

Institute for microbiology shows



TRACING THE CRIMINAL

Part six: More gram-negative
cocci and bacilli

Survey of topics

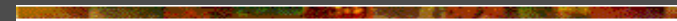
Clinical characteristics – G– cocci

Clinical characteristics – „other G– bacilli

Diagnostics of G– cocci

Diagnostics of „other G– bacilli“

Pictures of G– cocci and „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 penis...

Certainly, you know, what is the criminal



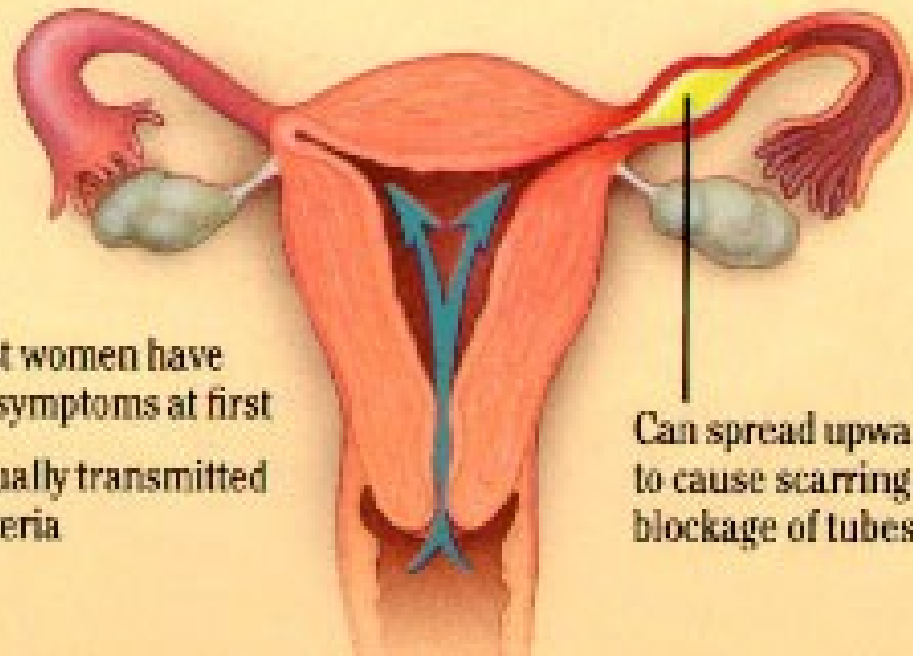
- Of course, it is *Neisseria gonorrhoeae*, (gonococcus) causing gonorrhoea.
- Gonorrhoea is urethritis, in females also cervicitis; asymptotically or symptomatically gonococci are found in pharynx and rectum, too.
- In females, it is not a colpitis (vaginal inflammation), so it is not recommended to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

Partner of Johnny of that night...

nova.medicina.cz

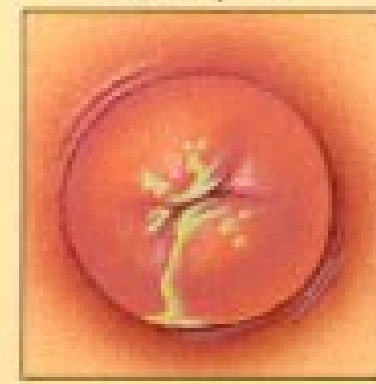


www.lcusd.net

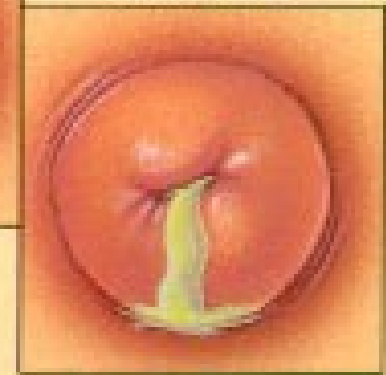


Can spread upwards to cause scarring or blockage of tubes

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**.
-

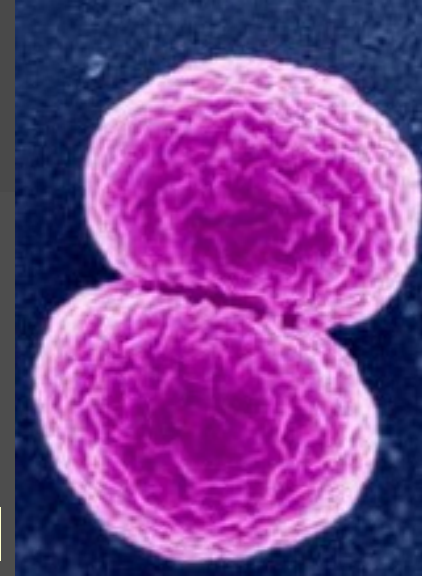
(continuing)



- 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...**

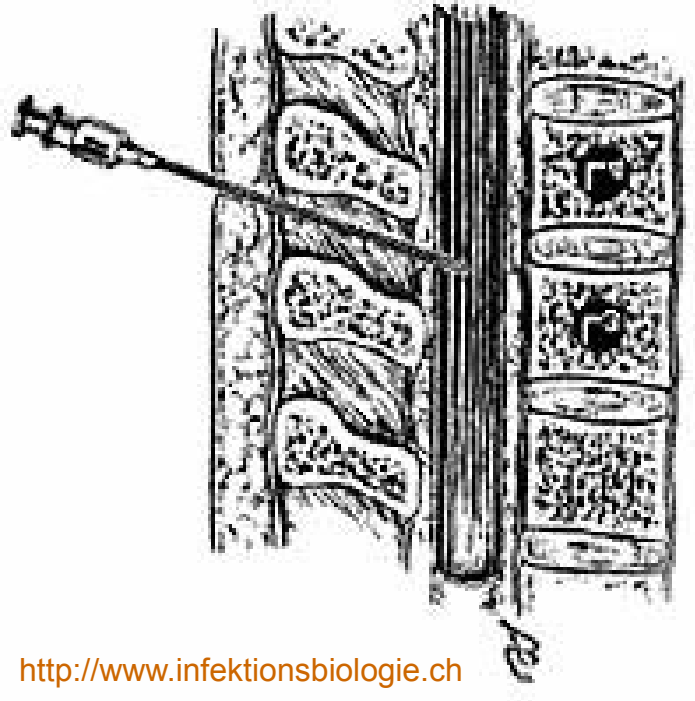
And the criminal is...

- ... ***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

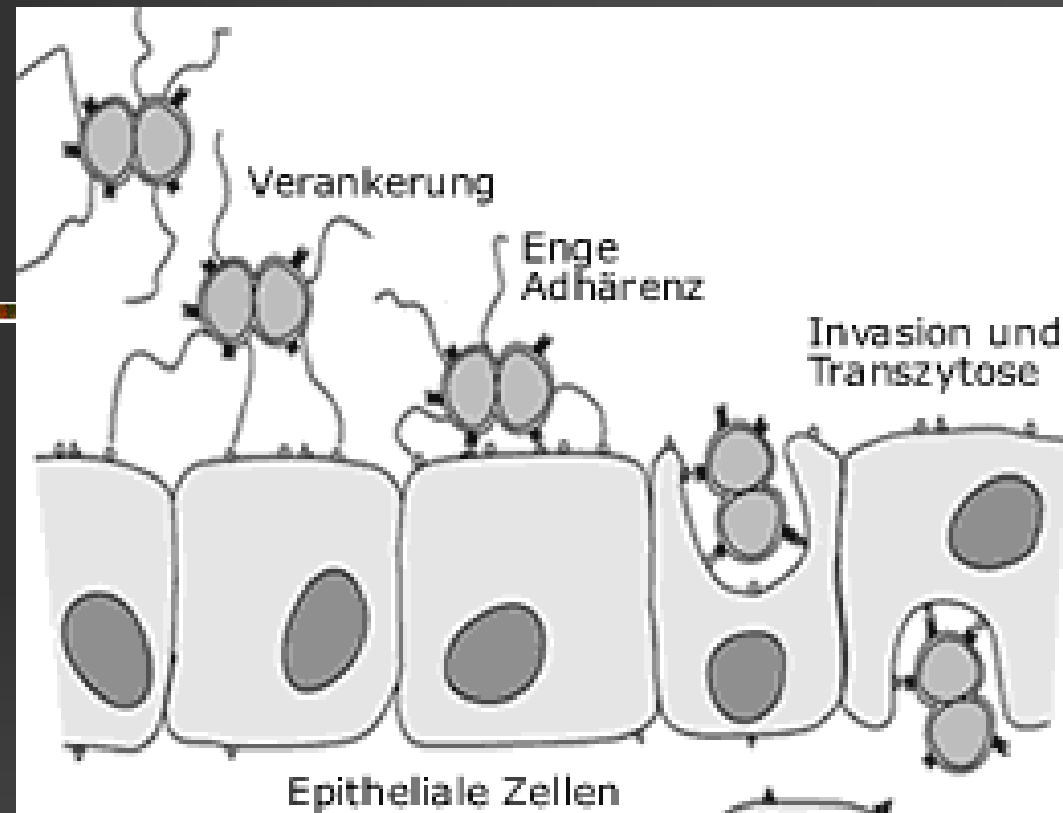


Why the infection comes sometimes, and sometimes does not

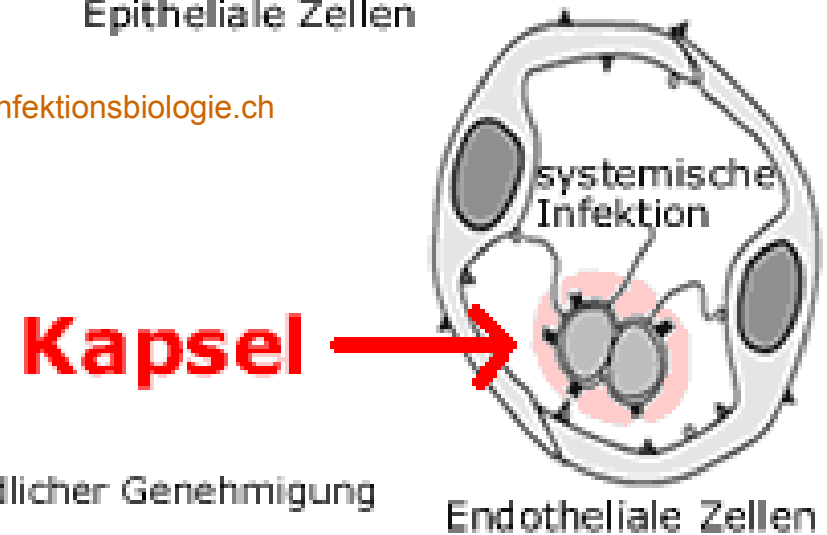
- The invasive infection is only present, when the strain is highly virulent (specific clones of the microorganism) and the host organism is ready to get infected
- Meningococcus is transmitted by a **narrow contact**. Invasive **infection is more likely when mucous membranes are damaged**, e. g. by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



<http://www.infektionsbiologie.ch>



<http://www.infektionsbiologie.ch>



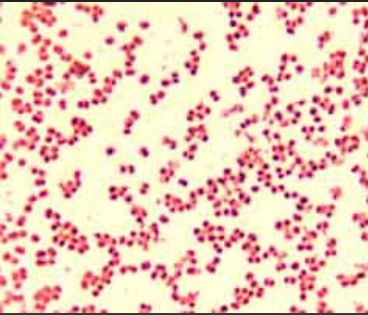
Mit freundlicher Genehmigung
© Dehio

Endotheliale Zellen

CSF sampling

How they come into the tissue

Meningococcal meningitis is worldwide very important infection



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



Let's compare neisseriae:



	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 practitioner'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 imediately. Later, a **susceptible pathogen** was found.

