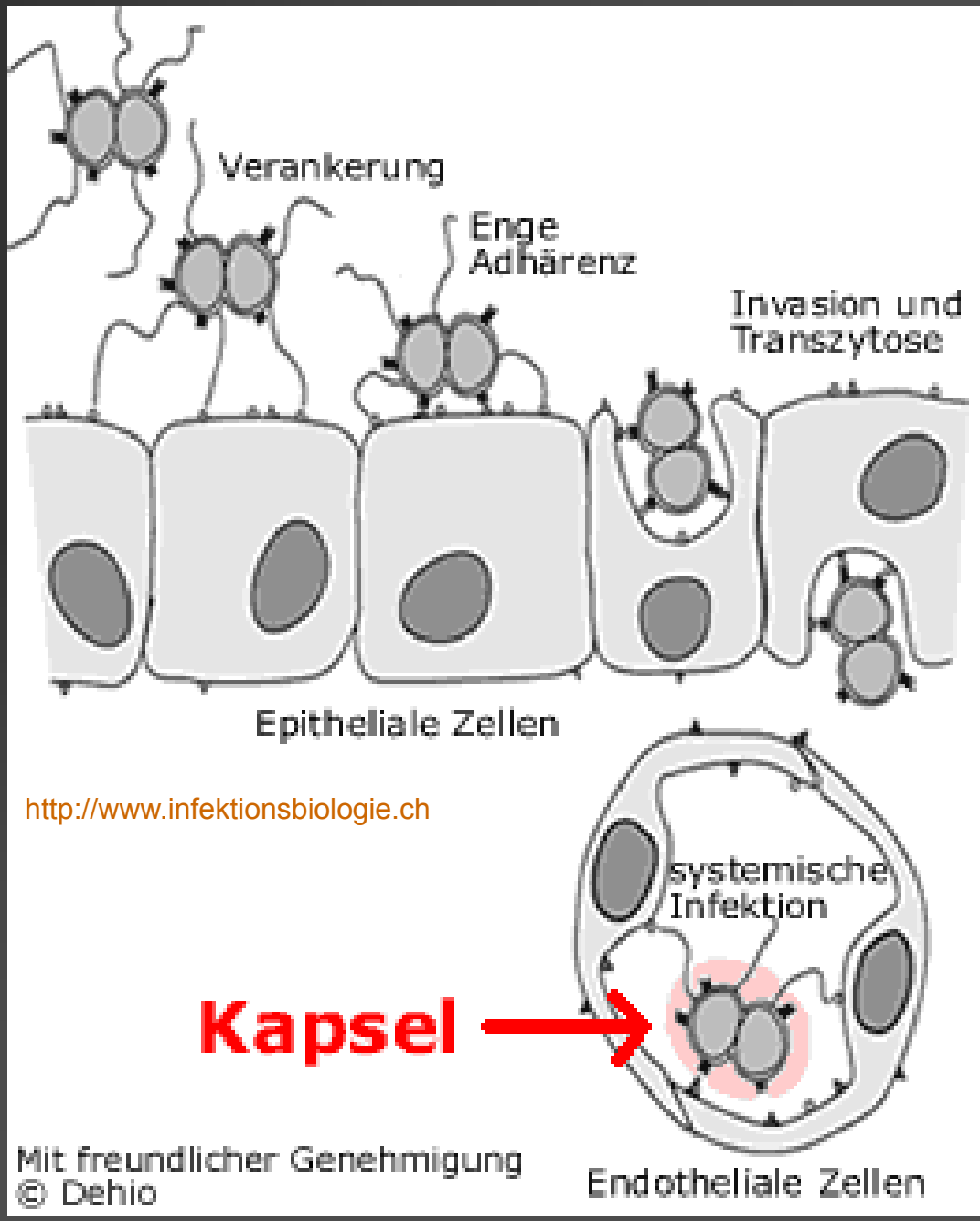
Why the infecion comes sometimes, and sometimes does not

- 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 narow contact. Invasive infection is more likely when mucous membranes are dammaged, e. g. by smoking or previous viral infection.
- Infection is often present after too big physical activity after long inactivity period

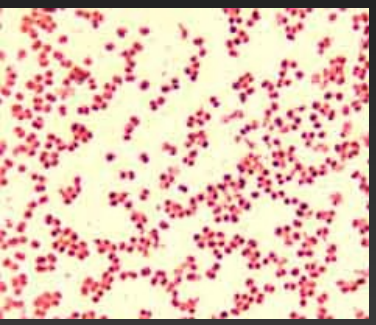
http://www.infektionsbiologie.ch

CSF sampling

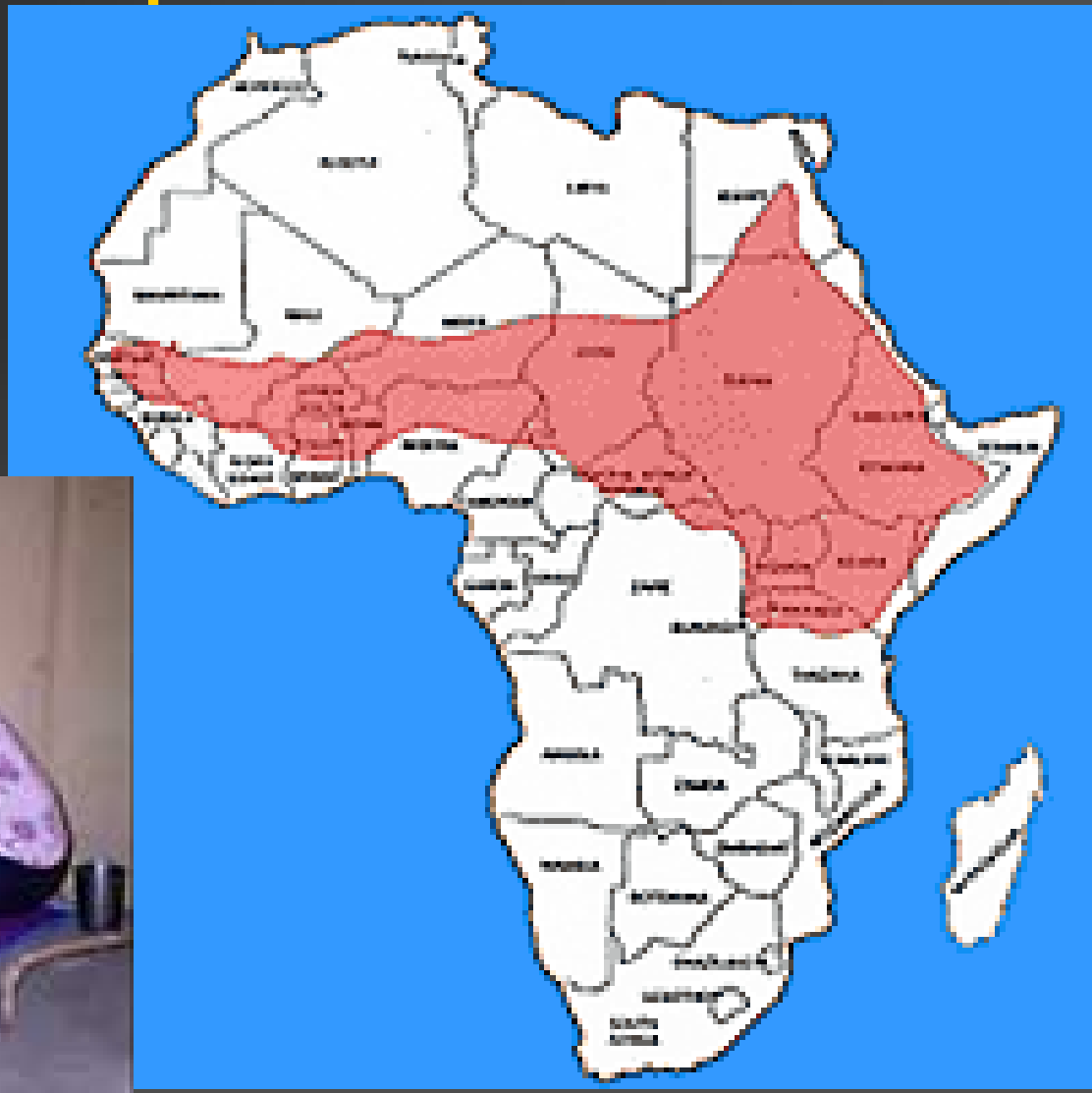
How they come into the tissue



Meningococcal meningitis is worldwide very important infection



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



all pictures: http://www.infektionsbiologie.ch

Let‘s compare neisseriae:

www.meningitis.de

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

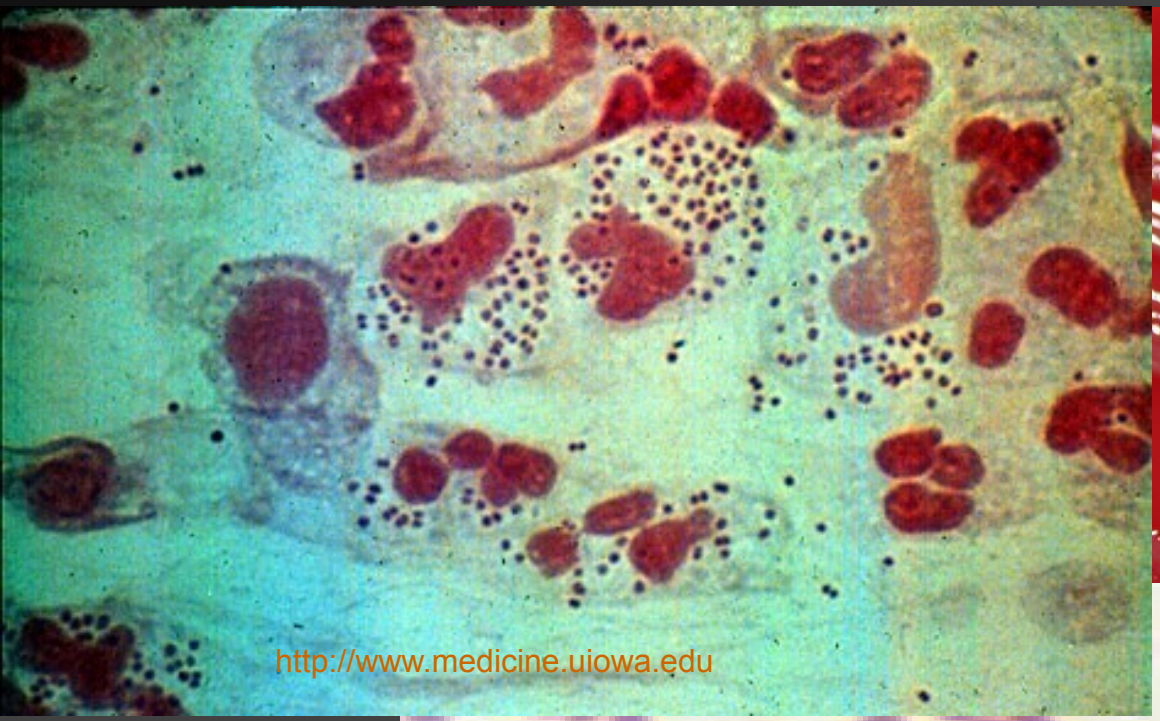
Story three

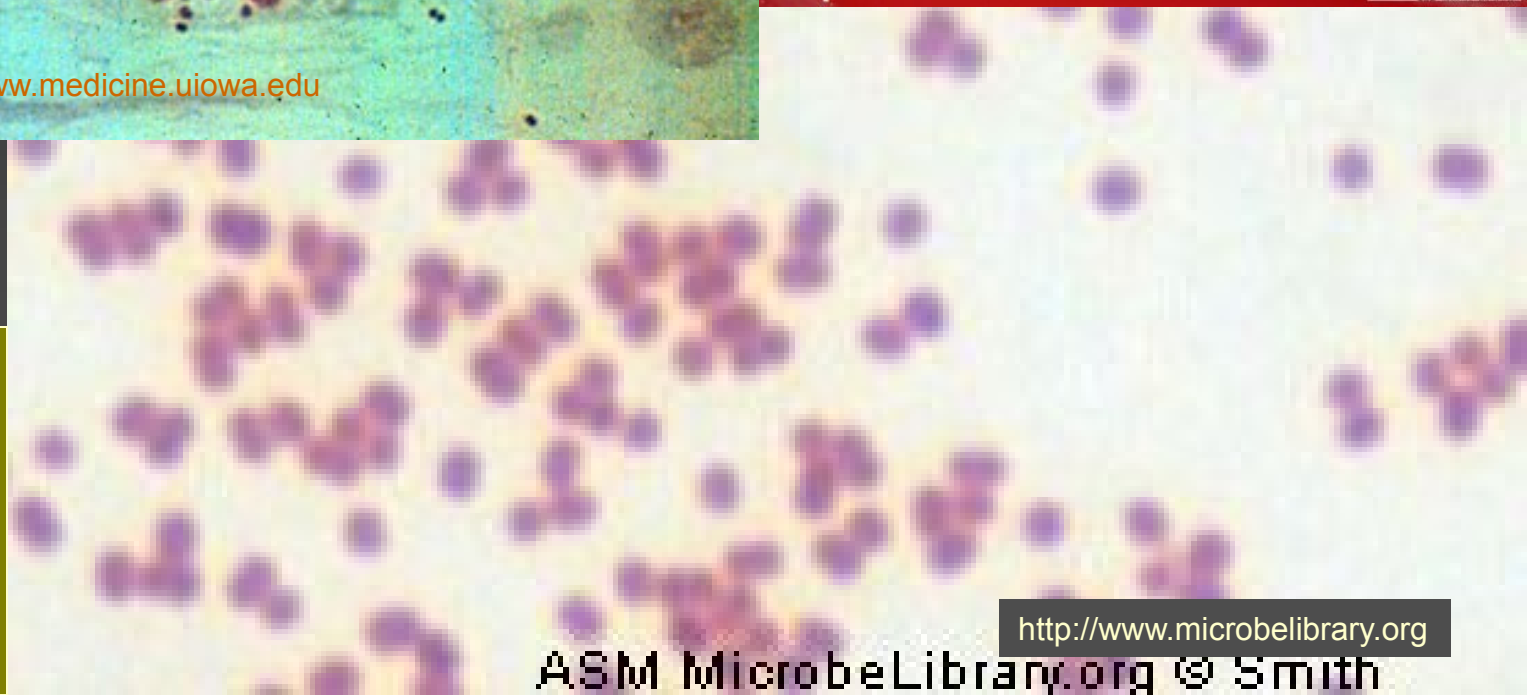
- Annie was **crying and touching her ear**. Her mother measured her temperature, and it was elevated.
- At general practicioner‘s, Annie was examined and diagnosis of **otitis media** was set
- As her **tympanon was already broken**, the pus was taken for examination
- **Amoxicilin** was used for threatment imediatelly. 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 catarrhalis