And this pathogen was



- ***Moraxella* (sooner *Branhamella*) *catarrhalis***
 - This organism is often present in small amounts even in healthy persons.
 - In pure culture causes **sinusitis, otitis media, pharyngitis** etc.
 - Her original 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
-

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

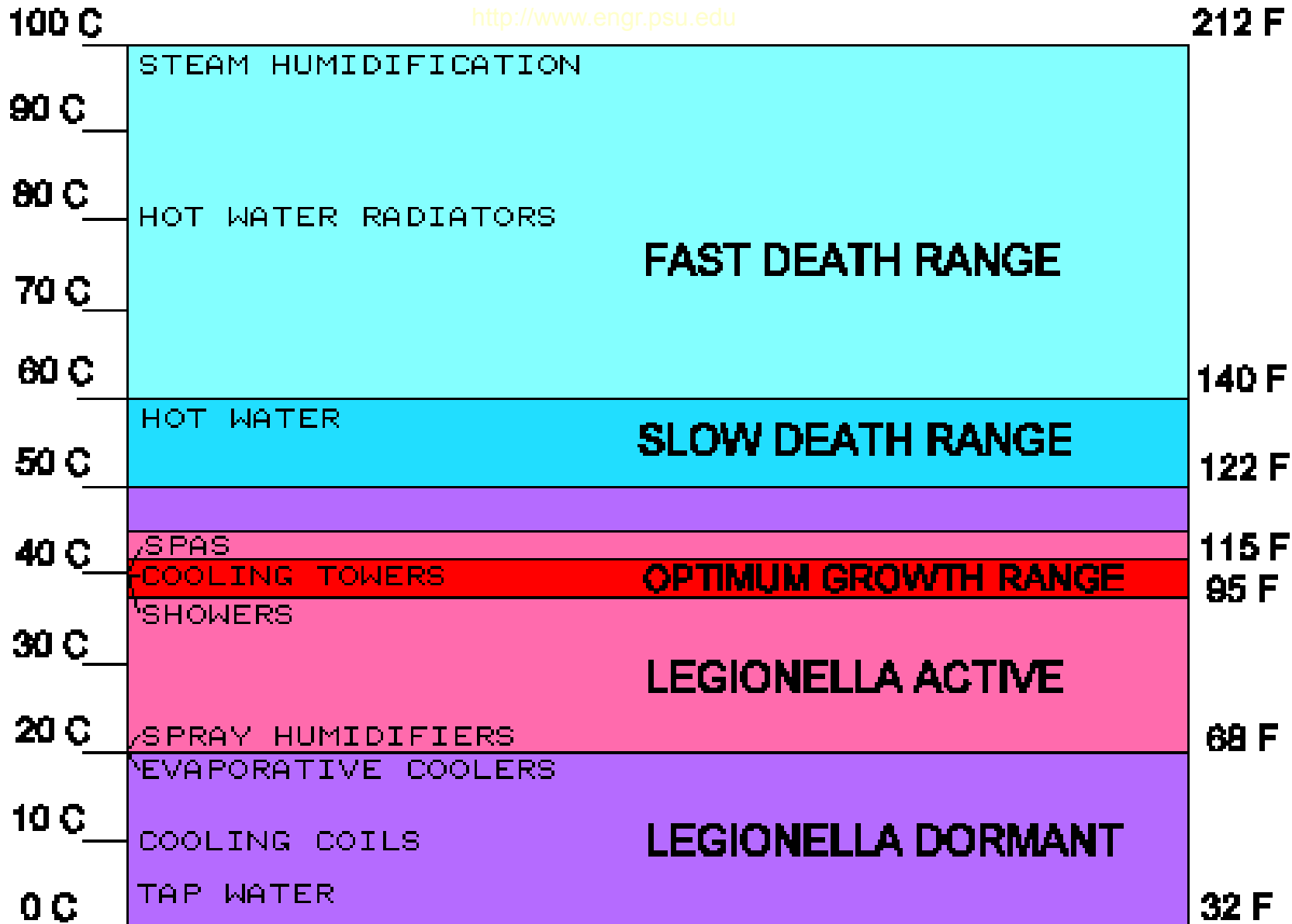
- It is a disease caused by *Legionella pneumophila*
- Legionaire's disease is a variant of disease that is quite severe; another variant, **Pontiac fever**, is rather mild
- The bacterium of other have reservoir in **water instalation, air condition**, etc.
- When building new hospital departments (but also senior houses, hotels, spa...) measures to prevent legionellosis should be taken, mostly at planing water pipe system (no blind branches).

Legionnaire's disease



Legionella and temperature

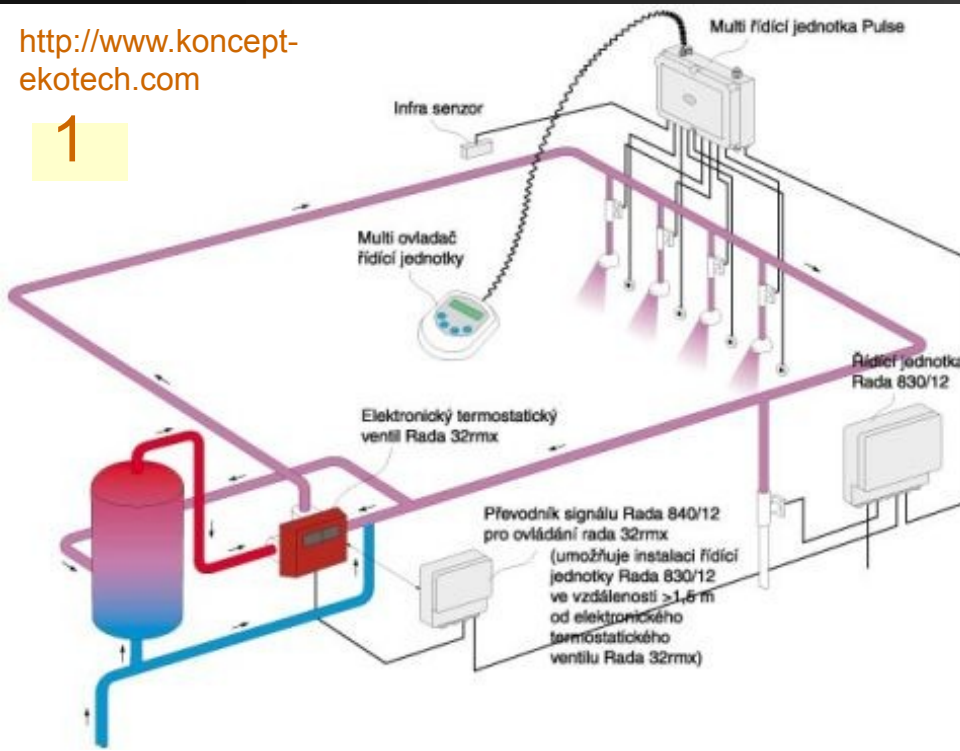
<http://www.engr.psu.edu>



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

The Legionella Control Association
 A Recommended Code of Conduct for Service Providers
Certificate of Registration

This is to certify that the following company has submitted a registration under the Conditions of Compliance as laid out in the Code of Conduct for Service Providers

Name of Company : AWT (Water Treatment) Ltd

Registration Number : 2000409 Certificate valid until: 31 August 2007

Registration under the following categories:

- Risk Assessment
- Cleaning and Disinfection
- Chemical water treatment services
- H&C water monitoring and inspection services
- Analytical services

This certificate is issued annually and is subject to verification via the website. Anyone wishing to confirm the current registration of any company is advised to contact the Secretariat quoting the company name and the registration number on the certificate.

Signed: *[Signature]* Chairman, Executive Committee
 Certificate Secretary

Legionella Control Association, 6, Sir Robert Peel Mill Building, Tolson's Enterprise Park, Farsley, Staffs, B71 3GD
 Telephone: 01827 338 053 www.conduct.org.uk

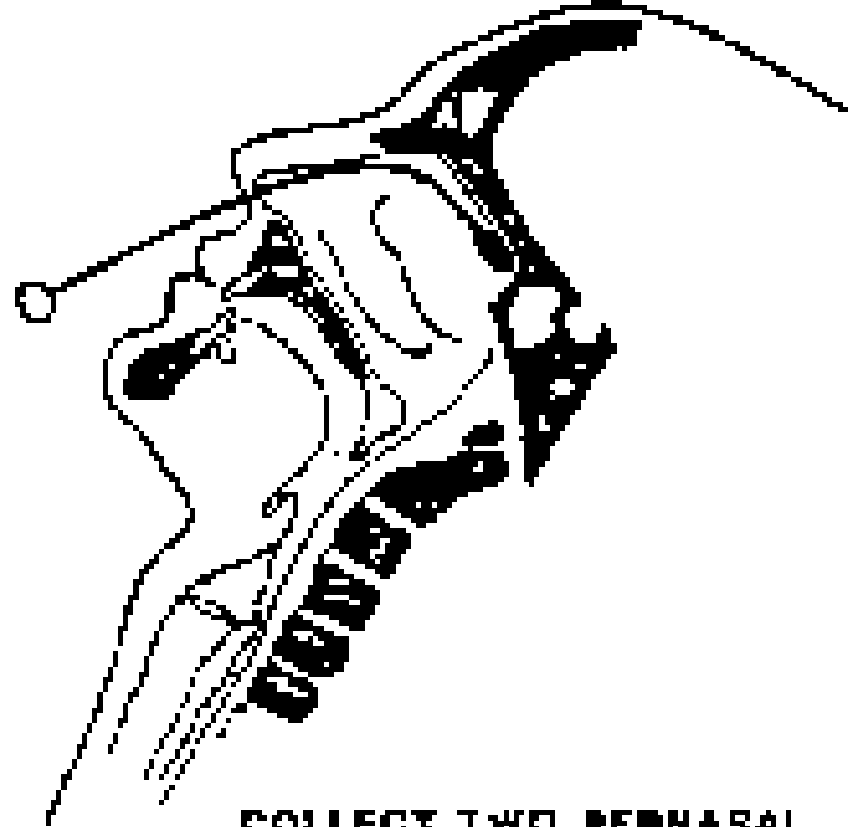


„Other Gram-negative bacteria“

- 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***
-

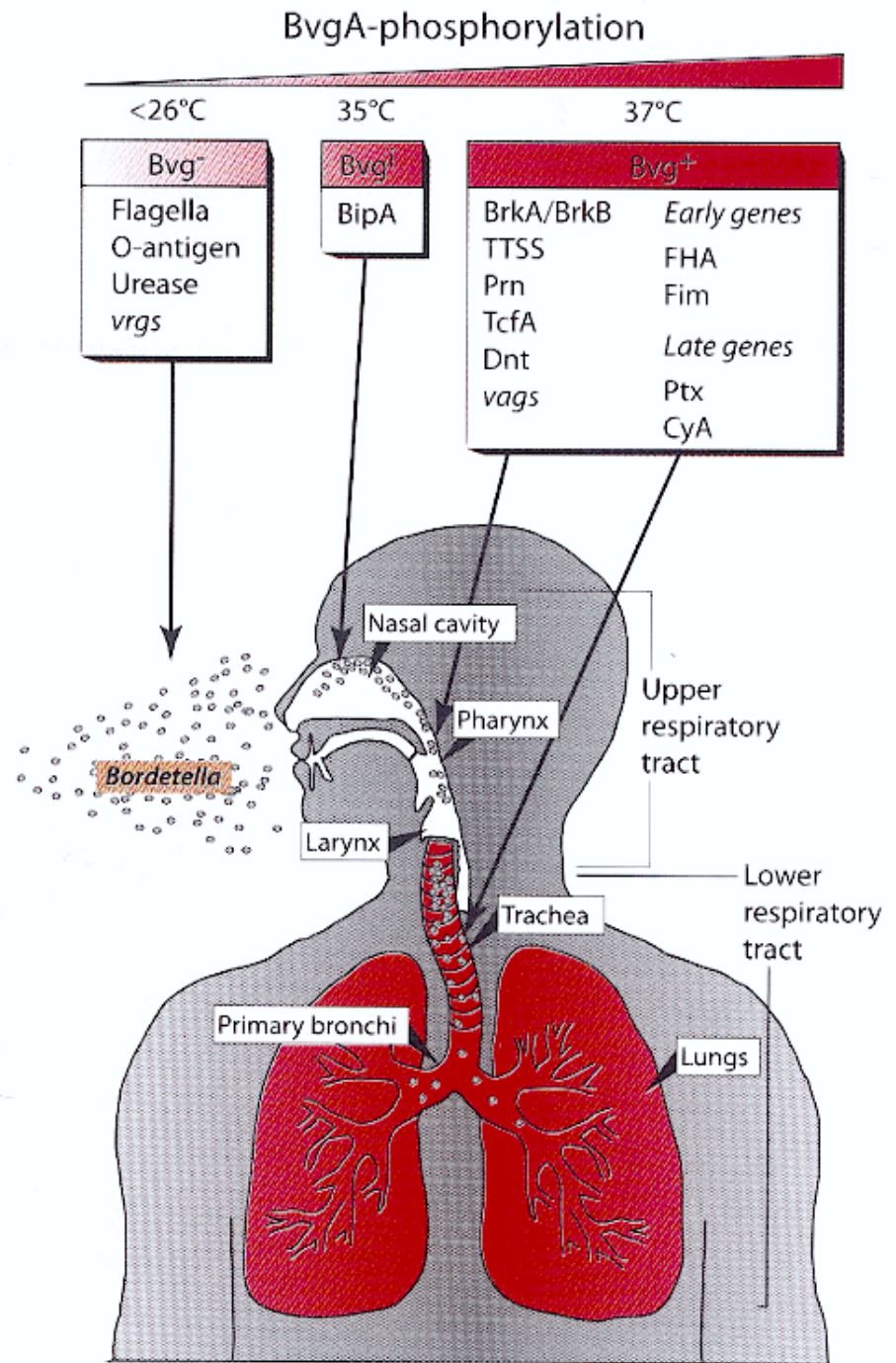
Genus *Bordetella*

- *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



COLLECT TWO PERNASAL
NASOPHARYNGEAL SWABS

Bordetella and temperature

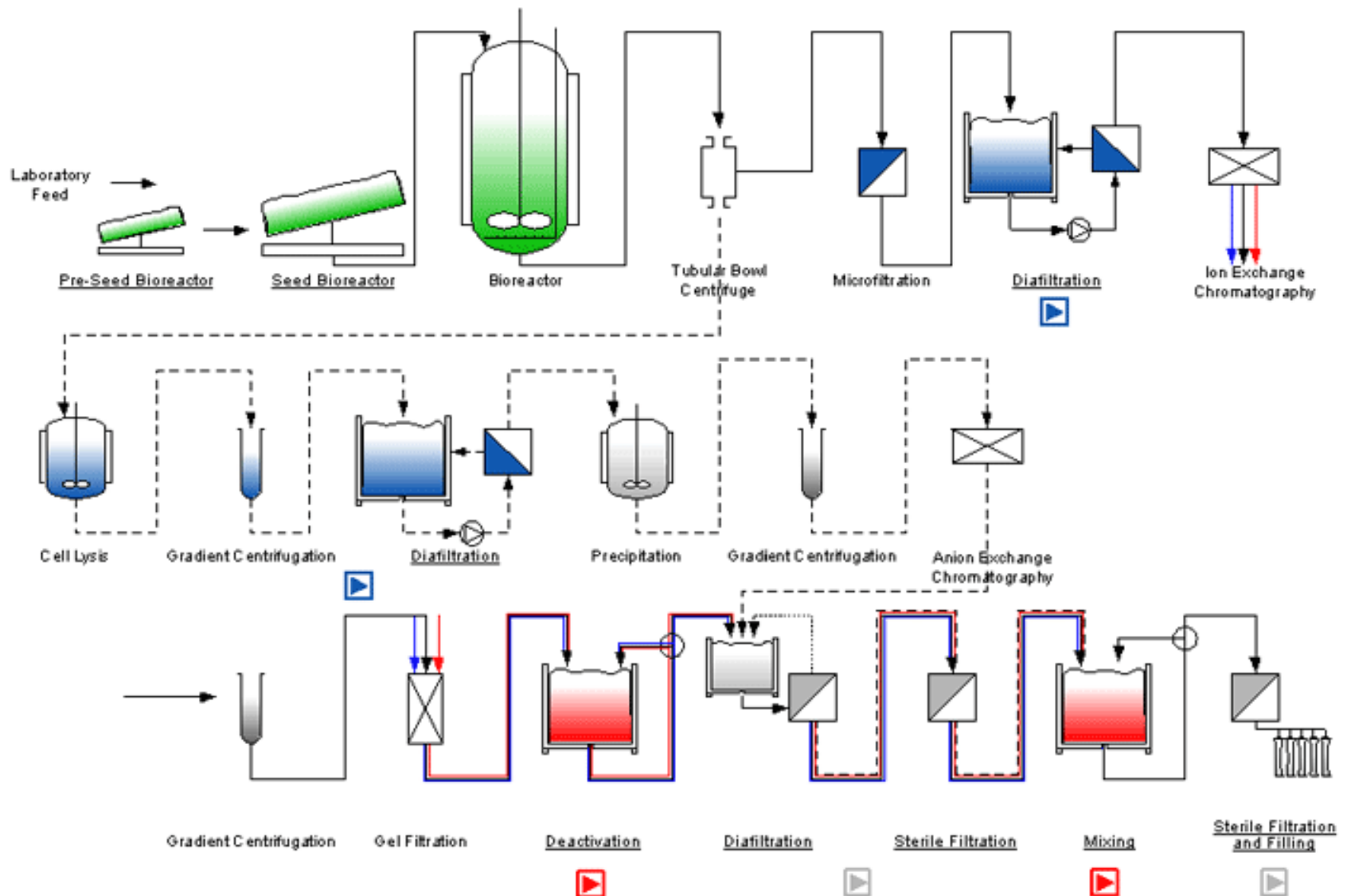


Bordetella
was
isolated in
1906 by
Jules
Bordet et
Octave
Gengou



Pertussis vaccine production

www.stedim.com



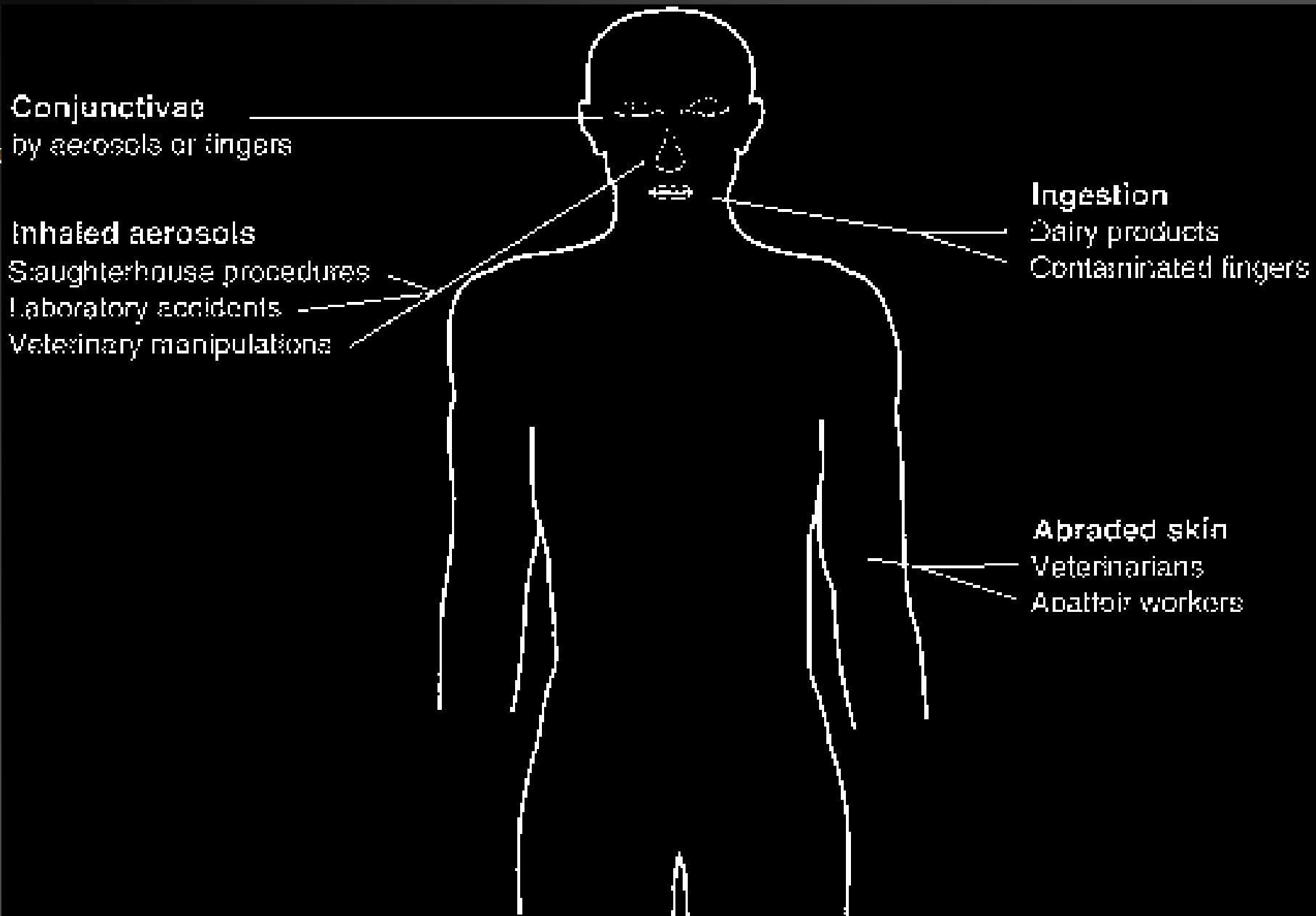
Bordetella bronchiseptica: cat infection



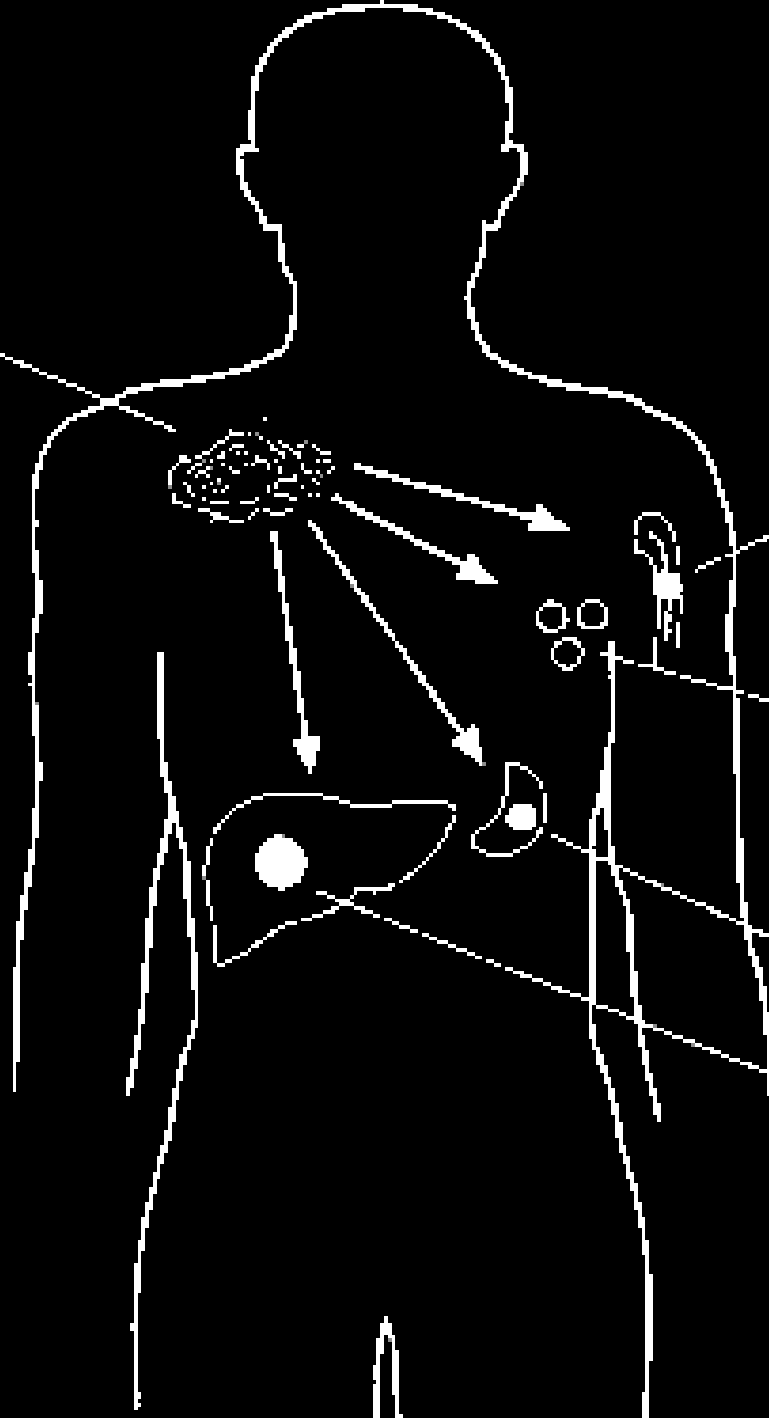
Genus *Brucella*



- 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 mellitensis*** from sheep and goats and ***Brucella canis*** from dogs
-



Brucella-infected
phagocytic cell



Bone marrow

Lymph nodes

Spleen

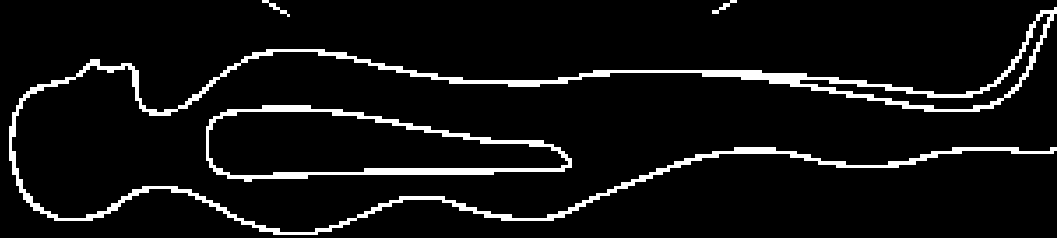
Liver

History and Physical Exam

Clinical

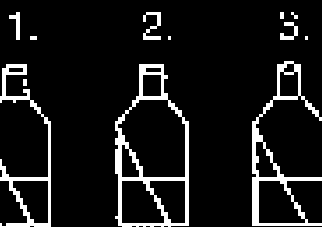
Occupation
Raw milk
Fresh cheese
Travel
Symptoms
Signs