Clinical characteristics – „other G– bacilli“

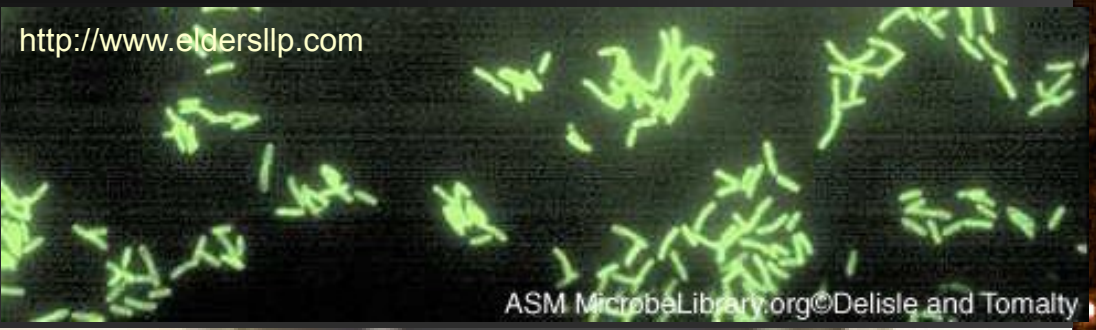
Story four

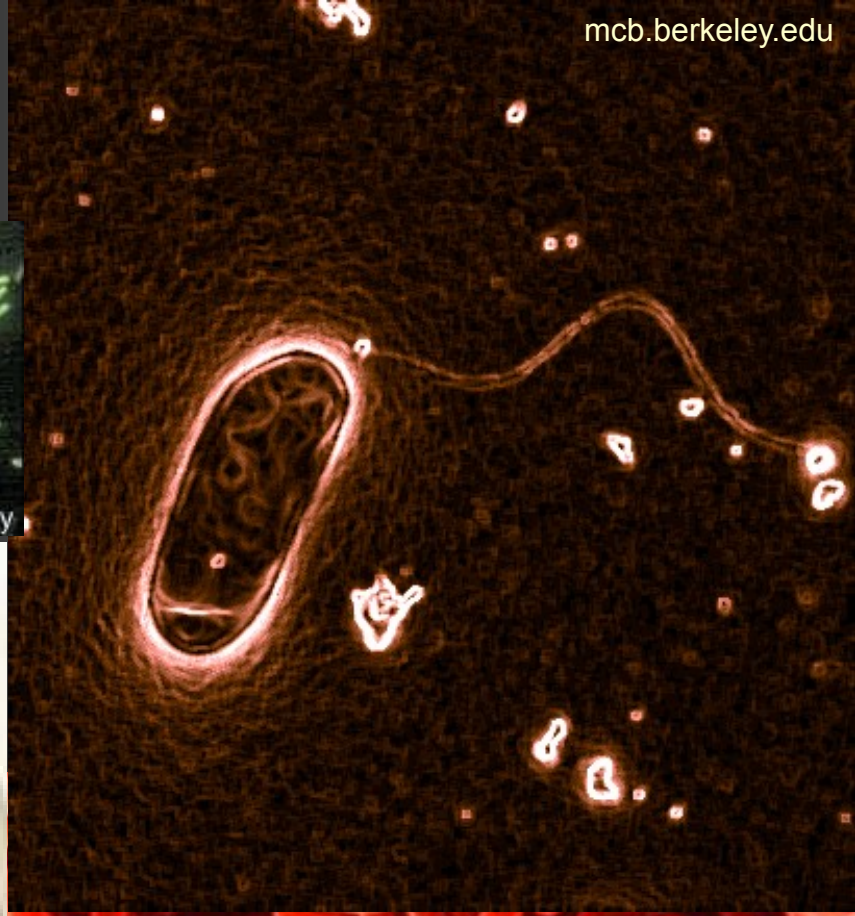
- There was a big movement in the hospital that day: three more patients, all of them **seniors**, became ill, and again it was the same – **breathing problems and fever**
- After a thoroughful examination, the laboratory found the pathogen not only in patient secretions, but also in hot **water pipes**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

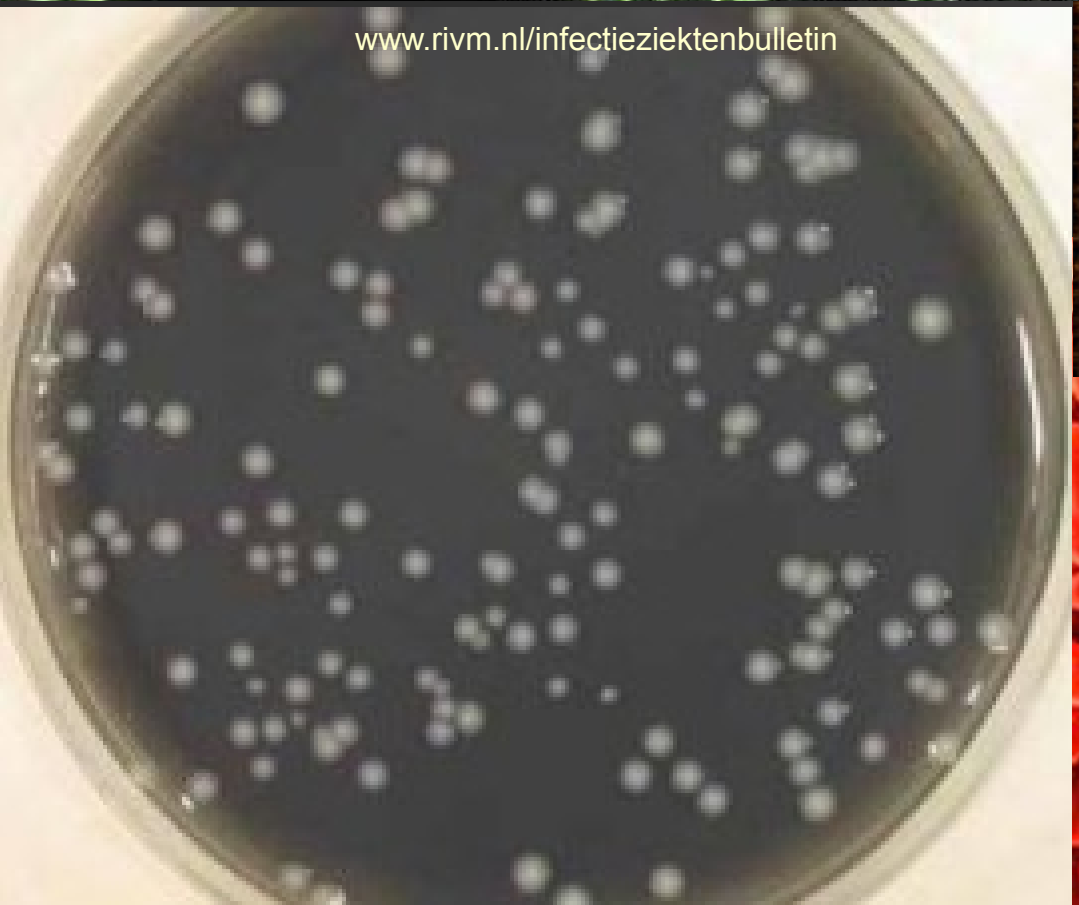
Legionaire‘s disease

- 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 ofther have reservoir in **water instalation, air condition**, etc.
- When building new hospital departments (but also senior houses, hotels, spa…) measures to prevent legionelosis should be taken, mostly at planing water pipe system (no blind branches).

Legionella


http://www.eldersllp.com
ASM MicrobeLibrary.org©Delisle and Tomalty

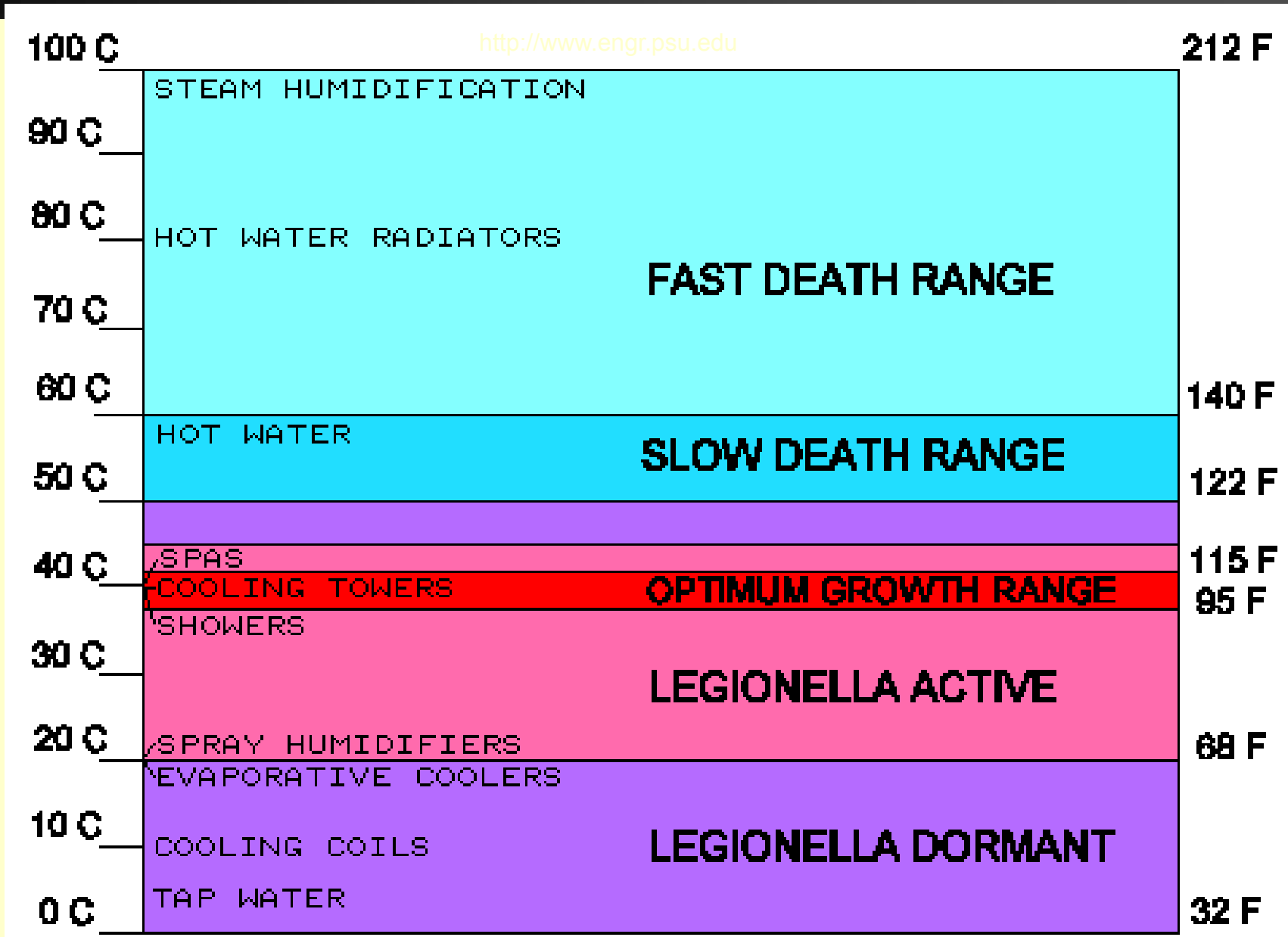

mcb.berkeley.edu


www.rivm.nl/infectieziektenbulletin


www.chemistryquestion.com

Legionaire‘s disease

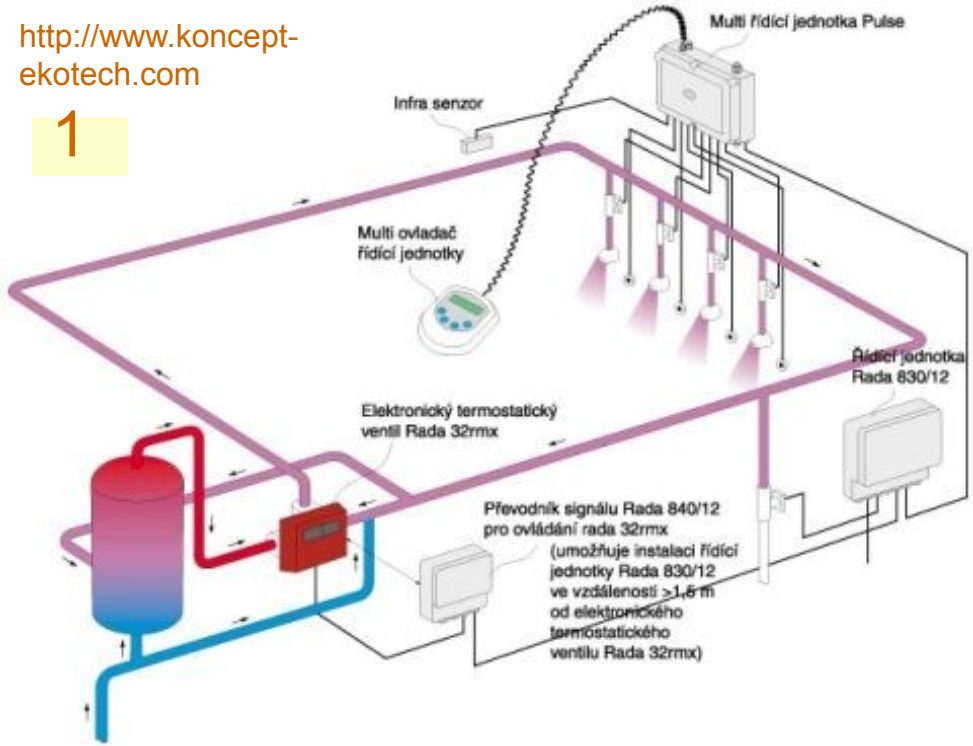
Legionella and temperature



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

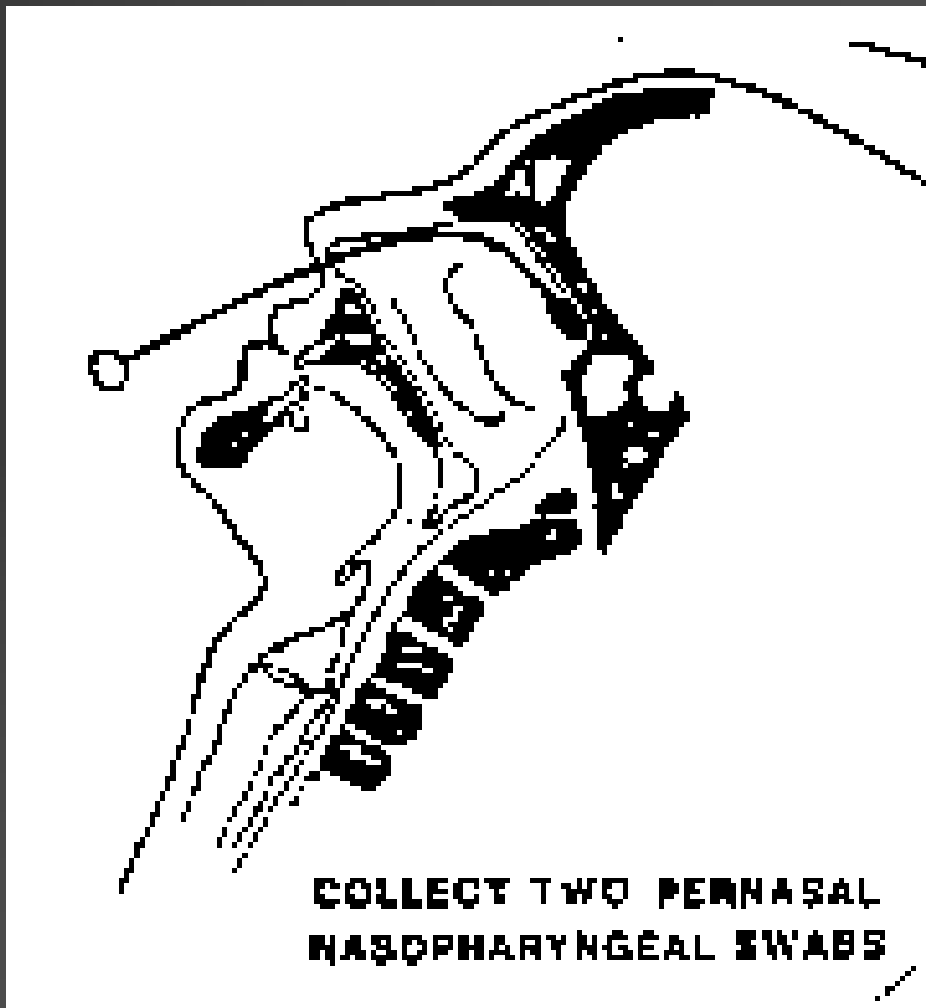
4

„Other Gram-negative bacteria“

- It is not a real „group“ or „familly“. 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*

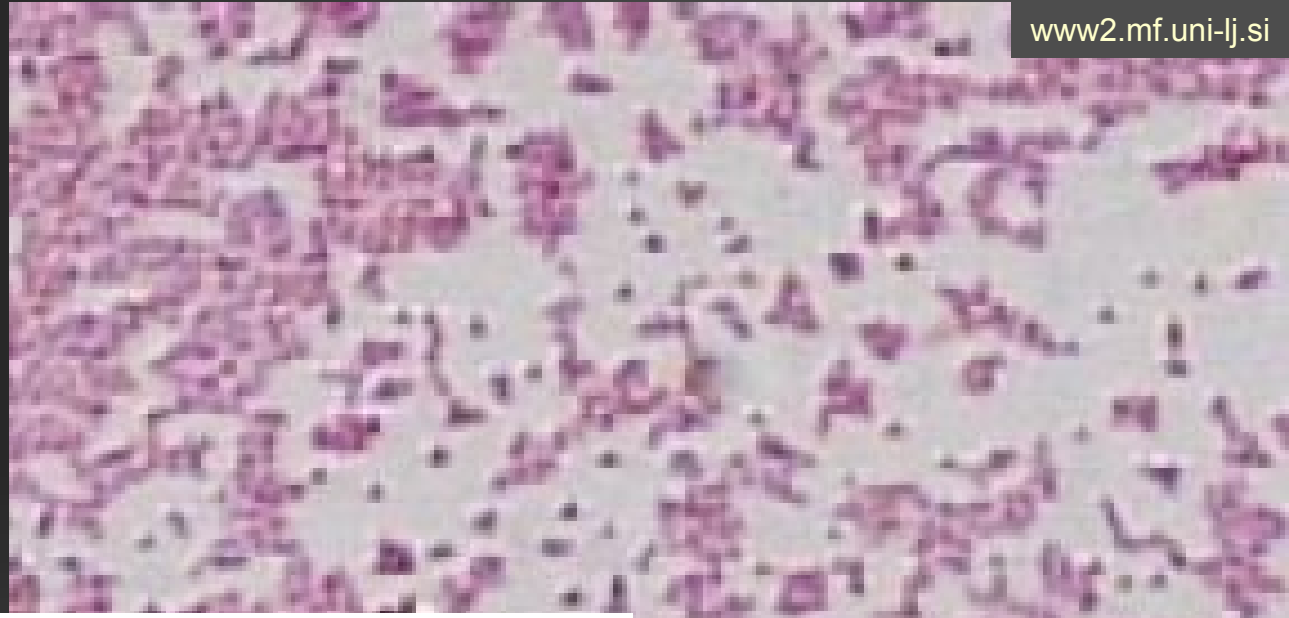
- ***B. pertussis* and *B. parapertussis*** cause whooping cough
- ***B. bronchiseptica*** causes various pathologies in humans and animals.
- Whooping cough is very rare due to vaccination
- **Pernasal swab** is used when necessary (swab from nasopharynx, taken throung nasal cavity)