Course of disease

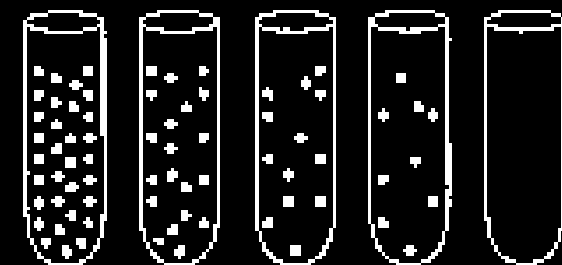


Laboratory

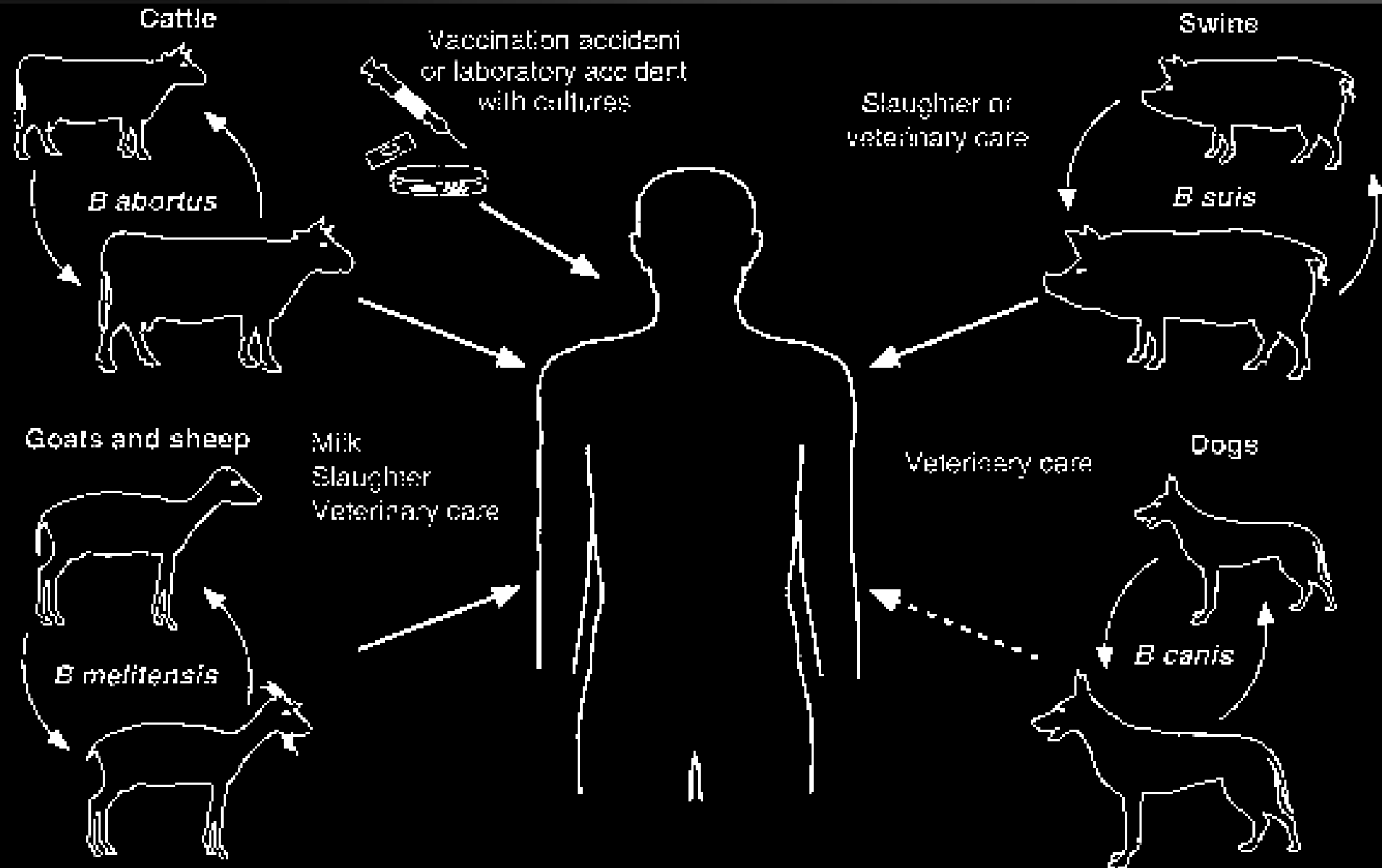
Blood cultures



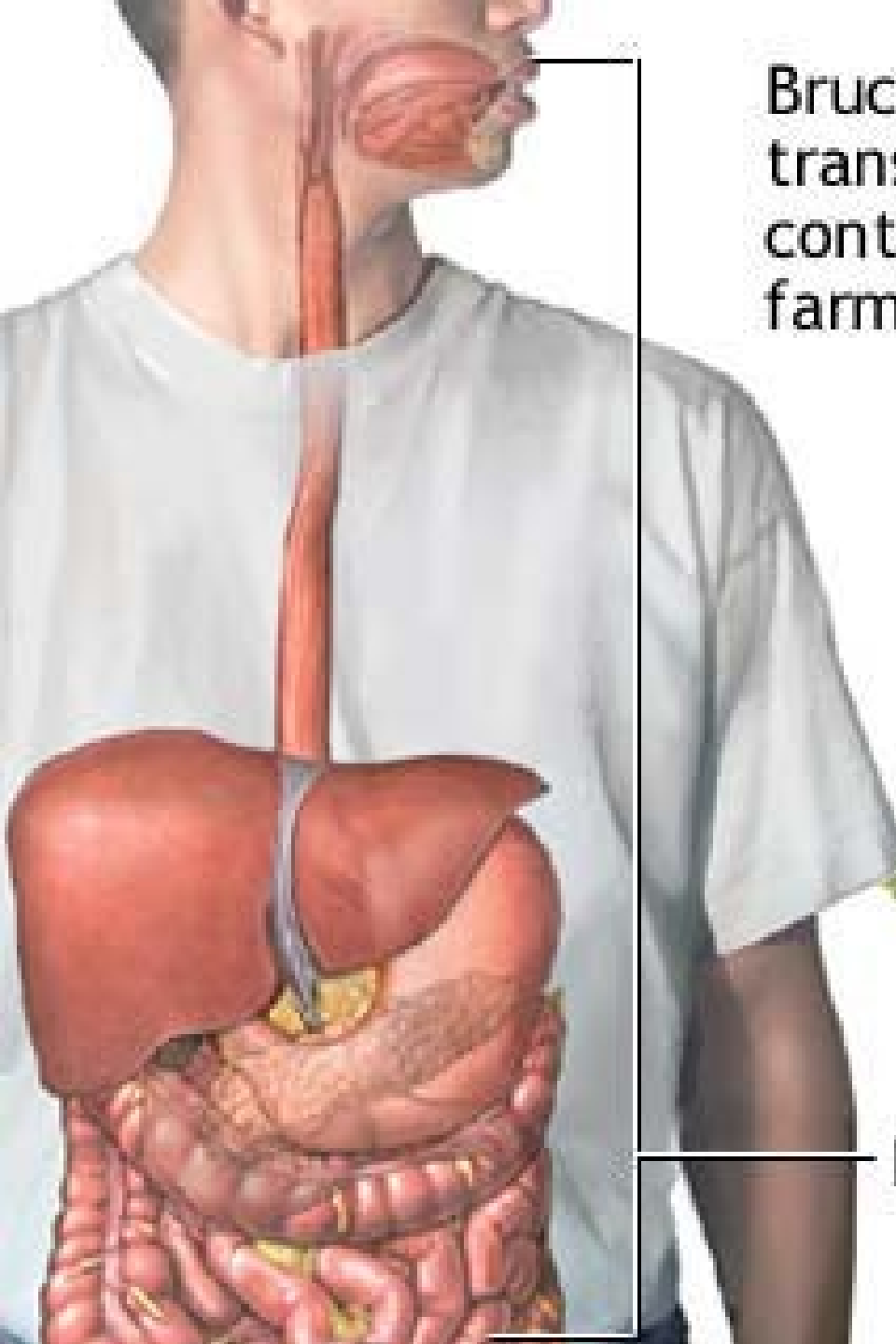
Quantitative serology



Remembering Brucella



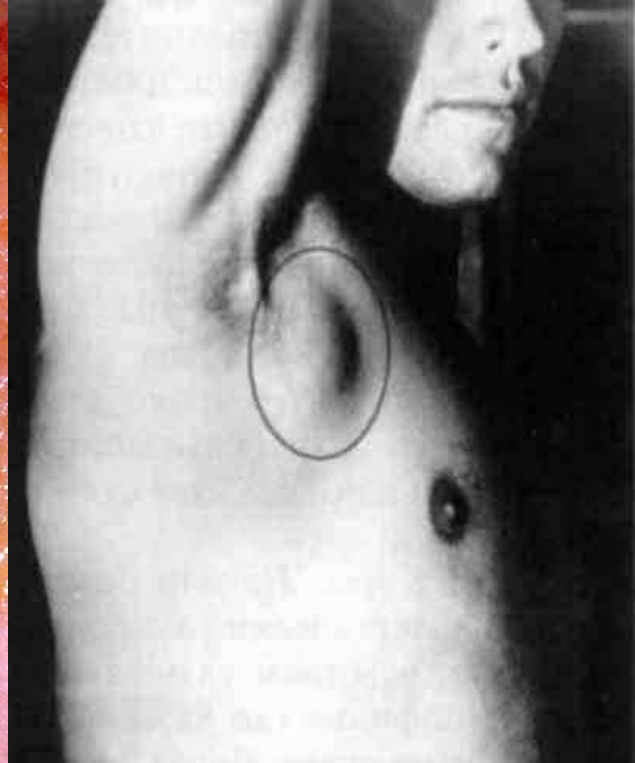
Brucella bacteria is usually transmitted to humans by contact with infected farm animals.



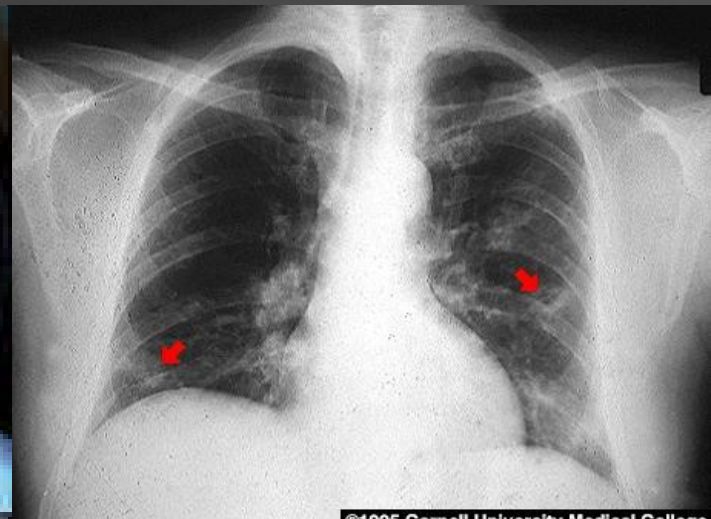
Digestive system

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**
-



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



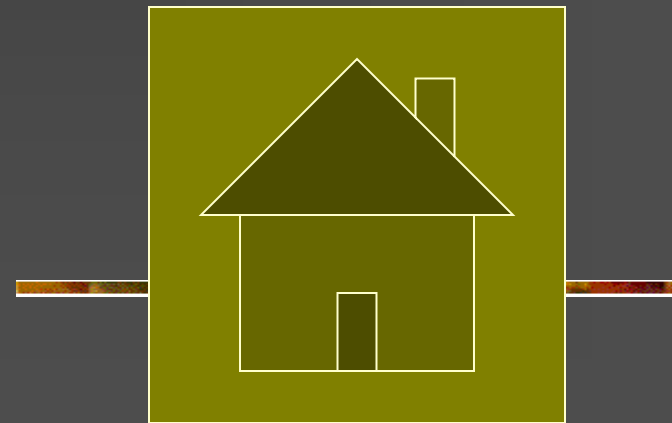
From report about epidemiological situation (IX/2008) – I (abbreviated)

- **Tularemia – A21:** Woman, born 1970 from Valtice, gardener. 20th June GP visit for T 38°C, sore throat, enlarged lymphonodes next to right clavícula. 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

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



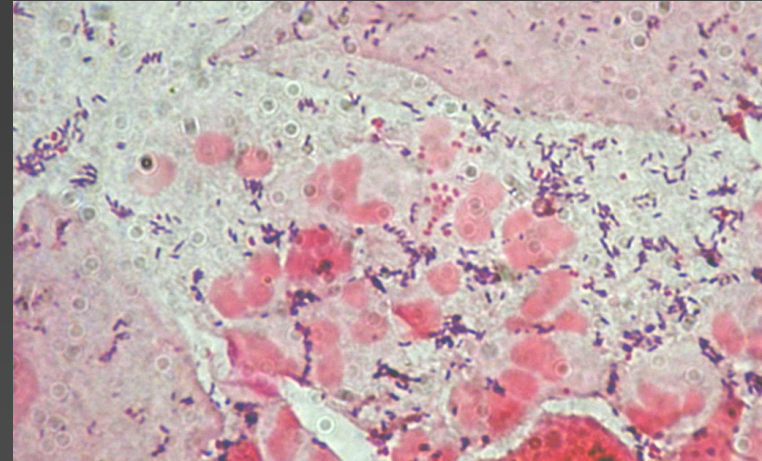
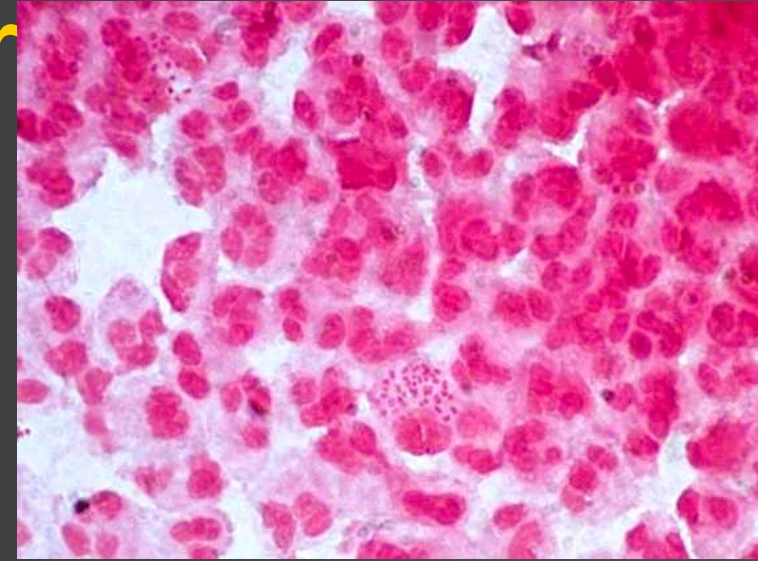
Diagnosics of G- cocci

Neisseriae and Moraxellae – characteristics

- **Microscopy:** G – diplococci, coffee bean shape, often intraleucocytar
 - **Culture:** tiny, colourless or yellowish (according to the species) colony, growing (species specific) on blood or chocolate agar
 - **Biochemical diagnostic:** catalase positive, oxidase positive, differentiation possible
 - **Antigen analysis:** very important in Meningococcus, serogroup B is not preventable by vaccination!
-