http://www.dsf.health.state.pa.us

Bordetella

www2.mf.uni-lj.si



Bordetella pertussis

Causitive agent of Whooping cough

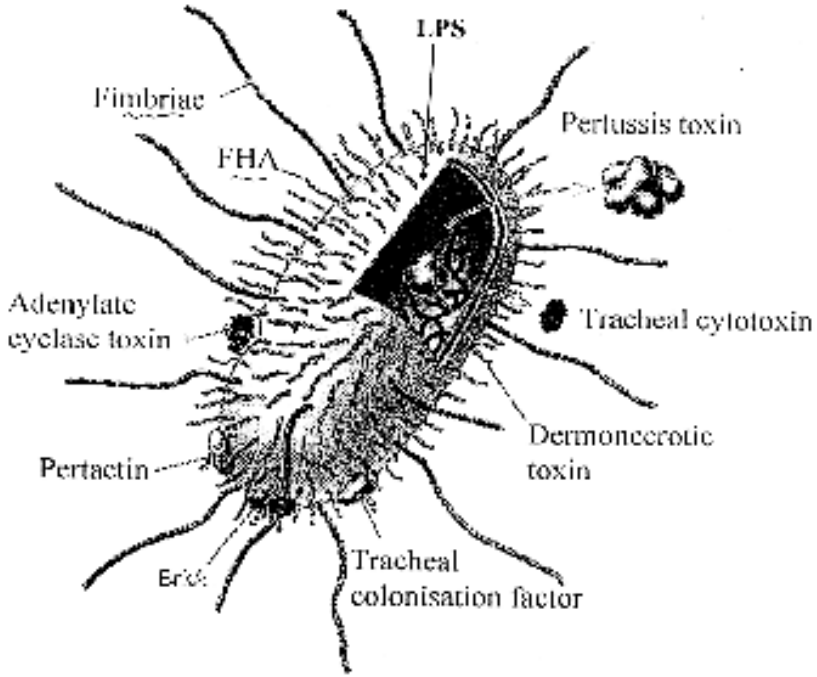
-Small gram negative bacterium

-Many virulence factors

Adhesins: **Pertactin**
FHA
Fimbriae

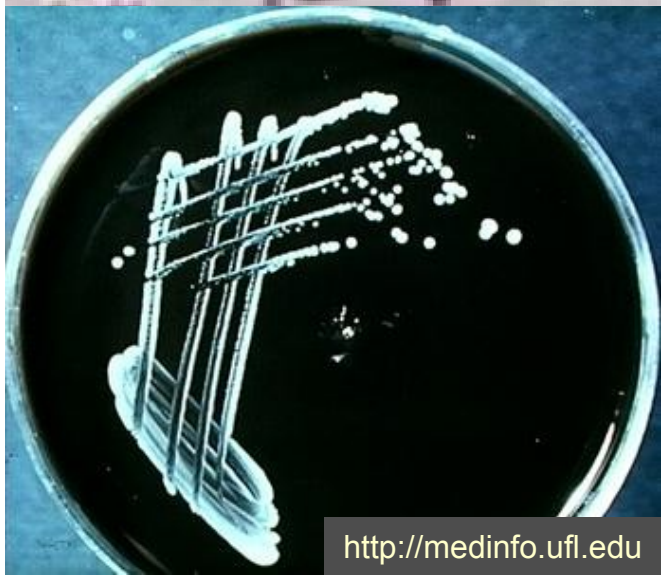
Toxins: **Pertussis toxin**
ACT
TCT

LPS



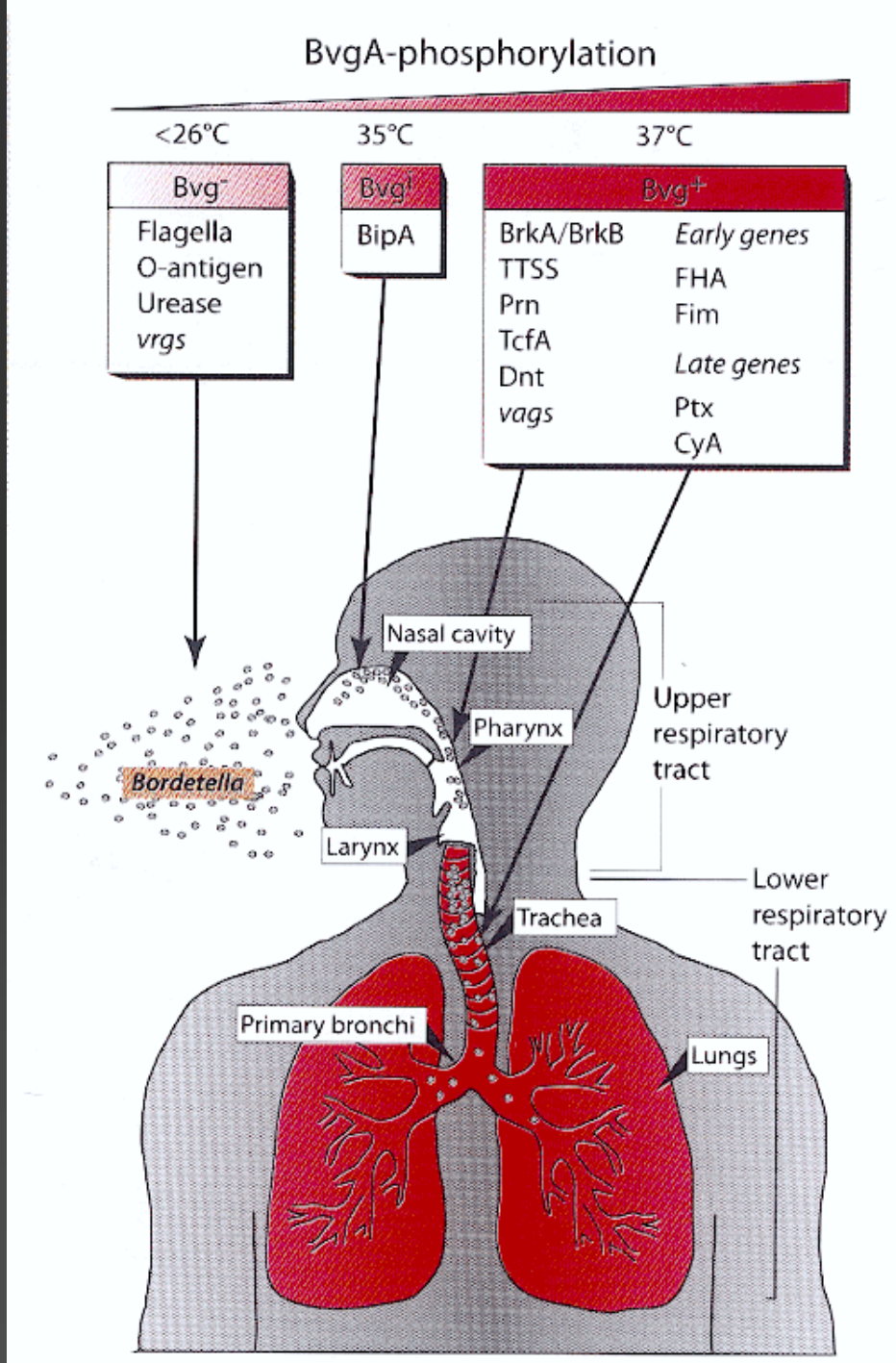
Alison Weiss, ASM News, 1997

www.gpo.or.th



http://medinfo.ufl.edu

Bordetella and temperature

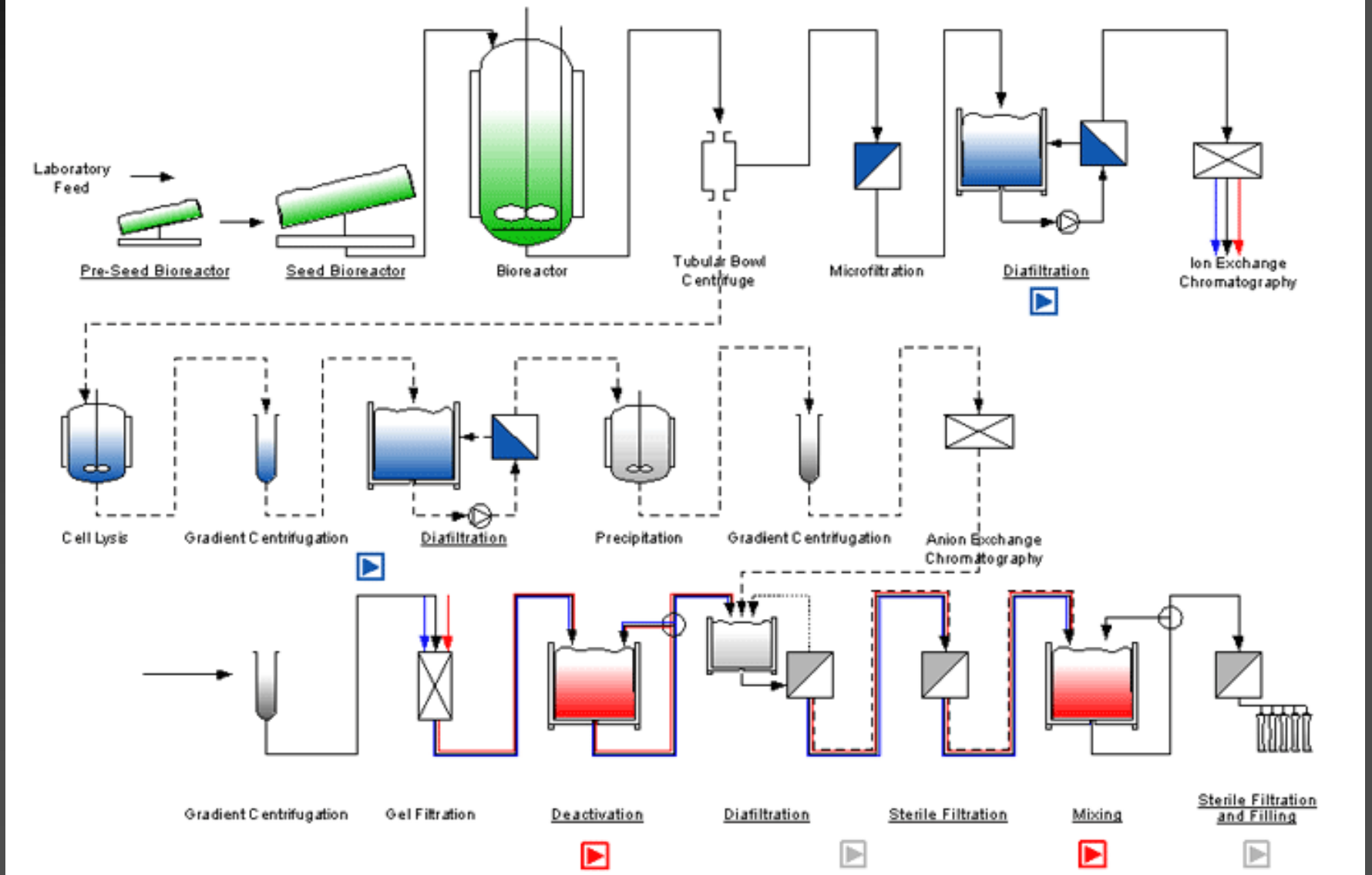


http://www.rivm.nl/infectieziektenbulletin

Bordetella was isolated in 1906 by Jules Bordet et Octave Gengou

Pertussis vaccine production

www.stedim.com



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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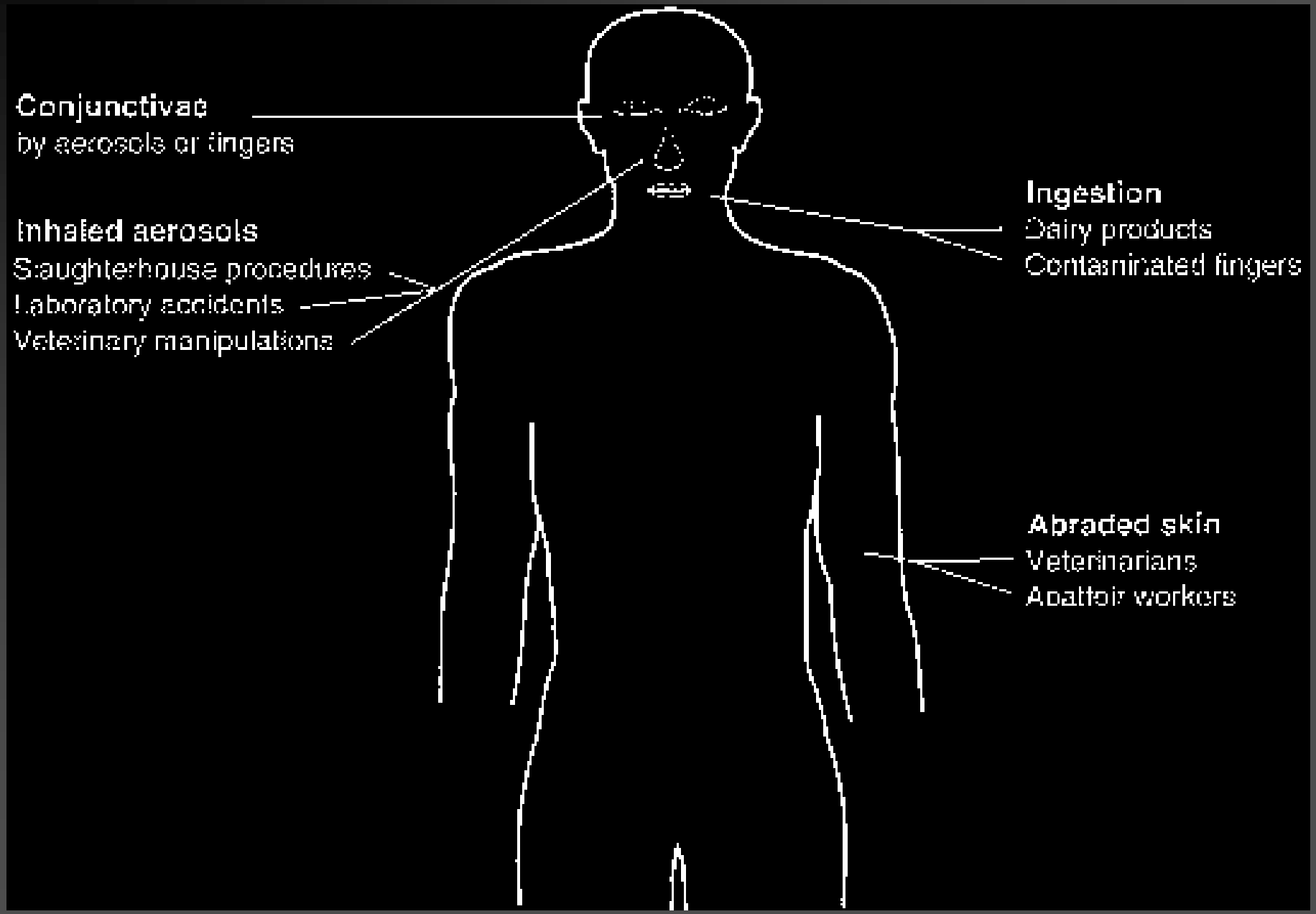
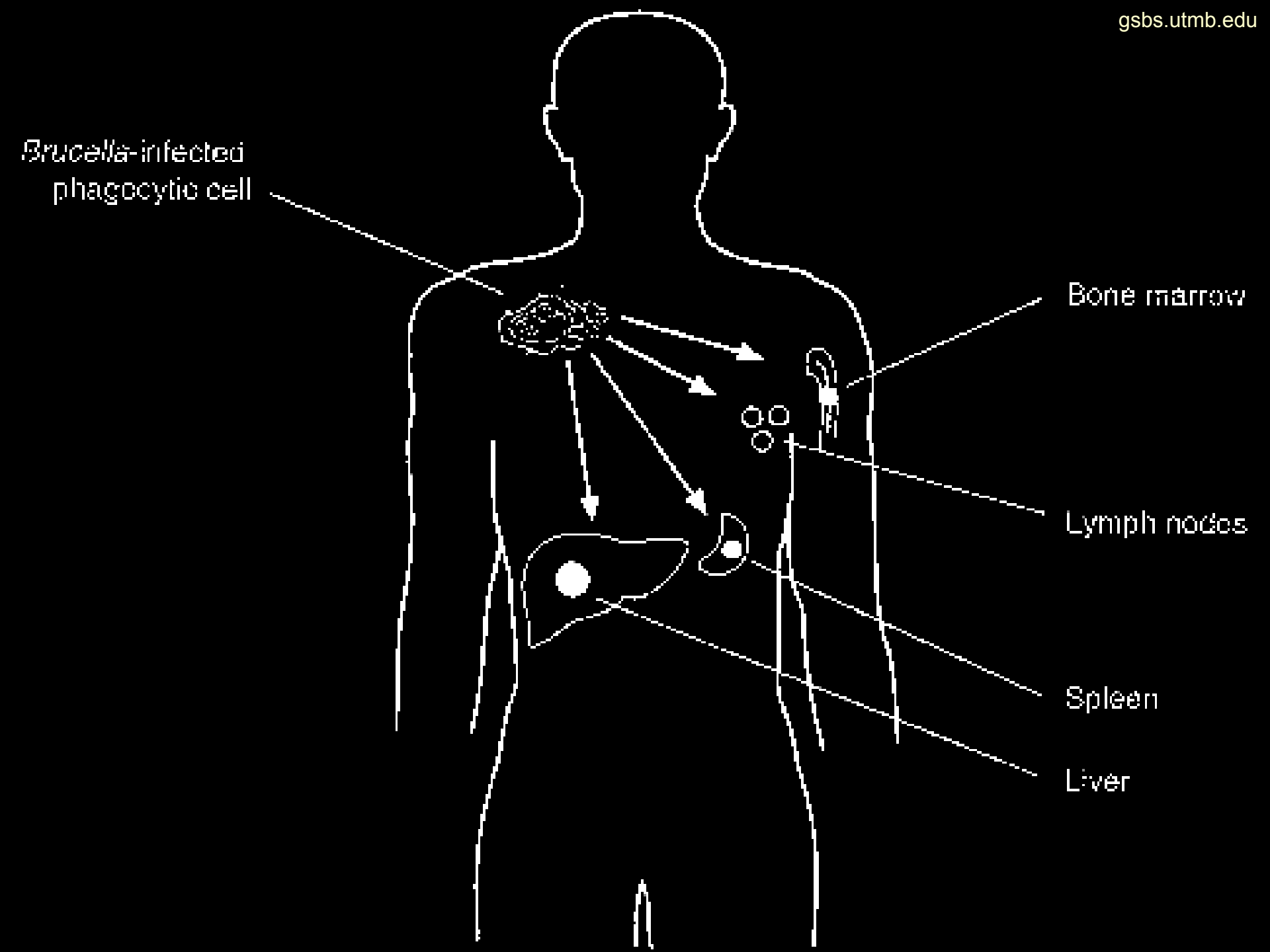
http://upload.wikimedia.org


Conjunctivae
by aerosols or fingers
Inhaled aerosols
Slaughterhouse procedures
Laboratory accidents
Veterinary manipulations
Ingestion
Dairy products
Contaminated fingers
Abraded skin
Veterinarians
Abattoir workers



History and Physical Exam

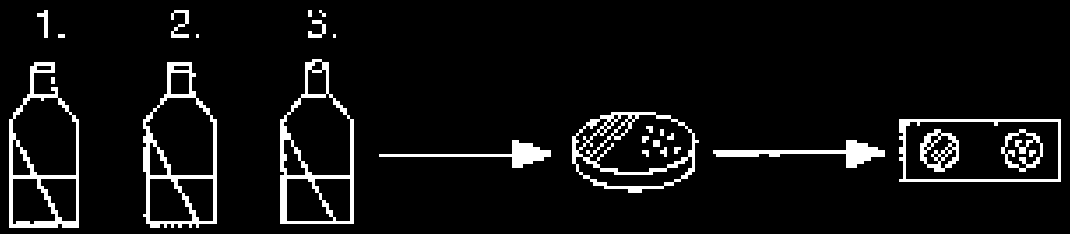
Clinical

- Occupation
- Raw milk
- Fresh cheese
- Travel
- Symptoms
- Signs

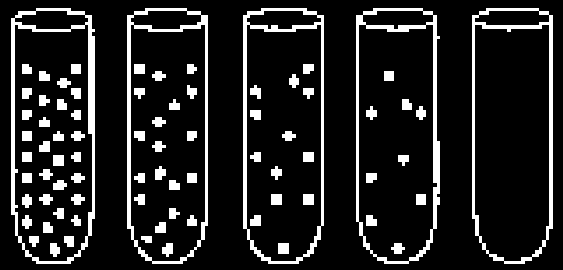
Course of disease

Laboratory

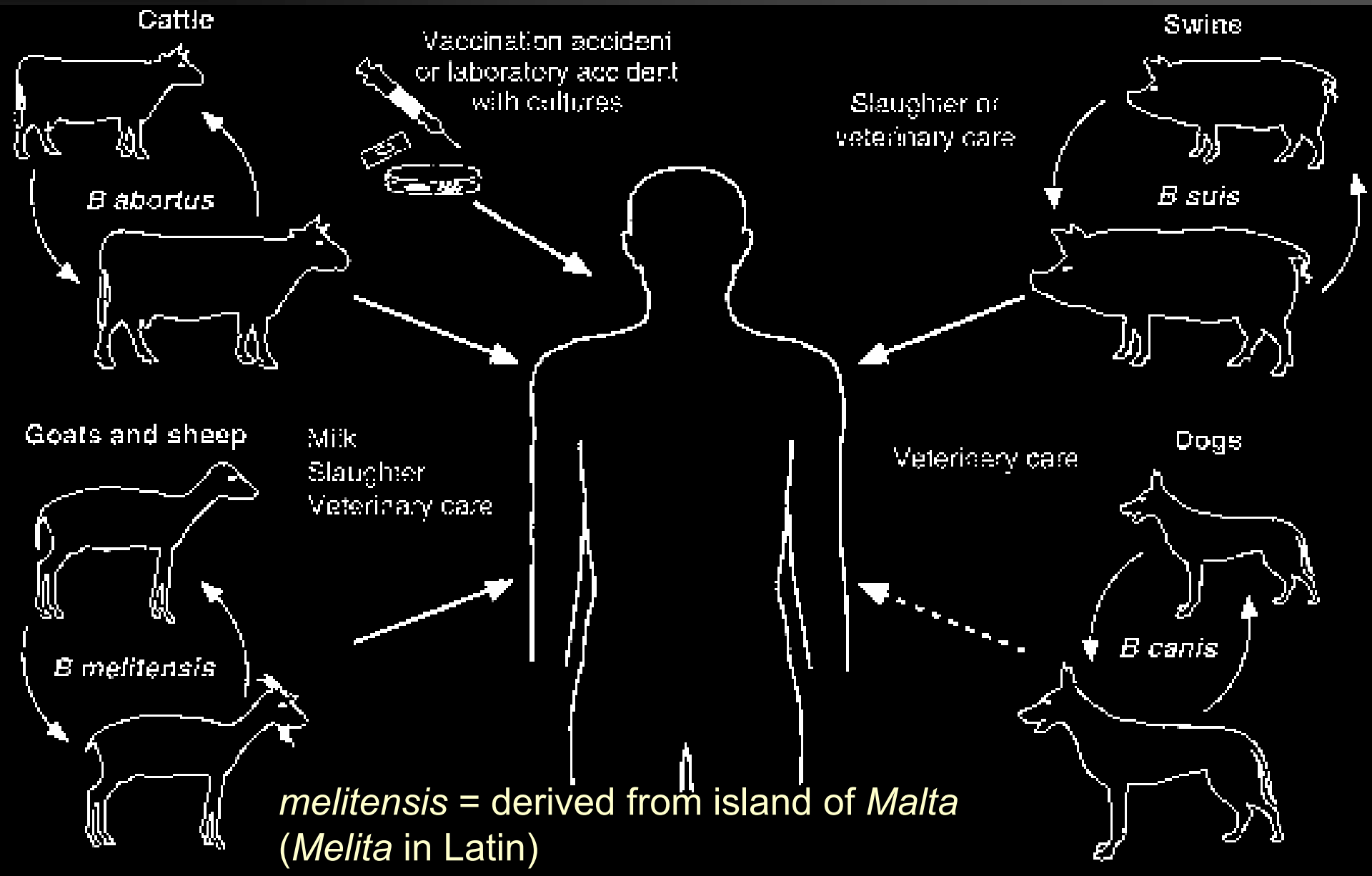
Blood cultures



Quantitative serology

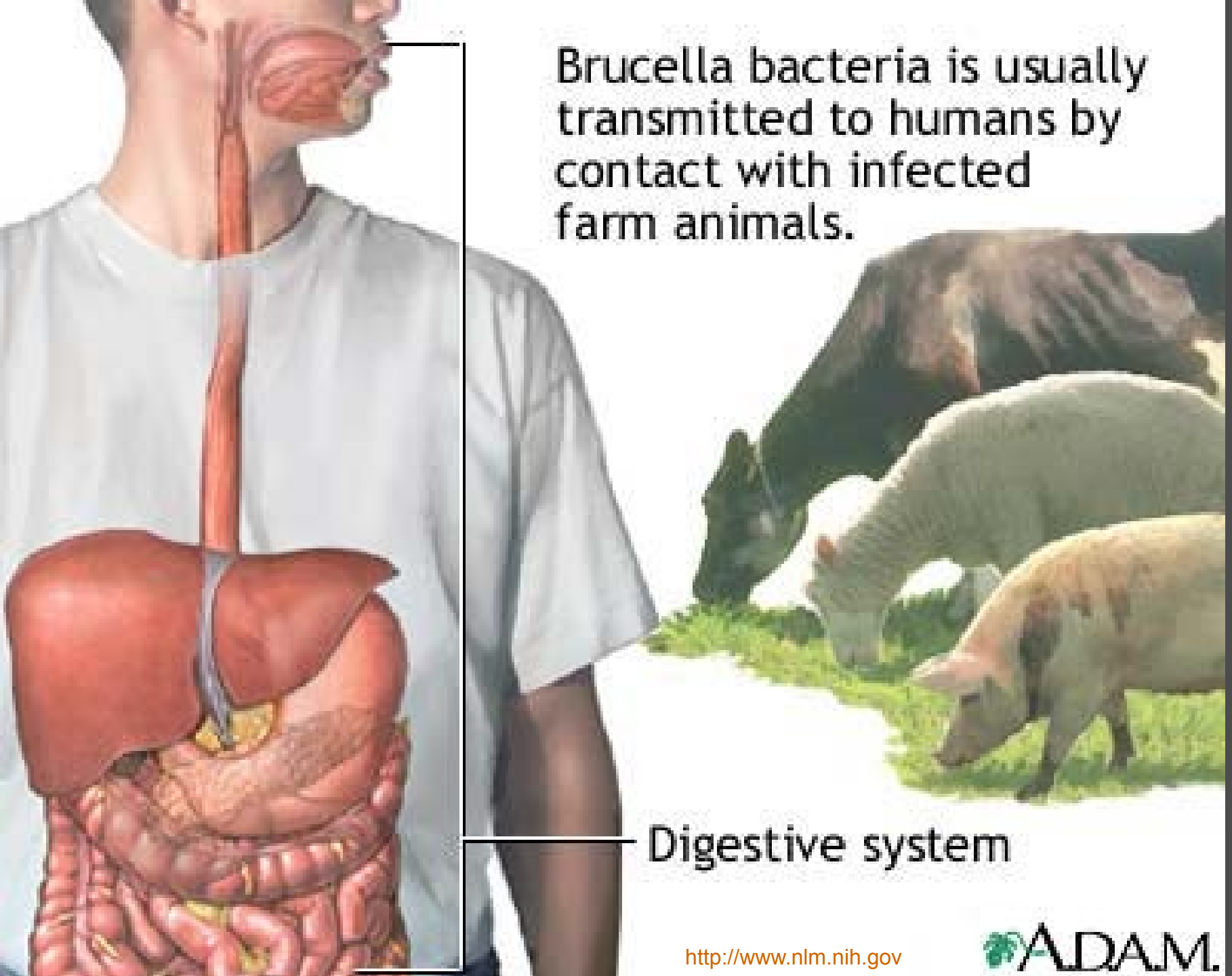


Remembering Brucella



melitensis = derived from island of *Malta* (*Melita* in Latin)

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



Digestive system

http://www.nlm.nih.gov

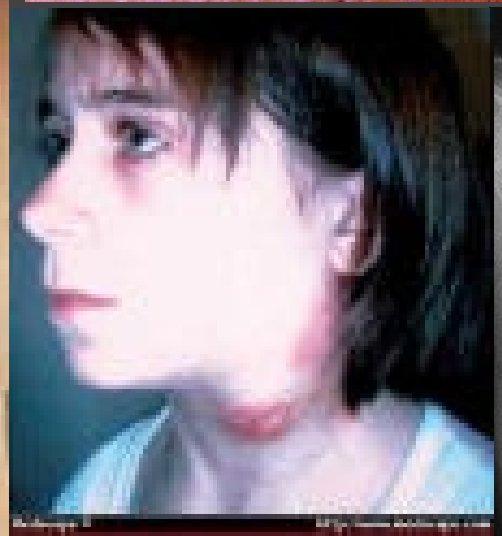
A.D.A.M.

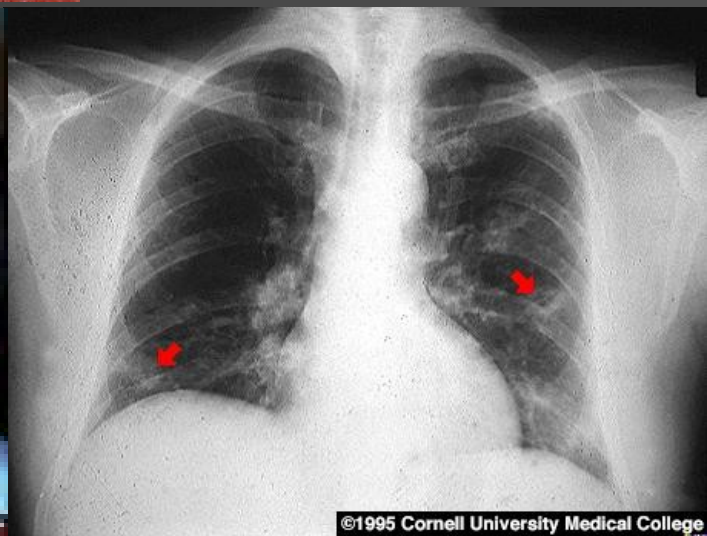
Genus *Francisella*

- 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 inhalate it, thus leading to **pneumoniae**

Francisella

http://www.antropozoonosi.it (4×)





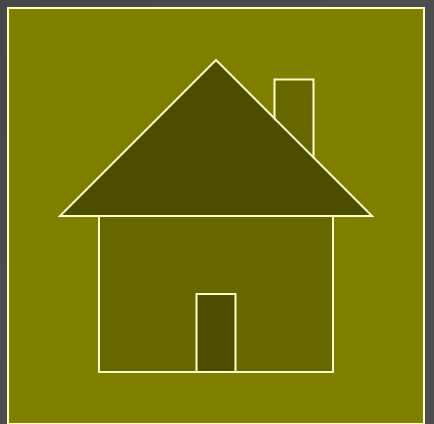
From report about epidemiological situation (IX/2008) – I (abbreviated)

- **Tularemia – A21:** Woman, born 1970 from Valtice, gardener. 20th June GP visit for T 38°C, sore throat, enlarged lymphonodes next to right clavicula. The lymphonode was extirpated. First serological examination negative, second (2nd July) positive
- **Brucellosis – A23:** Man, born 1972, Brno. 10th 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-vaccintion
- **Parapertussis (A37.1):** 3 cases of disease, coinfection, Brno environment, Hodonín

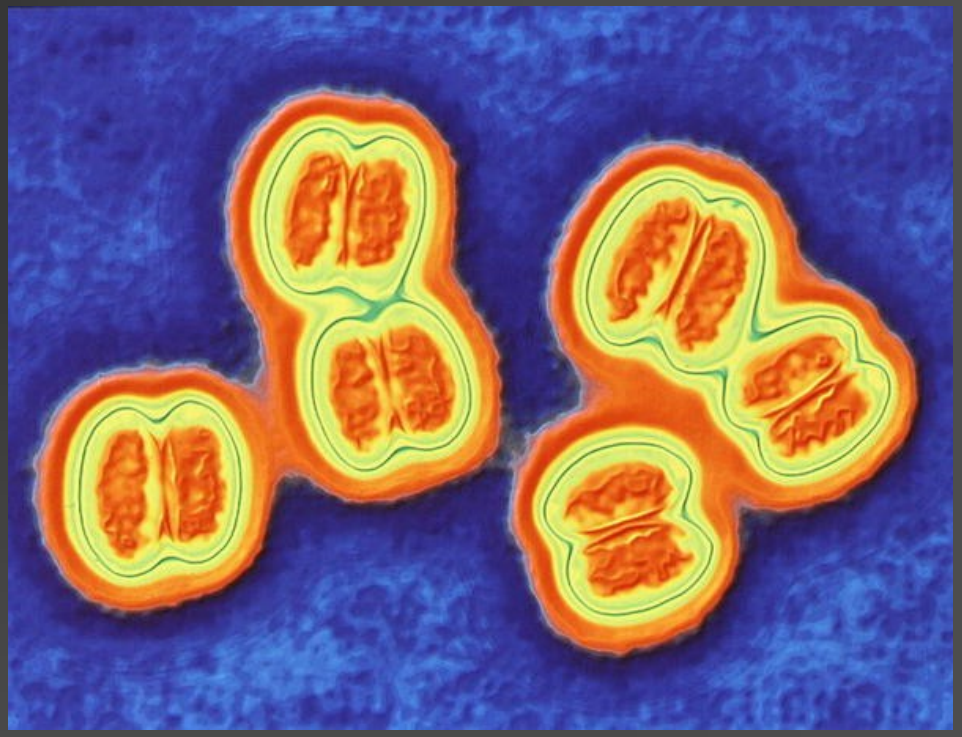


Diagnostics of G– cocci

Neisseriae and Moraxellae – characteristics I

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

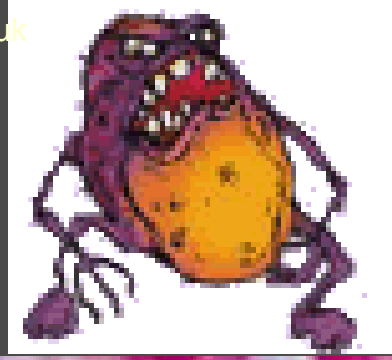
http://medicine.plosjournals.org

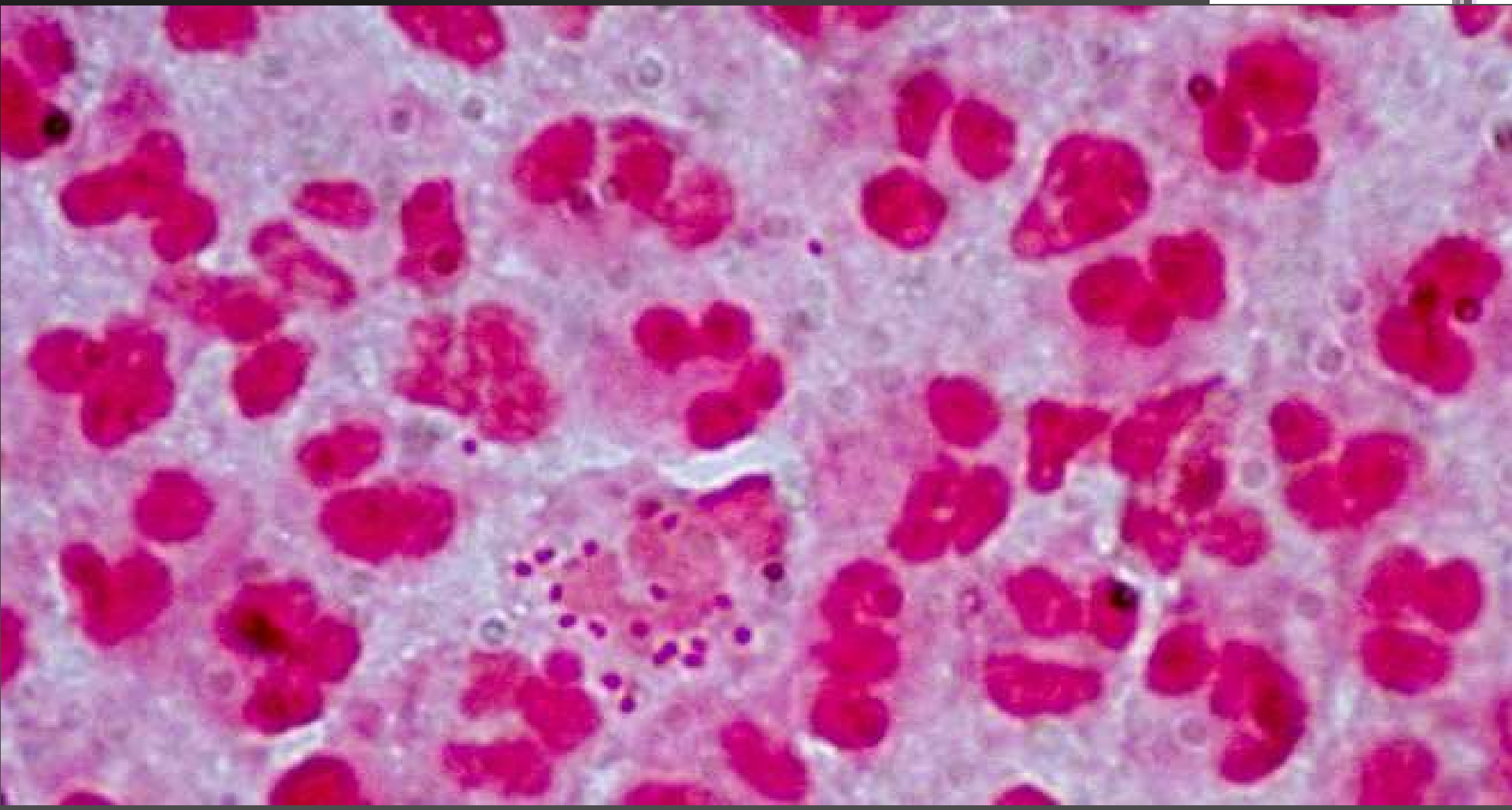


Some neisseriae a moraxellae may be prolonged and so they might be coccobacilli or bacilli (e. g. Neisseria elongata)

Gonococci

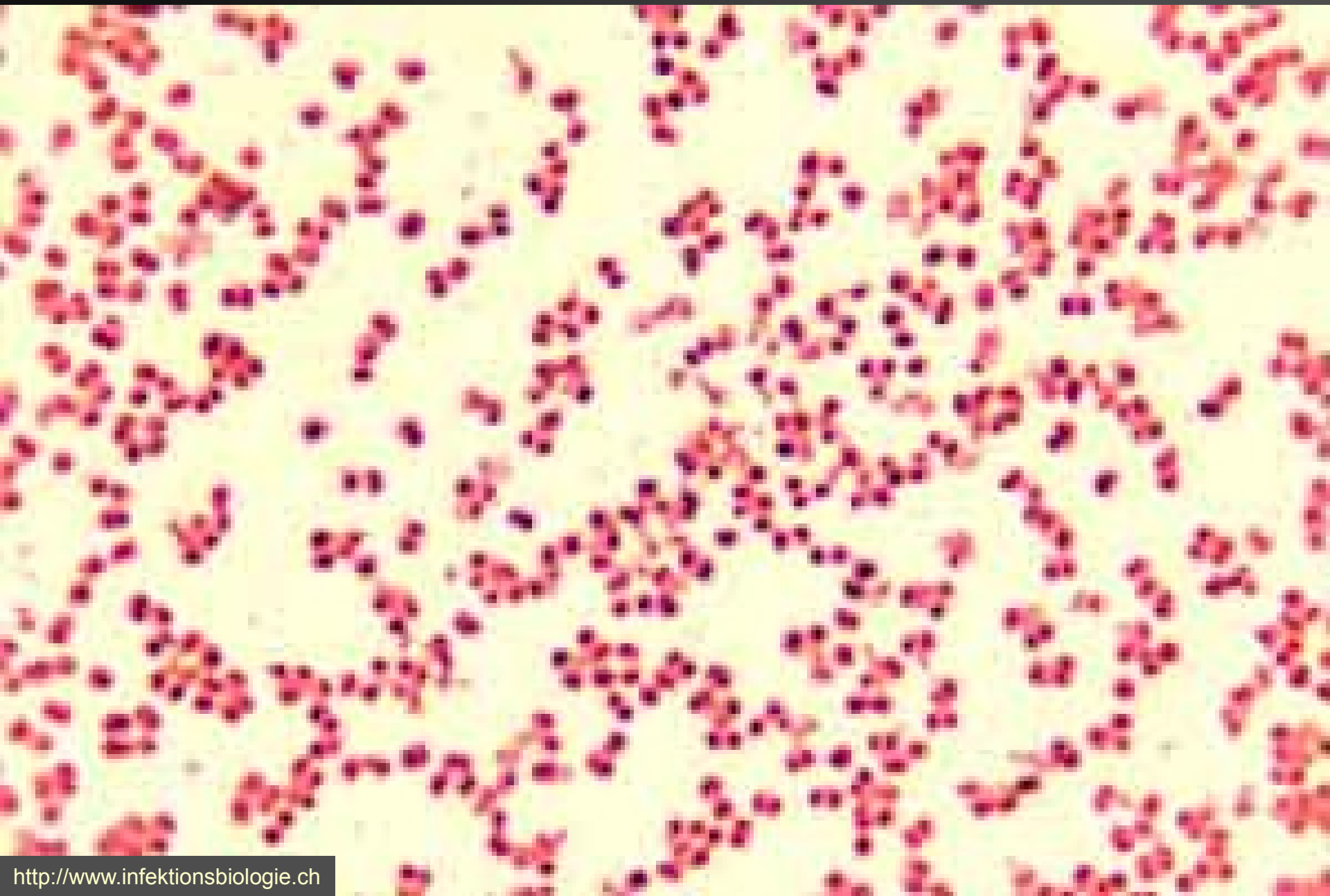
http://www.warwickshire.gov.uk





www.medmicro.info, photo O. Z.