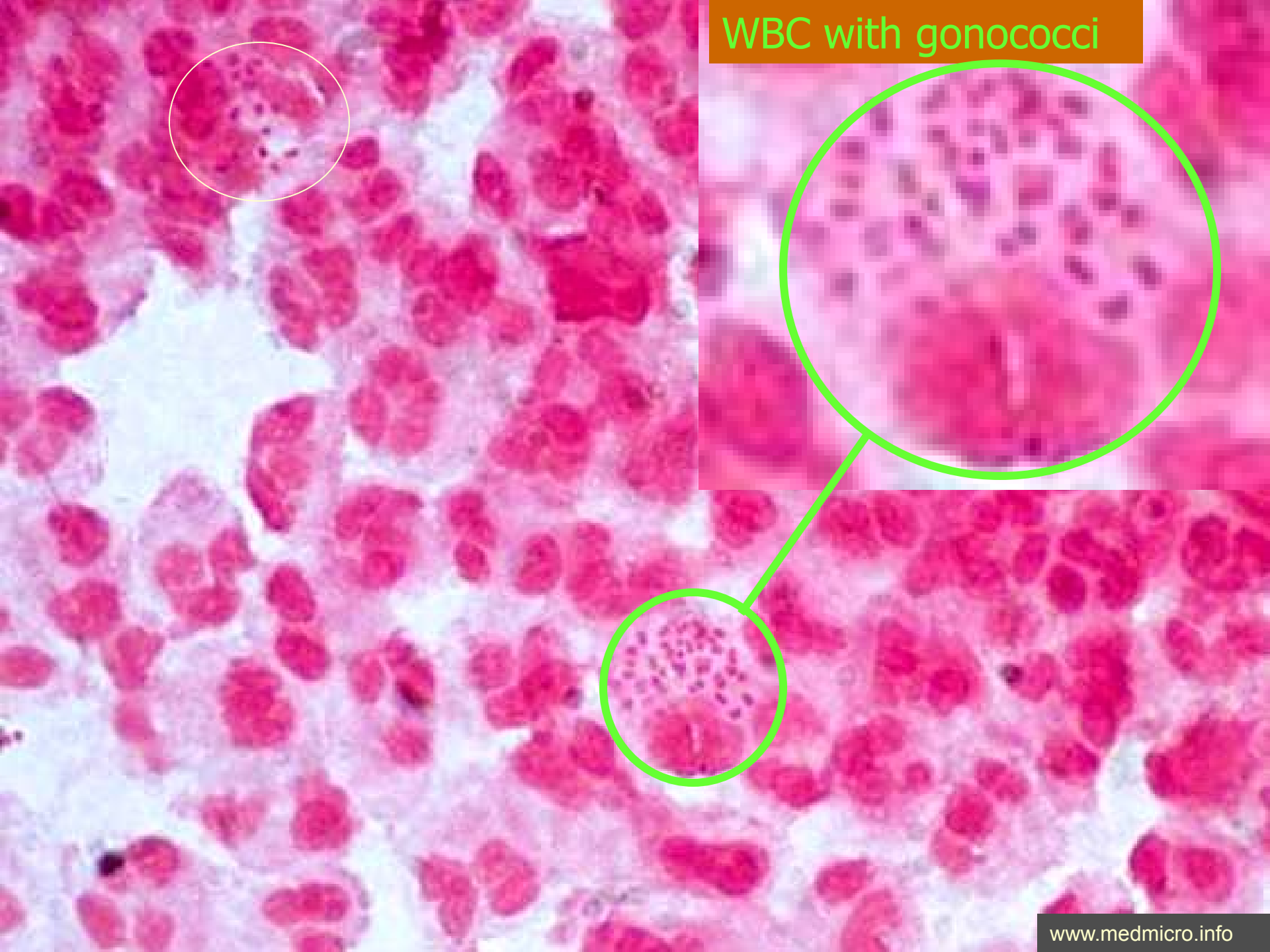
Observation of gonorr

- 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



Culture of *Neisseria* and *Moraxella*

Foto O. Z.



- There exist differences between G- cocci in culture properties. **Oral neisseriae and moraxellae** grow on blood agar (BA). **Meningococci** grow only on nutrient-rich variants of BA. **Gonococci** do not grow on BA at all, they require chocolat agar.

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. NEG.	GLU +	MAL +	FRU -	SAC -	ONPG -	TRI -	PS 24h
GGT +	NO ₃ -	NO ₂ (1)	NO ₂ + (2)	1395			

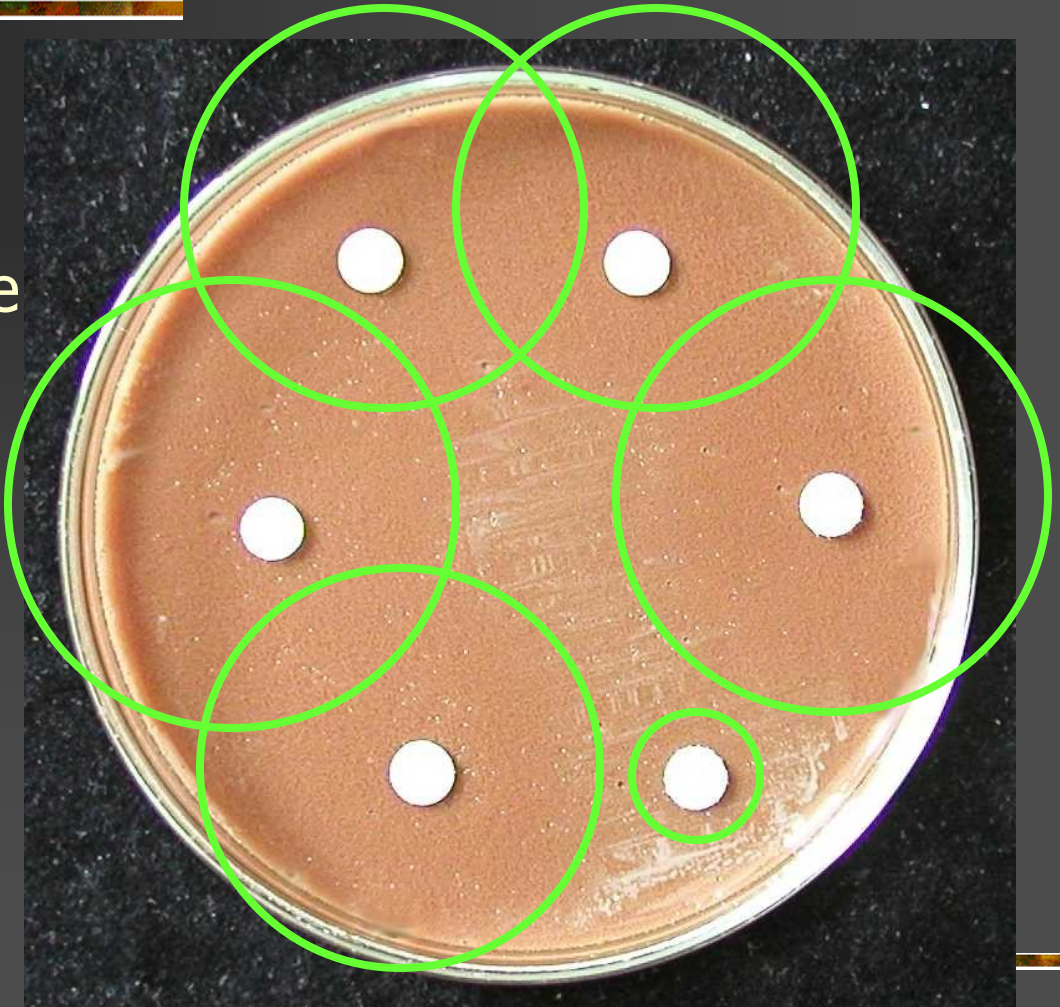
Antibiotic susceptibility testing in neisseriae and related bacteria

- 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!

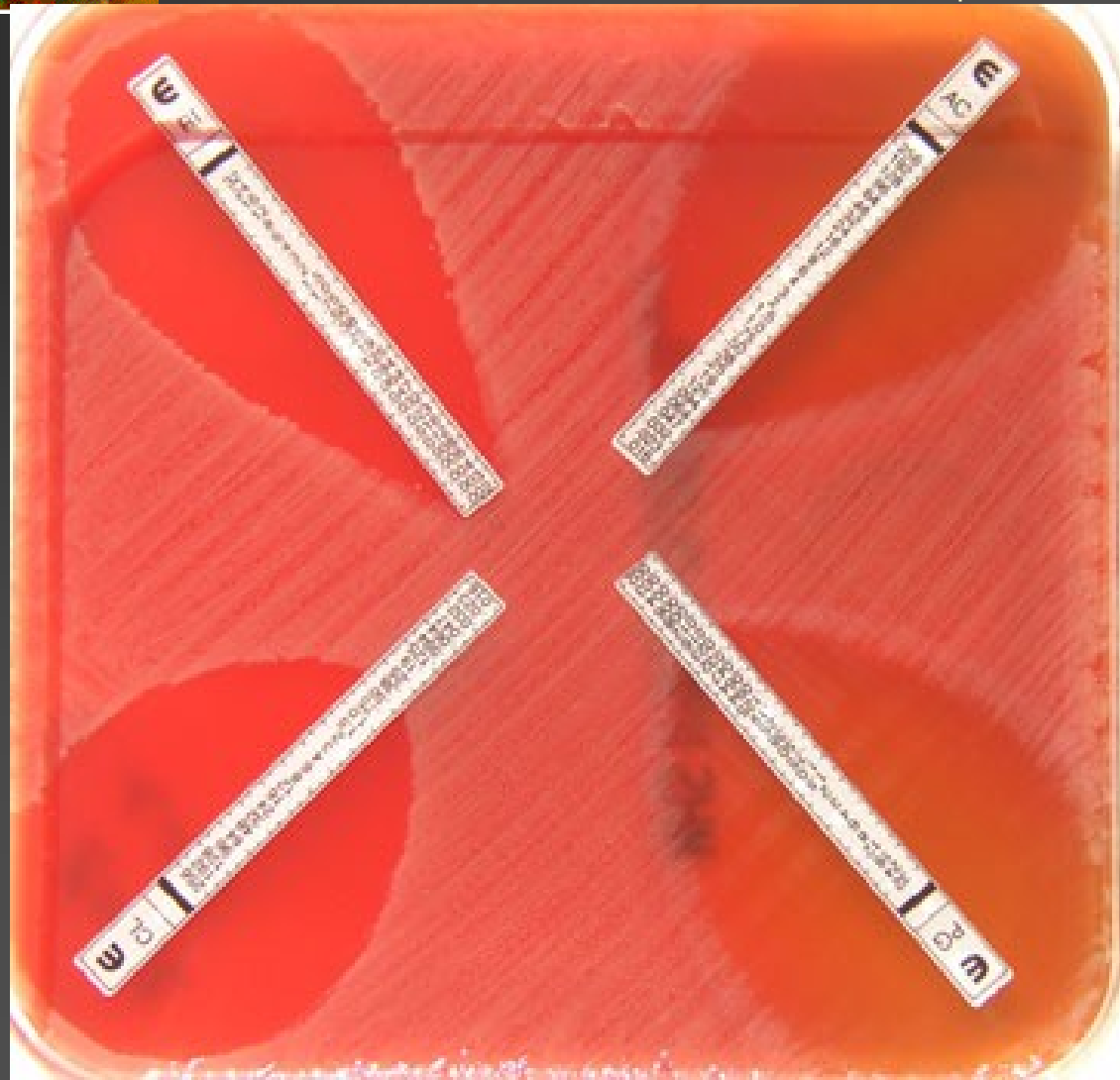


A set of antibiotics against pathogenic neisseriae

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



Antigen analysis

- Agglutination set for CSF is used for identification of pathogens. In case of a may be identified. In *Meningococcus* also the serogroups may be assessed.

www.baxter-e-commerce.com

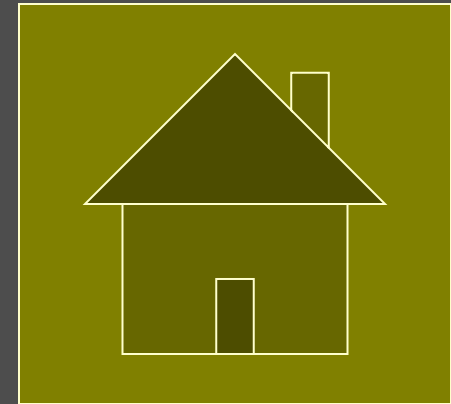


Photo O. Z.



Antigens detected at CSF antigen analysis

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



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 useful
 - **Indirect methods** used, mostly for tularemia
-

Diagnostics of *Bordetella*, *Brucella*, *Legionella* and *Francisella*

- To diagnostics of these bacteria, special media are used, or indirect diagnostics (antibody detection)
 - Each subtask describes one of the genera mentioned here
-

Inoculation on BG agar

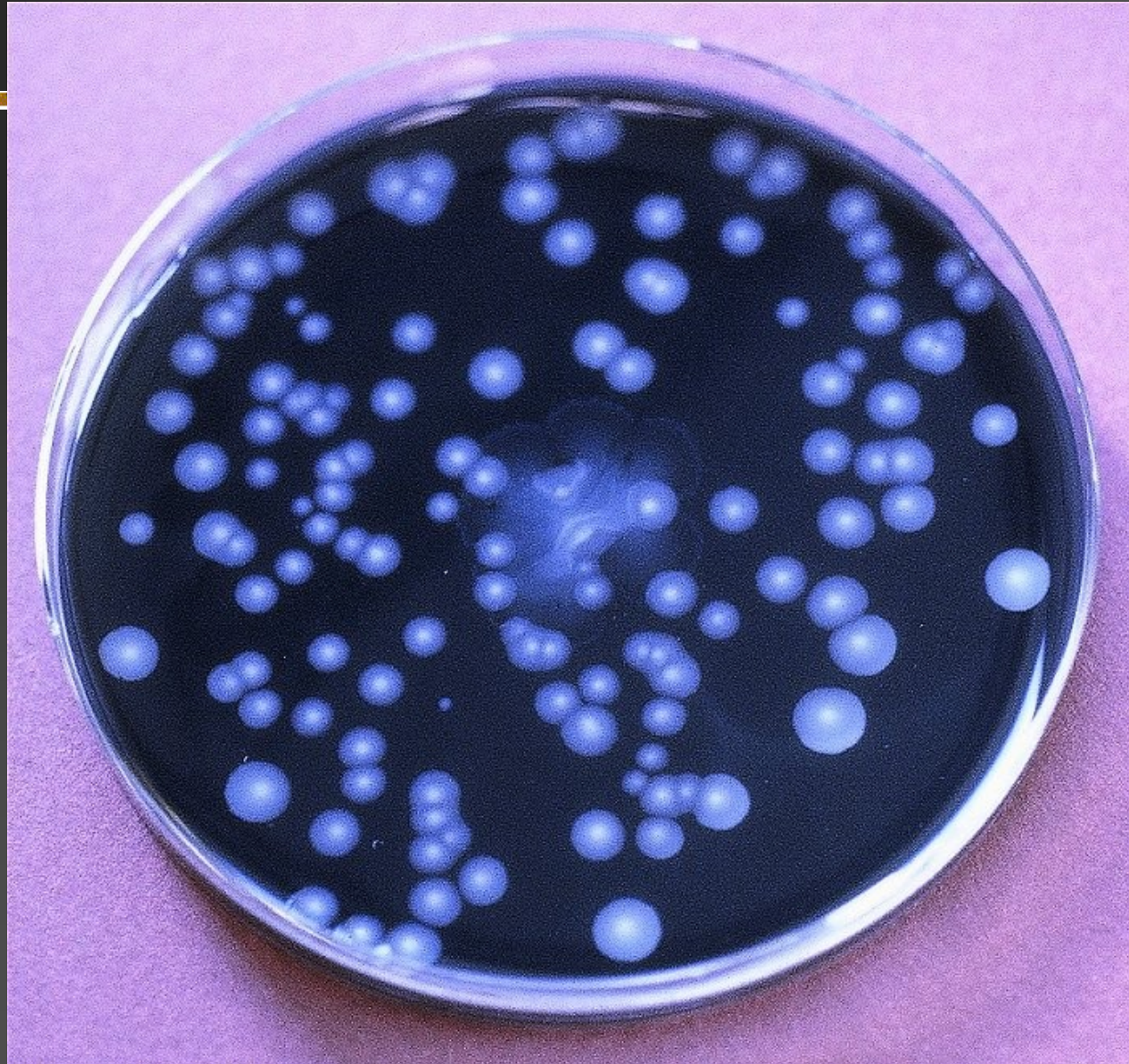
This strange way of inoculation is used, as the experience showed, that it increases successful diagnostics.

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



BCYE medium for Legionella

Buffered
Charcoal
Yeast
Extract



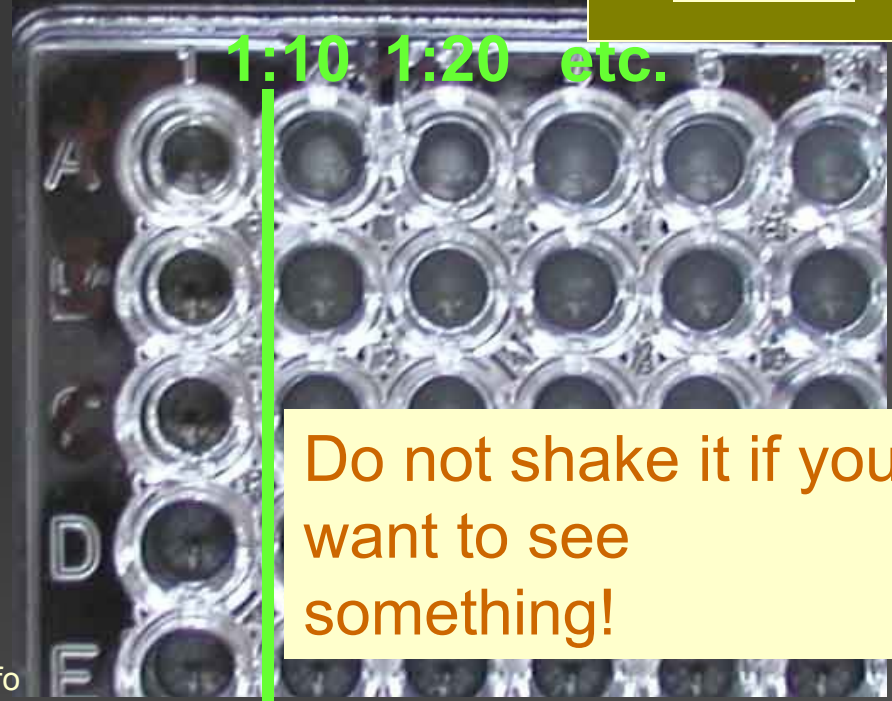
Francisella diagnostics: Reading the agglutination set



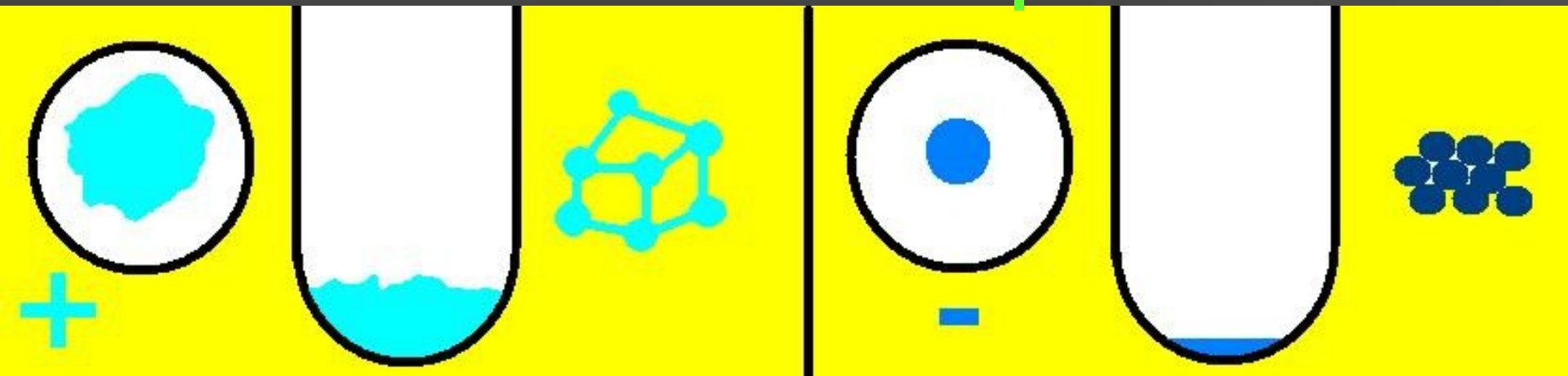
Titre is counted, i. e. the highest dilution giving still a positive reaction

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

www.medmicro.info

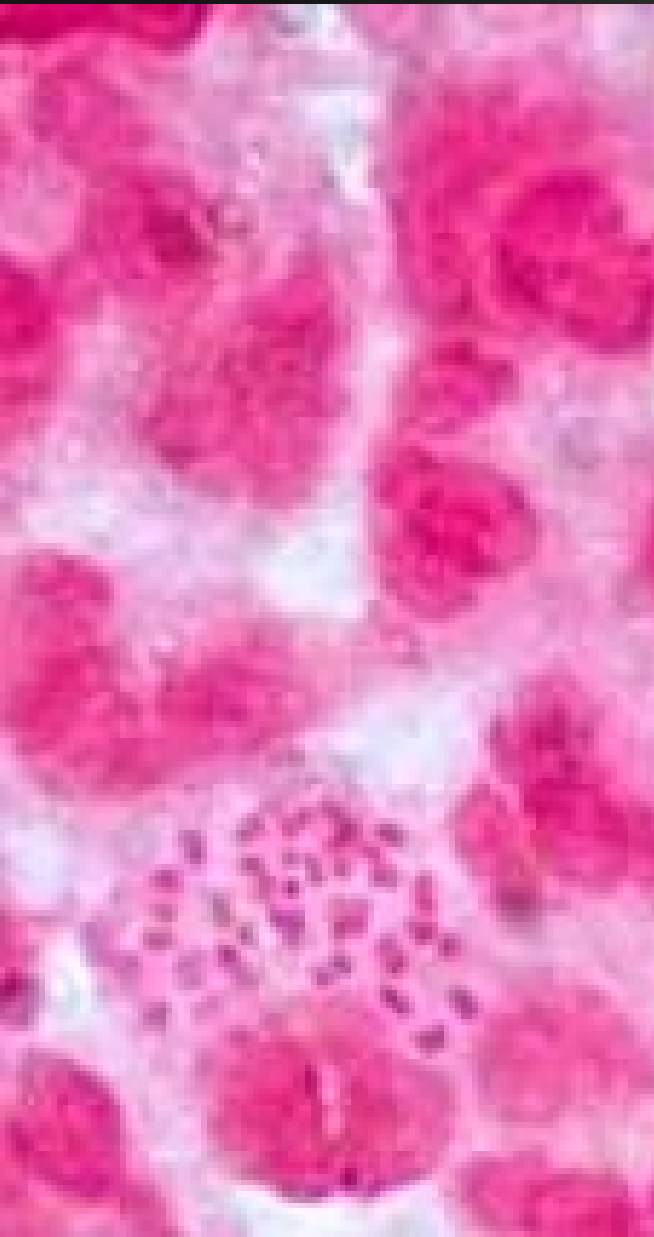


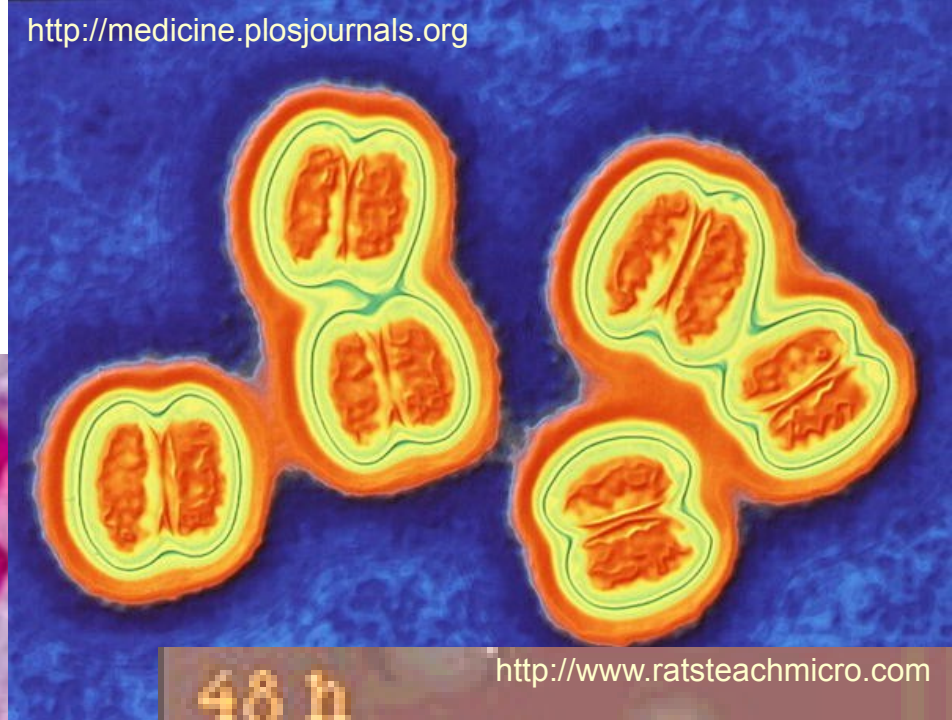
Do not shake it if you want to see something!