Meningococcus



Microscopic Pictures Of Neisseria (Gram-negative Diplococci)

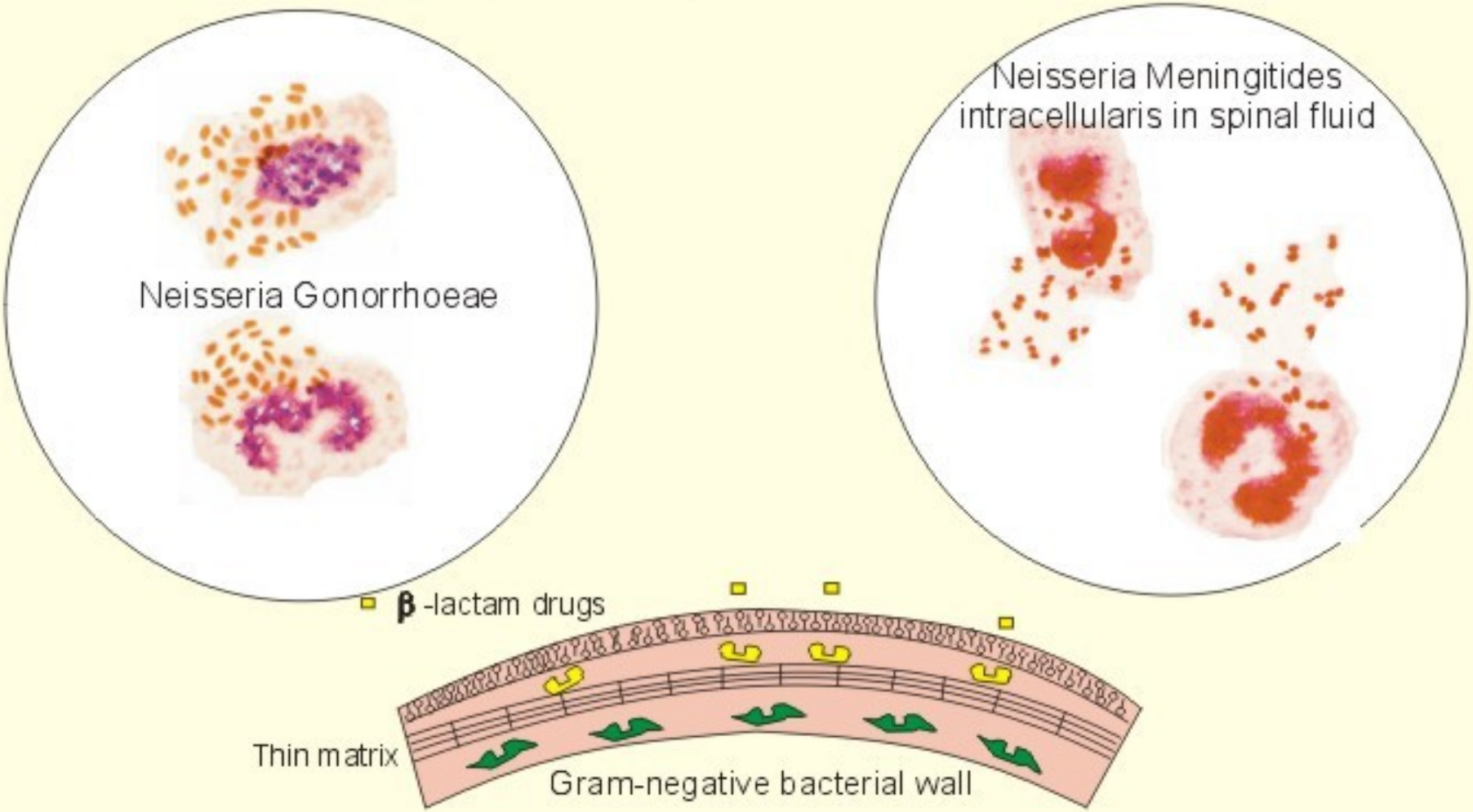
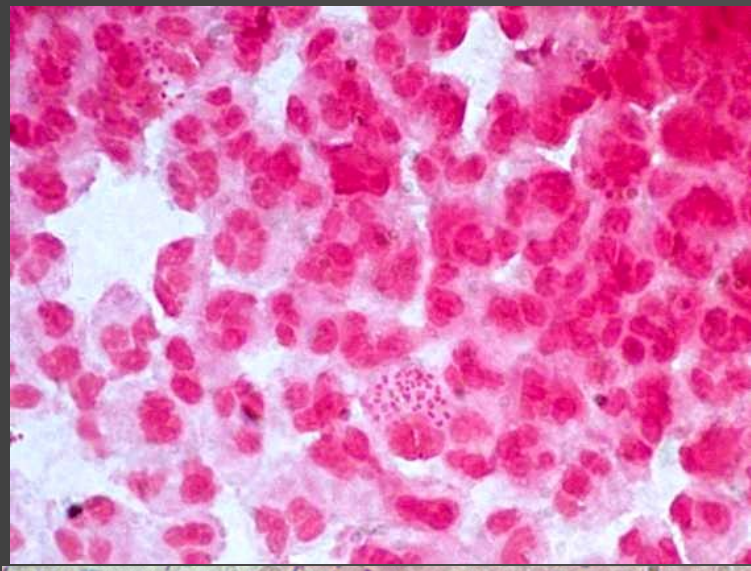


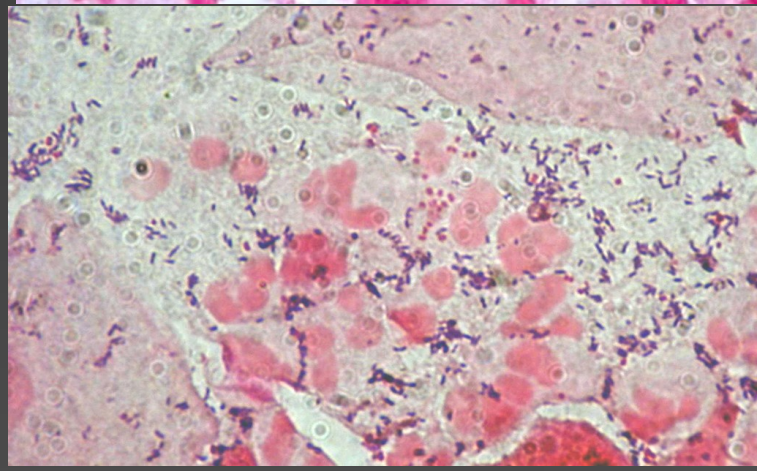
Fig. 33-3

KMc

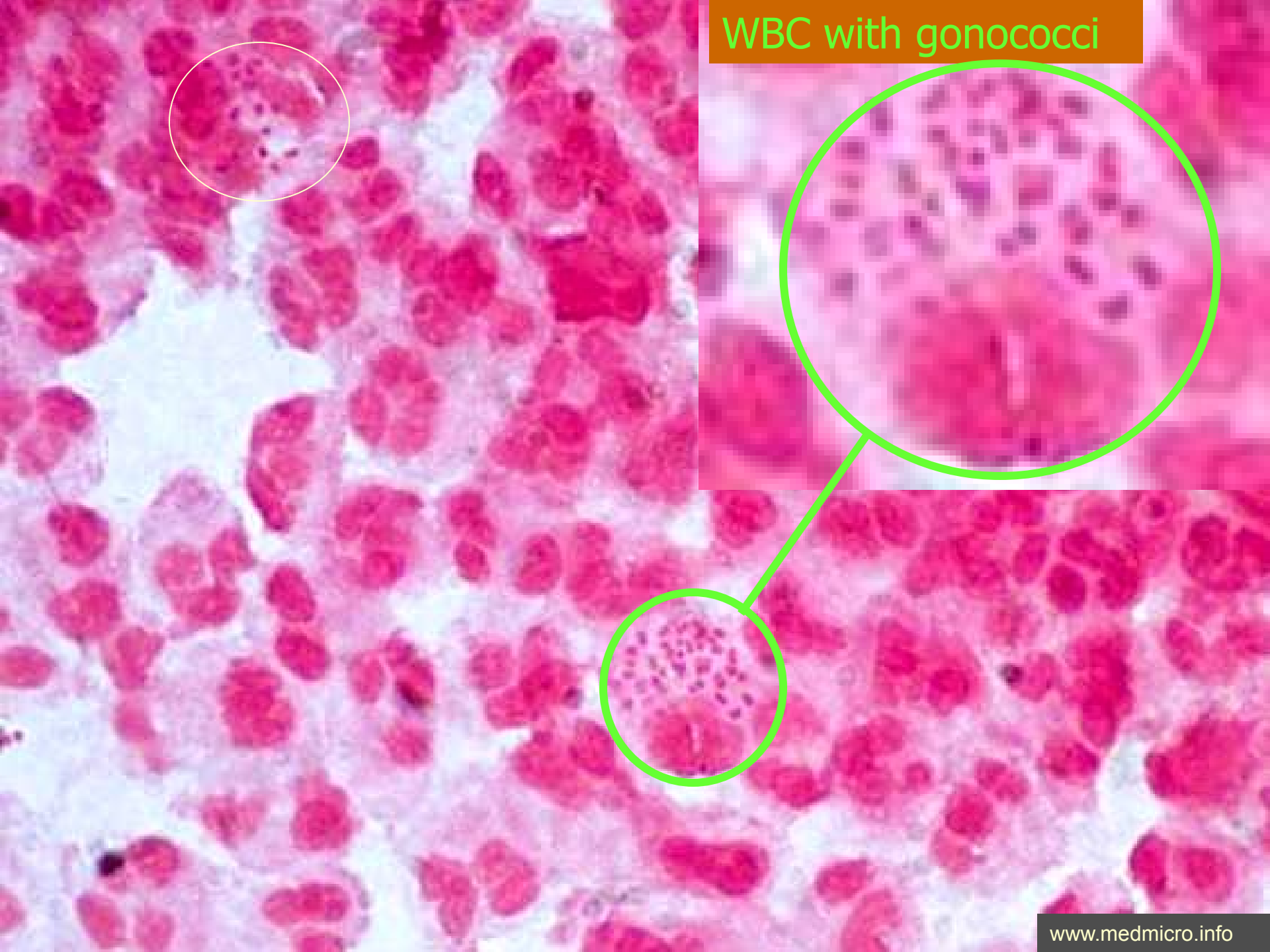
Observation of gonorrhoea smear

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





In females, the microscopy is slightly different from males.