Pictures of G– cocci and „other G– bacilli“

Criminal photos: Gonocococcus



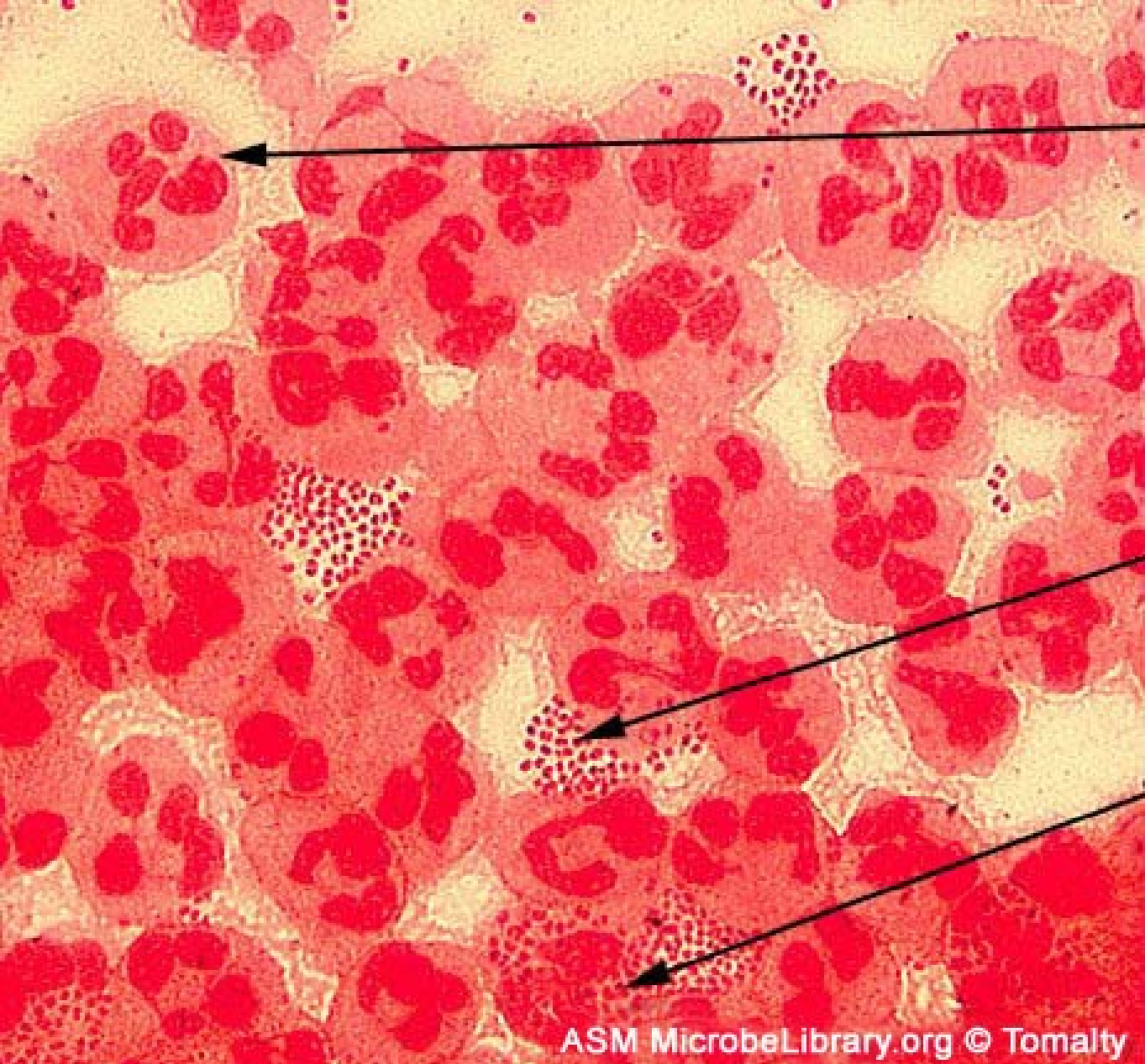


48 h



Gonococci





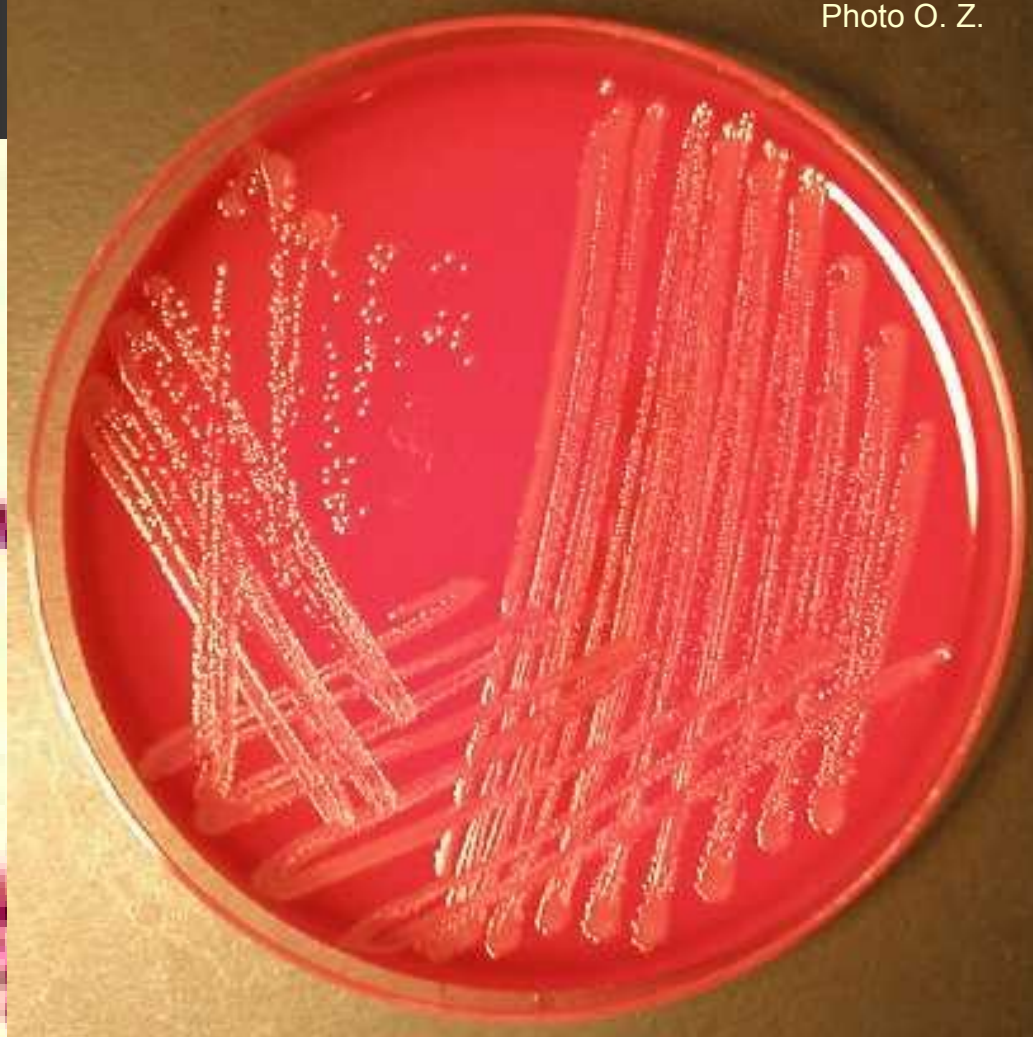
polymorphonuclear leukocyte

Extracellular gram-negative diplococci

Intracellular gram-negative diplococci

ASM MicrobeLibrary.org © Tomalty

Meningococcus



Microscopic Pictures Of Neisseria (Gram-negative Diplococci)

Neisseria Gonorrhoeae



Neisseria Meningitidis
intracellularis in spinal fluid



■ β -lactam drugs

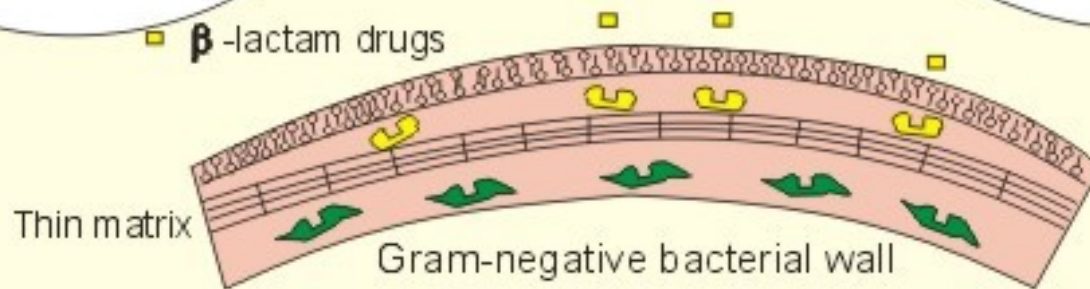
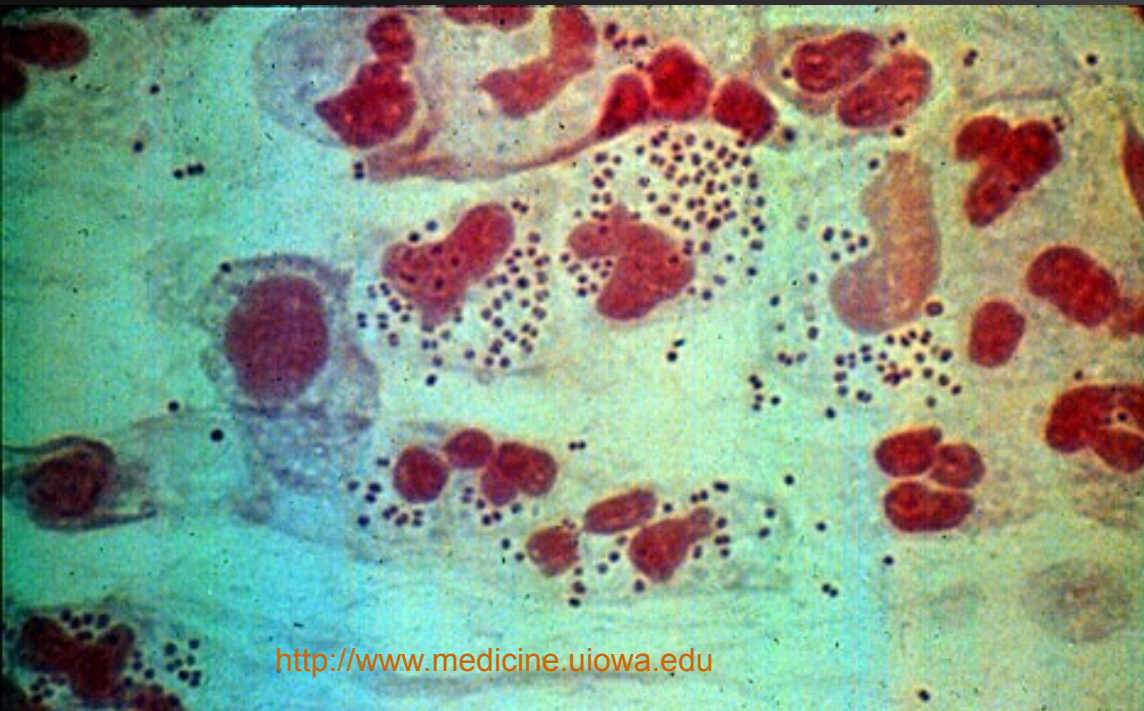


Fig. 33-3

Moraxella catarrhalis



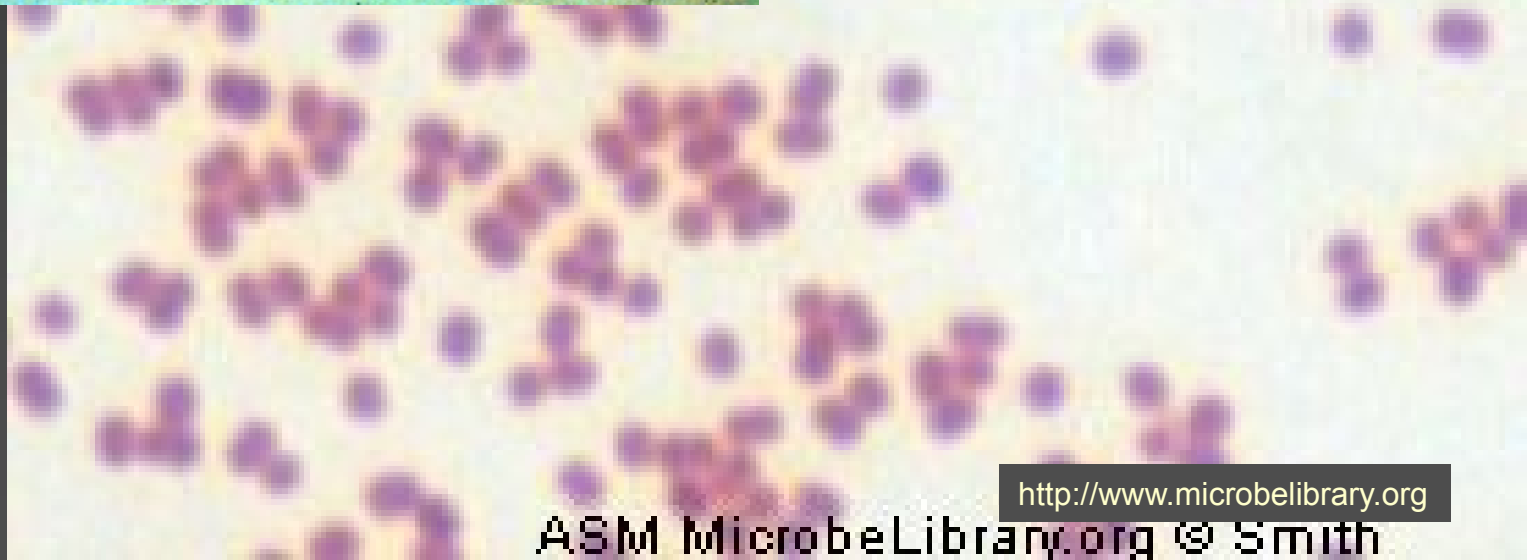
<http://www.medicine.uiowa.edu>



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

medianovo.de

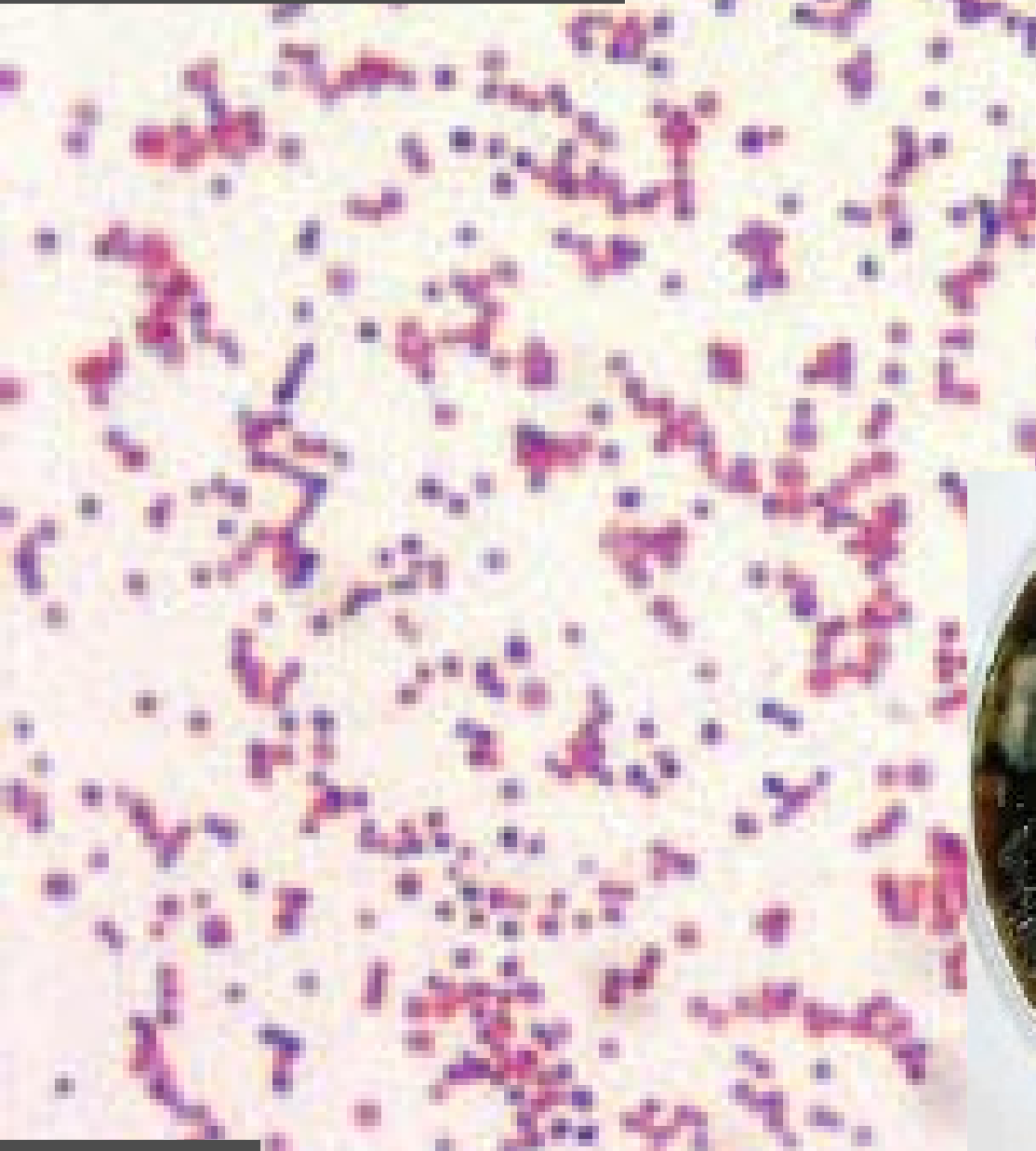


<http://www.microbelibrary.org>

ASM MicrobeLibrary.org © Smith

Francisella

www.infektionsnetz.at

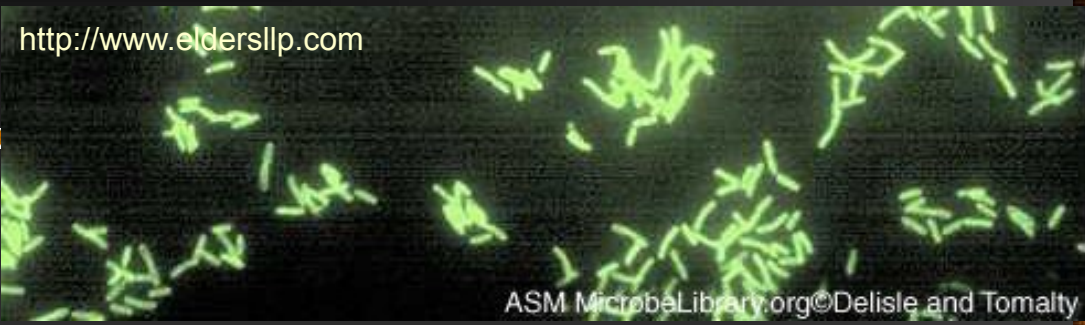


www.wnysmart.org/tularemia

www.stedim.com

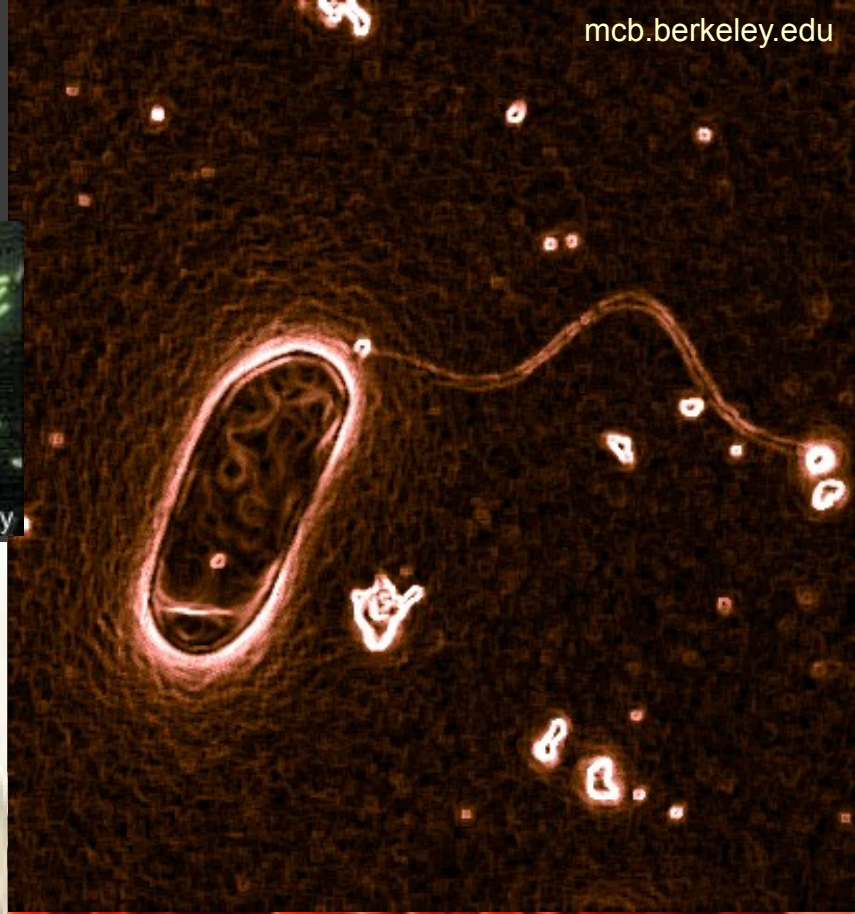
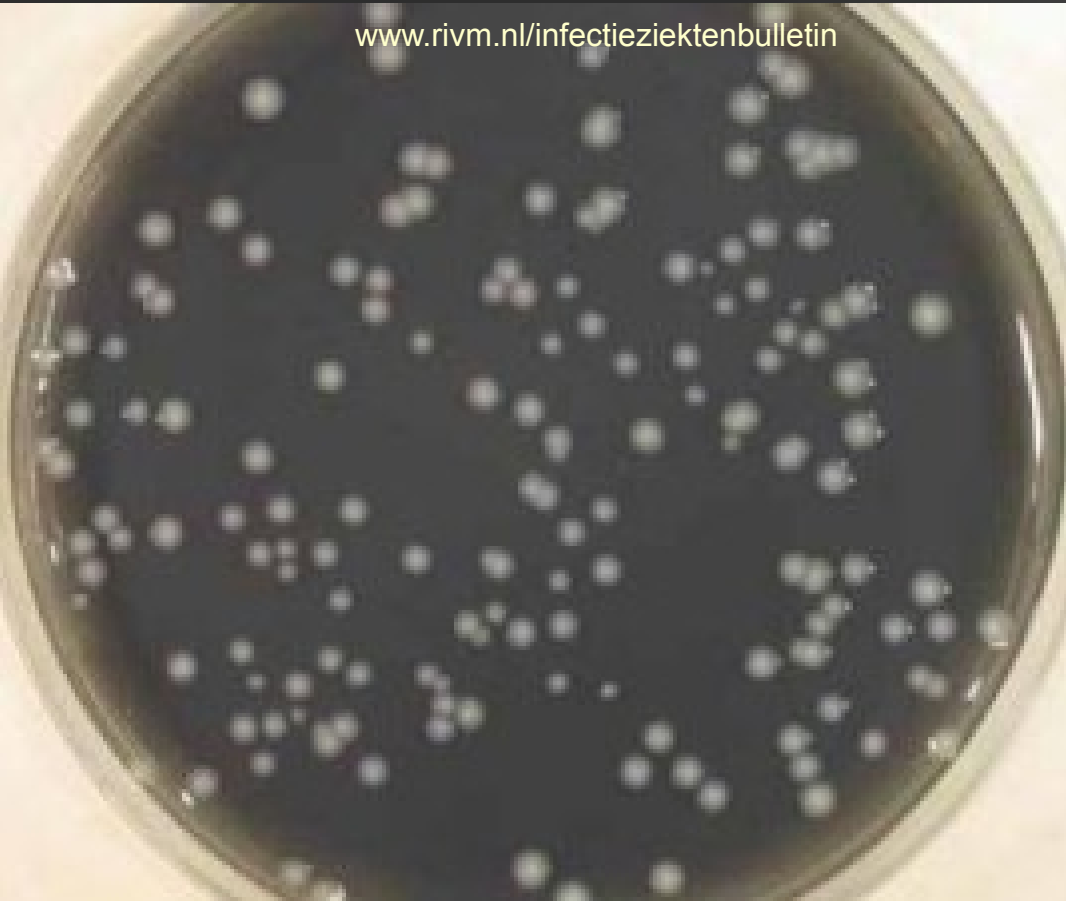
Legionella

<http://www.eldersllp.com>



ASM MicrobeLibrary.org © Delisle and Tomalty

www.rivm.nl/infectieziektenbulletin



www.chemistryquestion.com

Bordetella

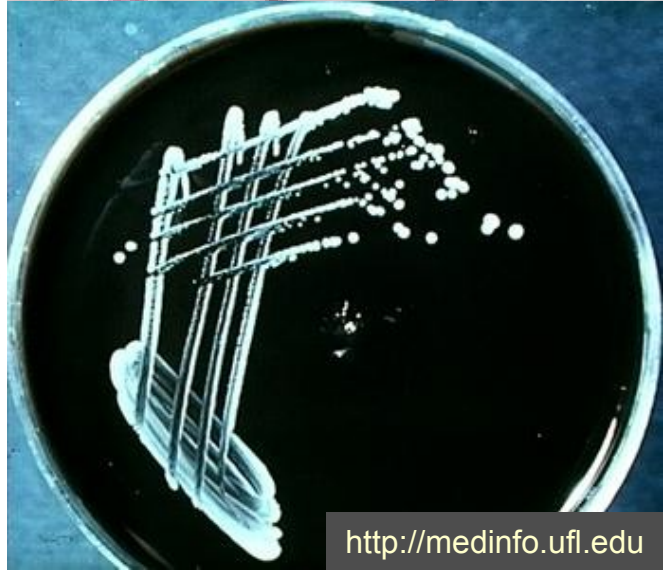
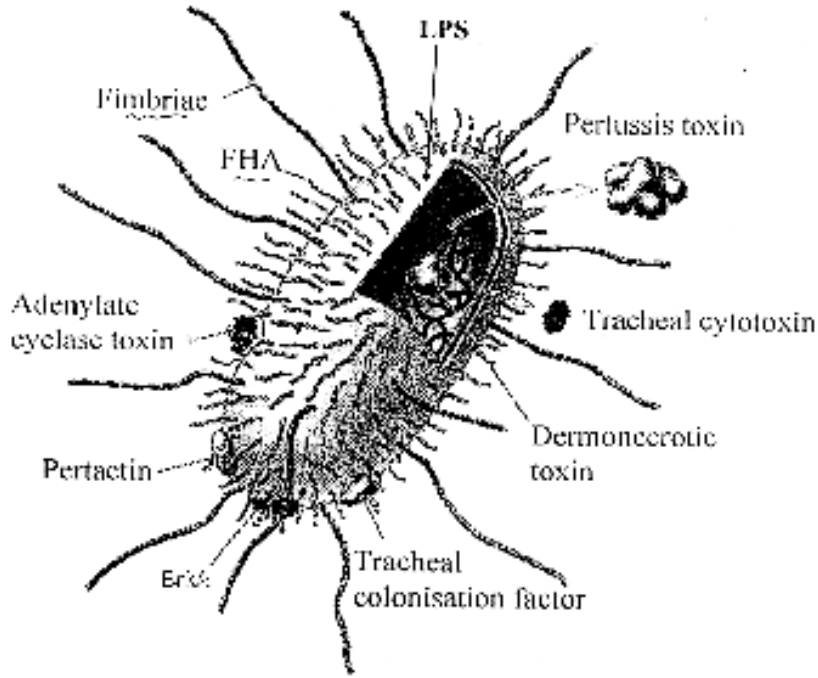


Bordetella pertussis

Causitive agent of Whooping cough

- Small gram negative bacterium
- Many virulence factors

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



Alisan Weiss, ASM News, 1997

Brucella



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

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The End