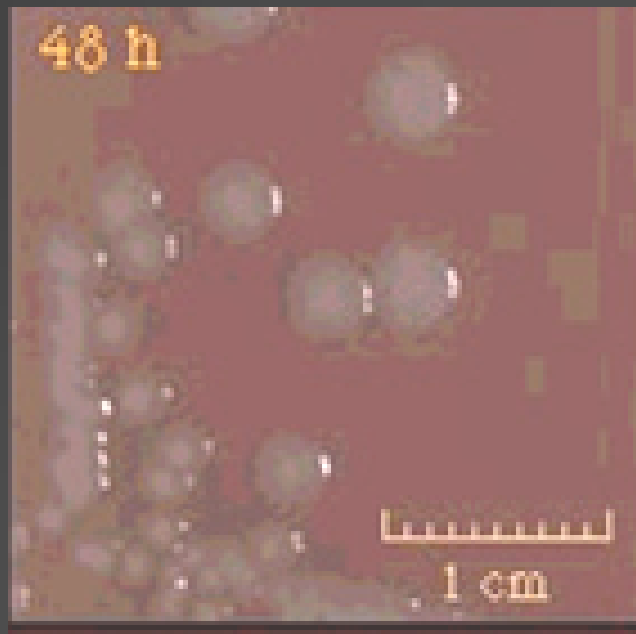


Neisseriae and Moraxellae – characteristics II

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

Gonococcus →

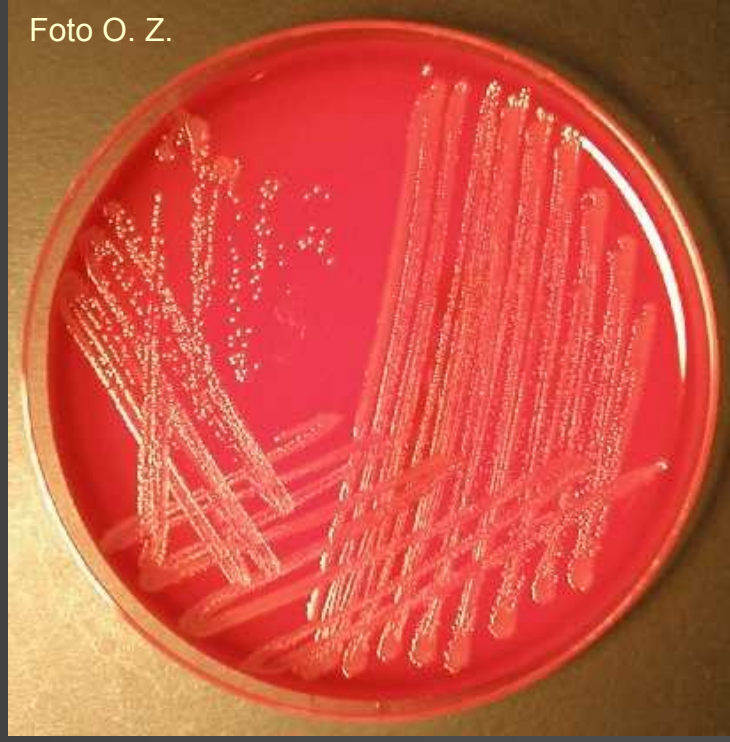
← Meningococcus



Culture of *Neisseria* and *Moraxella*

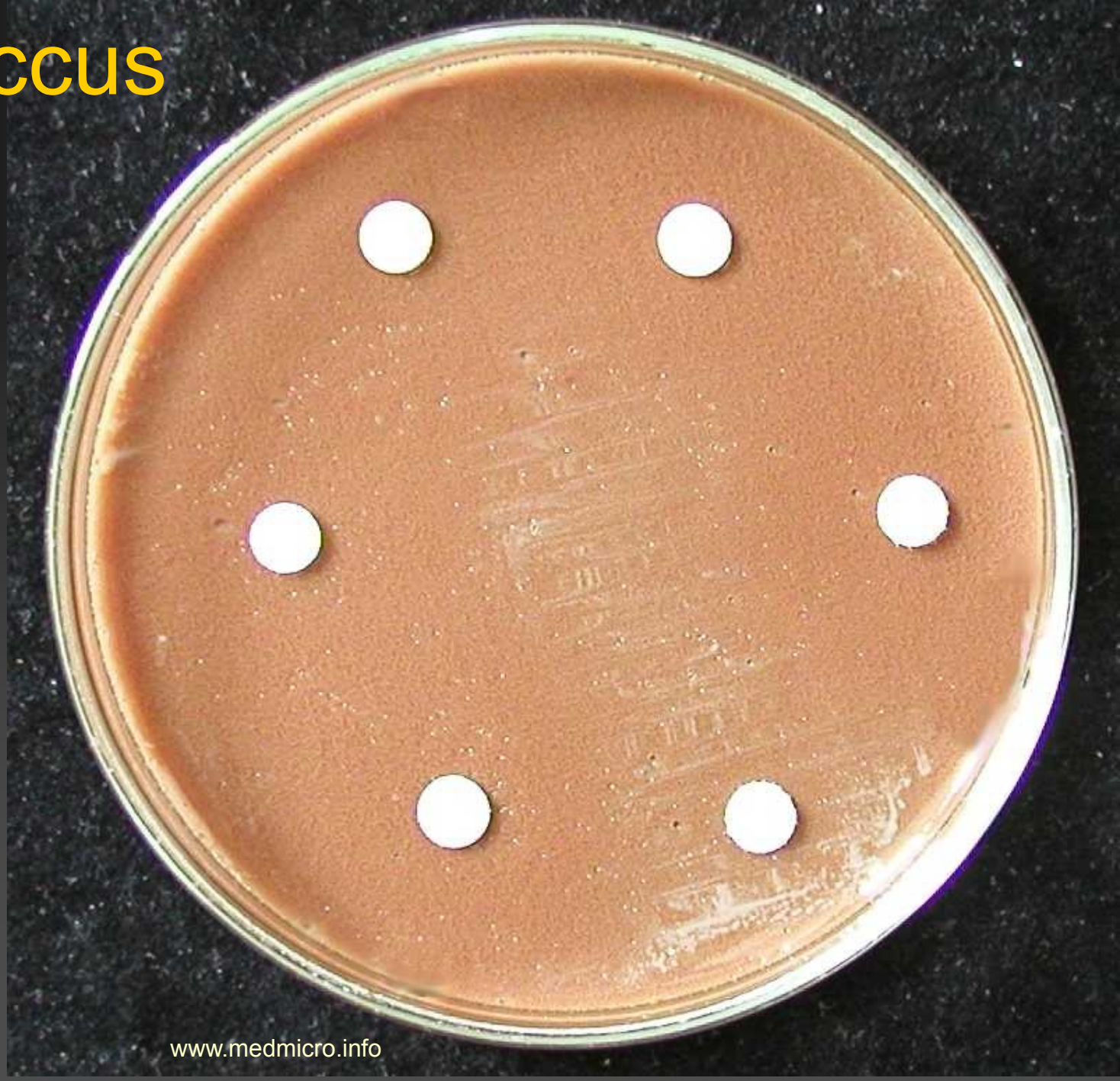
There exist differences between G– cocci in culture properties:

- **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

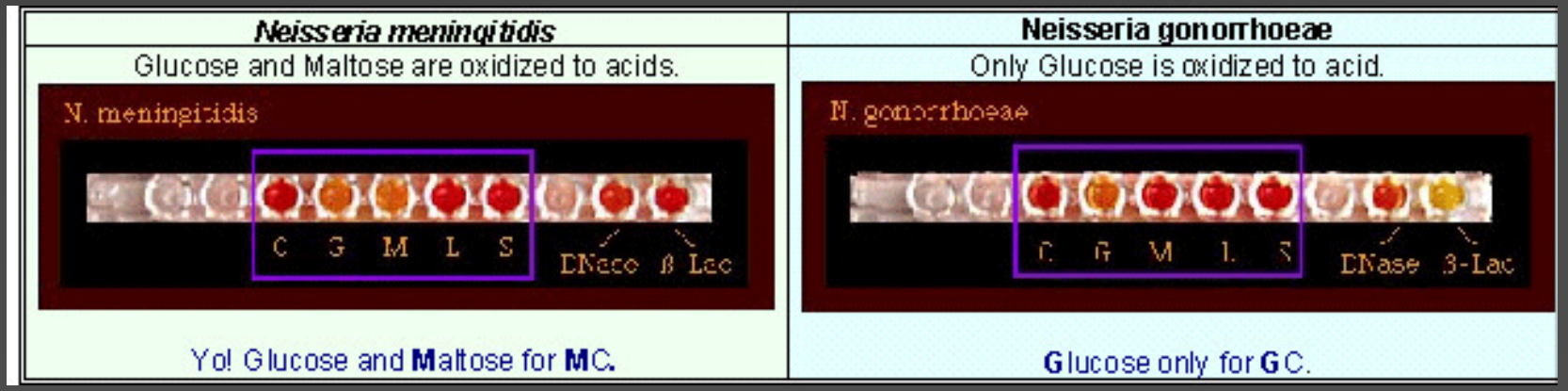
- **Biochemical diagnostic:** all of them are catalase positive, oxidase positive; *Moraxella catarrhalis* also positive in a specific test, called INAC (indoxyl-acetate test)
- **Antigen analysis:** performed usually by means of by latex agglutination, very important in meningococci for differentiation 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 simillar 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.



Neisseriae and Moraxellae – differential diagnostics 1

- **Gram staining:** G – (diplo)cocci
- **Oxidase** differenciates some other G– cocci (e. g. *Acinetobacter* – a G– non-fermenter – is also often coccoid)
- **Growth on various media** differenciates
  - **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 differenciation of oral neisseriae
- **Antigen analysis** (determination of meningococcal serogroup in invasive infections)

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 recomended 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)
- **phagyngeal swab** in Amies (no smear)

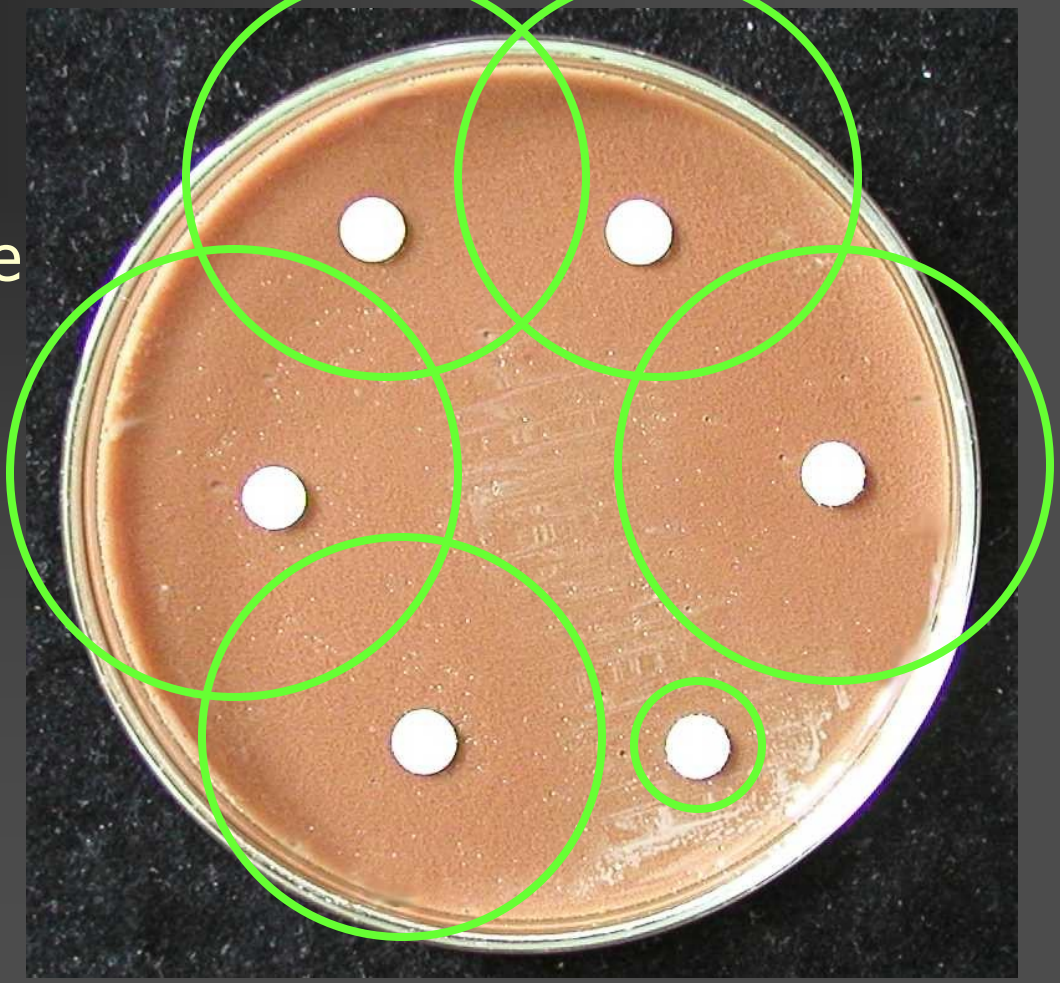
Antibiotic susceptibility testing in neisseriae and related bacteria

- 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 cefriaxone.

In neisseriae, zones are often large and confluent.

When the zones are so large that is 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!



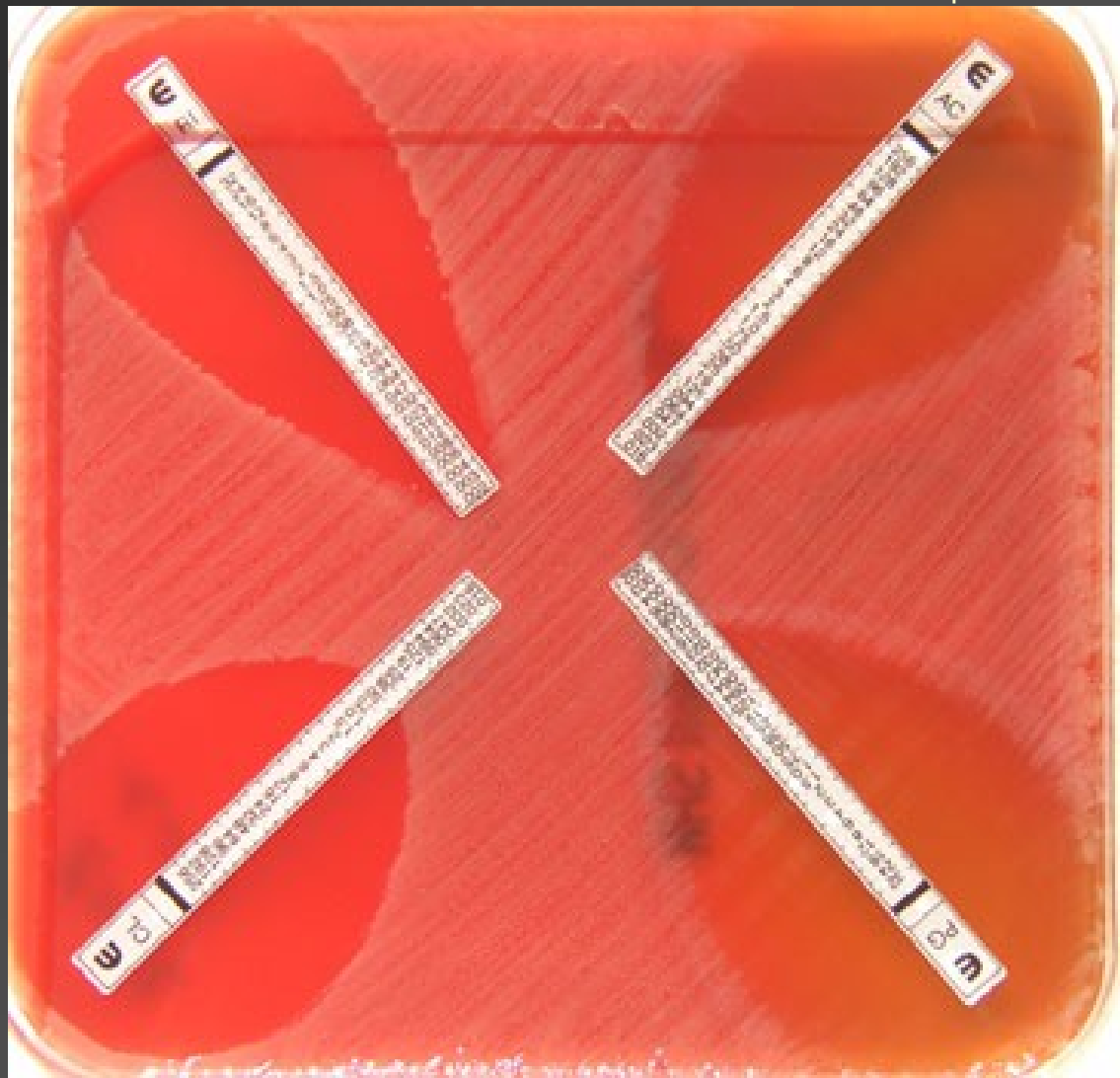
www.medmicro.info, photo O. Z.

A set of antibiotics againts pathogenic neisseriae

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

Somewhere, E-test is used

www.actu-pharo.com



Antigen detection / antigen analysis

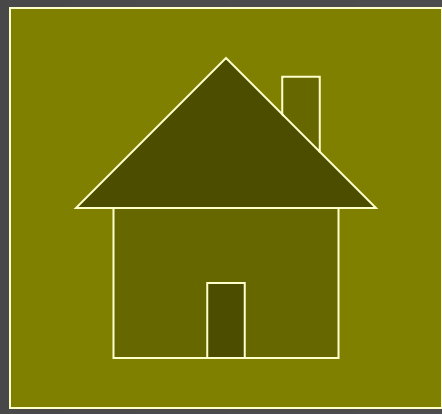
- Agglutination set for CSF agglutination is used for identification of pathogens. In case of a may be identified.
- In *Meningococcus* also the serogroups may be assessed. Therefore we can use it also for antigen analysis of an already cultured strain.

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**

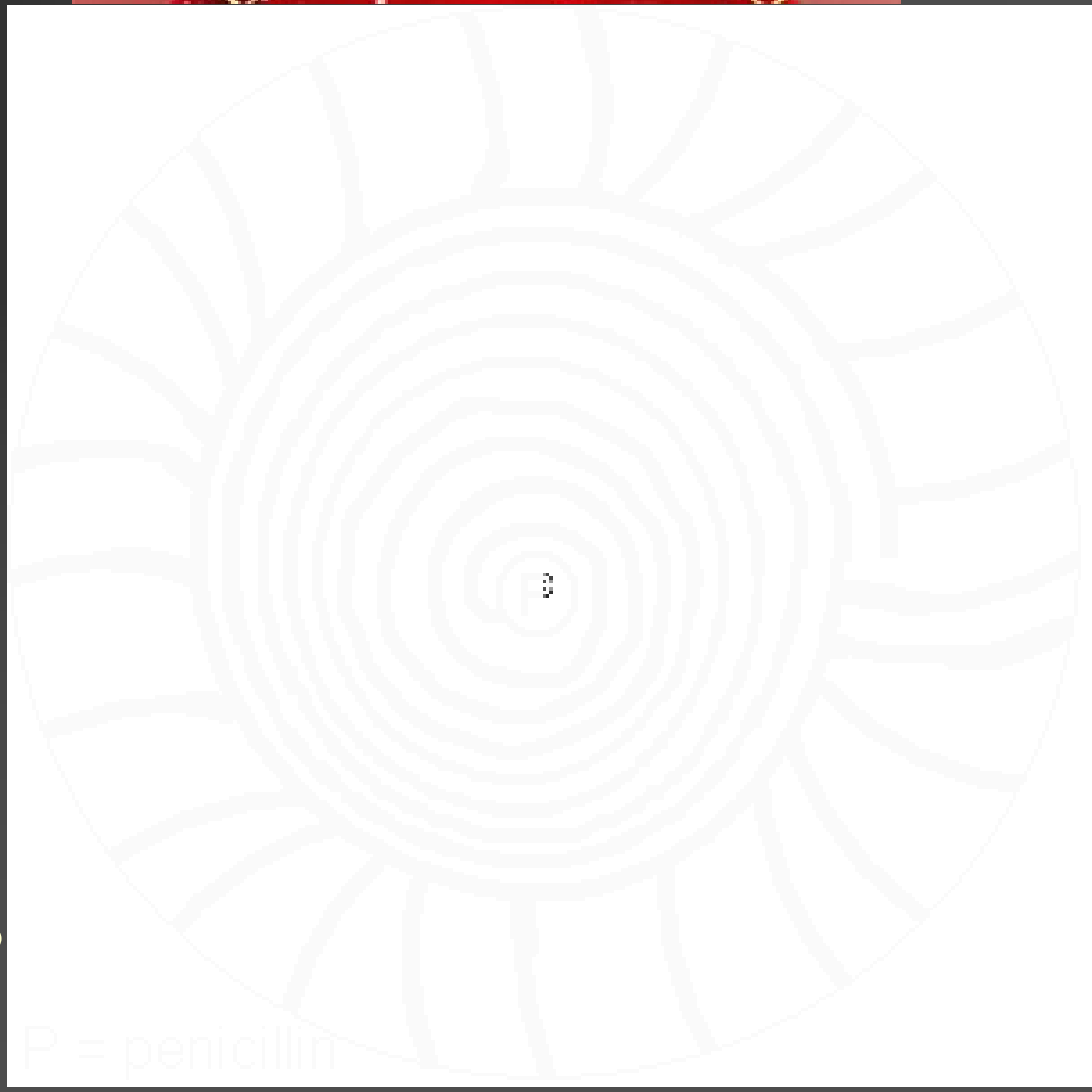
Diagnostics of „other G– bacilli“

„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 usefull
- **Indirect methods** used, mostly for tularemia
- **Differential diagnostics** is not algoritmic here. Usually specimens are sent to the laboratory with suspicion for legionelosis, whooping cough, Bang disease etc.

Bordetella: Inoculation on BG agar

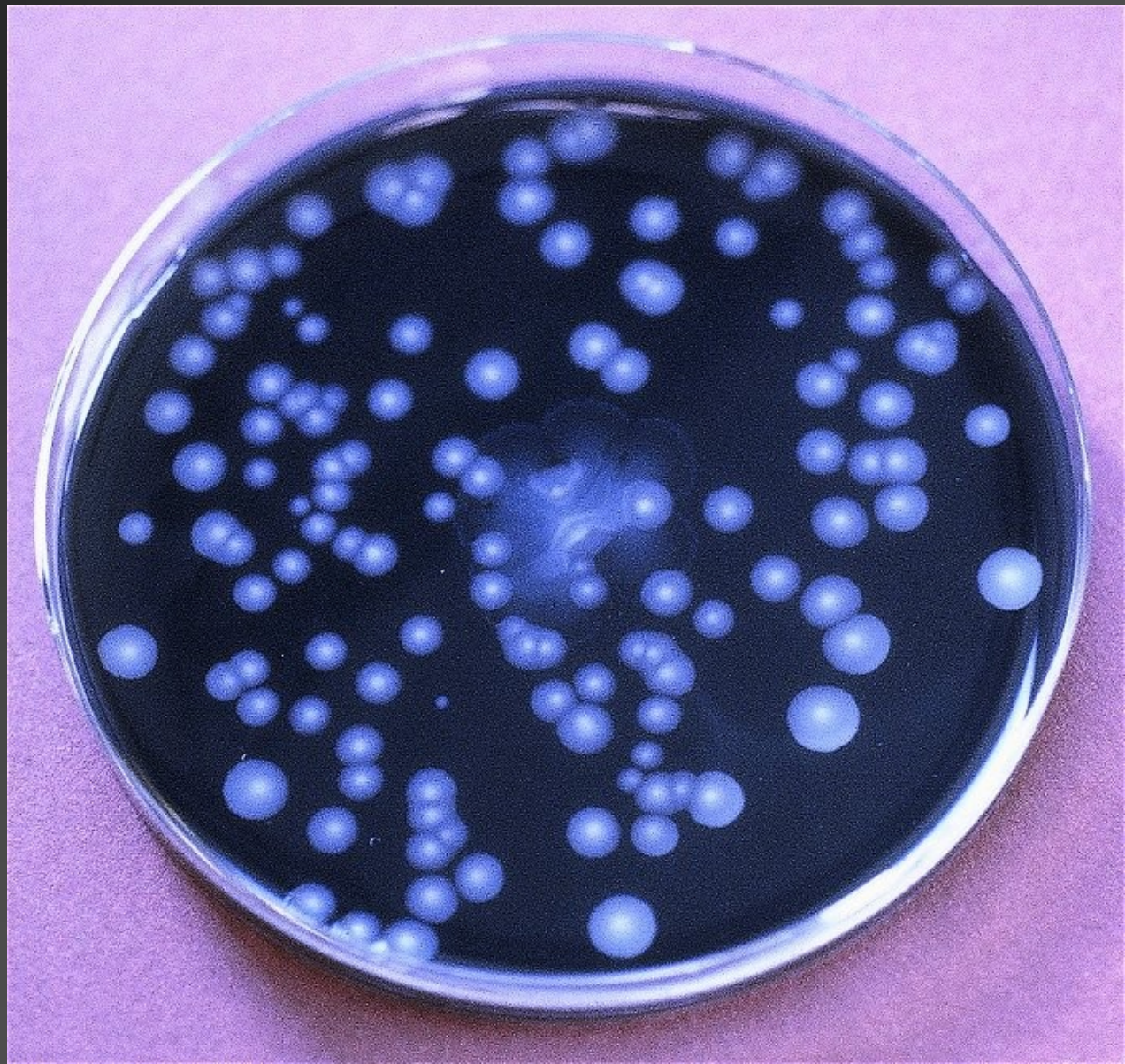
This strange way of inoculation is used, as the experience showed, that it increases successfull diagnostics.



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

BCYE medium for Legionella

Buffered
Charcoal
Yeast
Extract

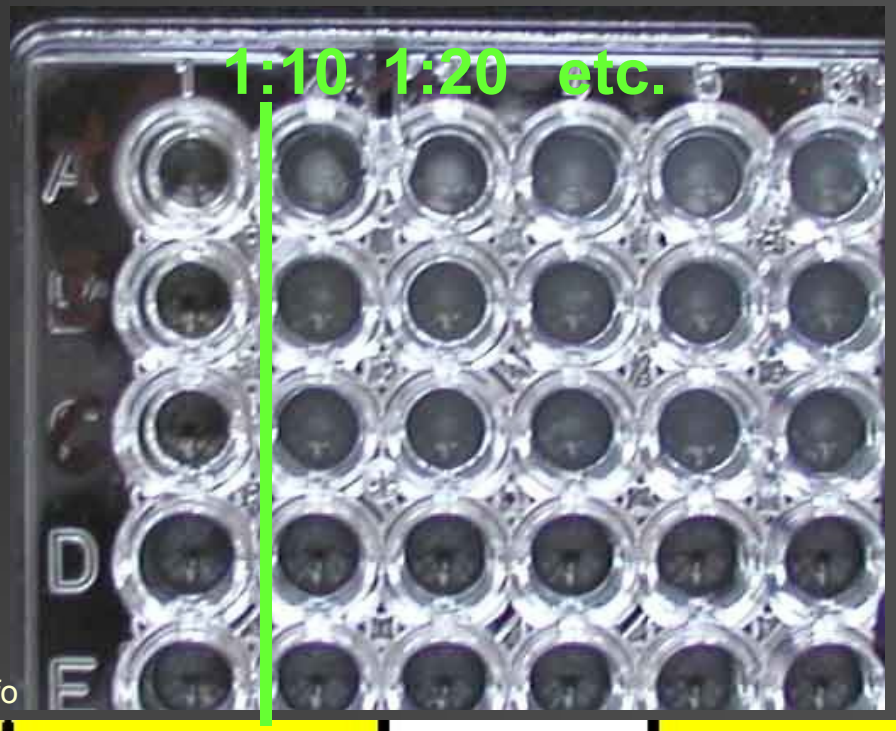


http://medecinepharmacie.univ-fcomte.fr

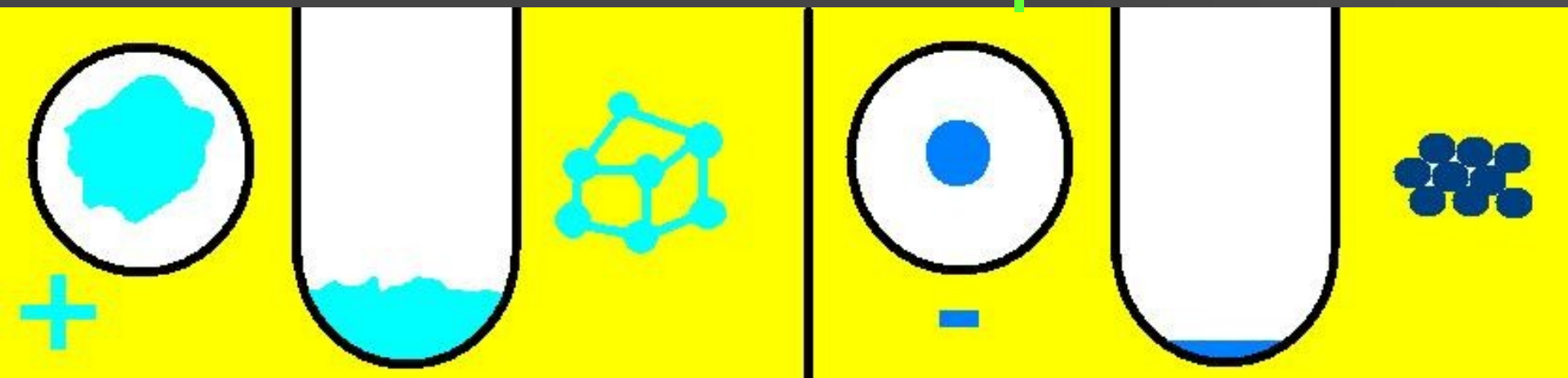
Francisella diagnostics: Reading the agglutination set

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

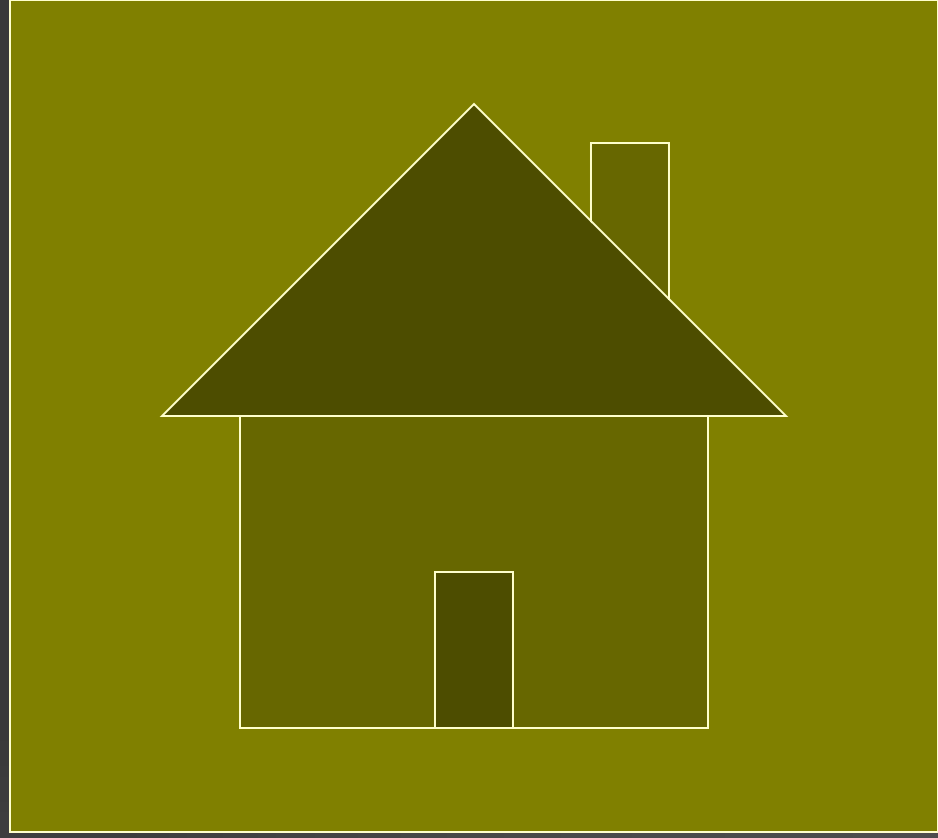
Any titer (i. e. everything except negative results) is interpreted as suspicious!



www.medmicro.info

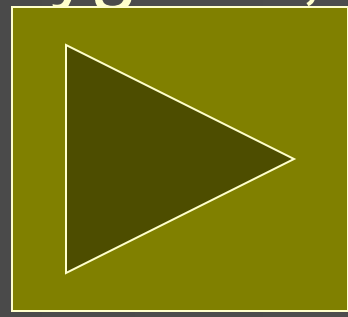


The End



How to build the buildings for health care to prevent legionellosis

- to ensure **properties of the building** for the healthcare (enouth space for personel, 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 legionelisus.
- In many cases an outrbreak 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