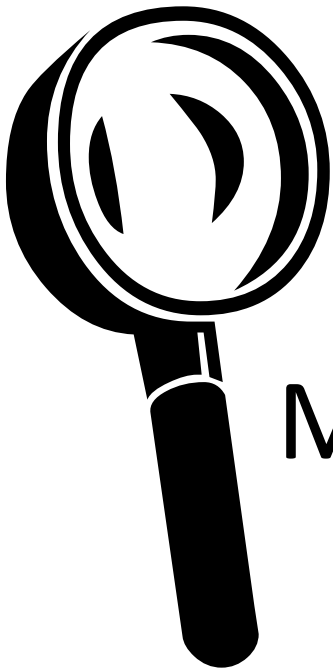


Institute for microbiology presents

# TRACING THE CULPRIT



Part six:

More gram-negative cocci and  
bacilli

# Summary

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 culprit

- Of course, it was *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.

# Story two

- Lucy studied for four weeks for the examination of physiology. She did not leave the house at all and only sat and read. 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 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 said that it is a **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 culprit**. Some of his strains are present in **throat of healthy persons...**

# And the culprit 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 innocent and studies say that about ten percent of population are throat carriers of meningococcus.
- Virulence is related mostly with **protein antigens**
- **Polysaccharide 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, according to guidelines, is still **classical penicillin**. Nevertheless, we would rather use it for less serious infections. For meningitis, we would use 3<sup>rd</sup> generation cephalosporins (**ceftriaxon** – good access to the CSF), or other antibiotics (macrolids, tetracyclins, quinolones)

# 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 weak. Long time there was no vaccination against this group. Recently, a new vaccine exists, but its effectiveness is only 74 % (preliminary data)

# 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

# Let's compare Neisserias:

	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“ Neisserias	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
- AMOCLEN (**amoxicilin**) was used for treatment immediately. Later, a **susceptible pathogen** was found.

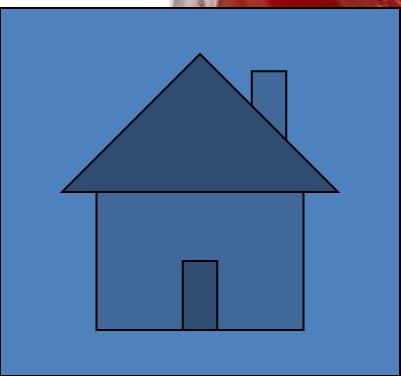
# And this pathogen was

- *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*

Foto Mikrobiologický ústav



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 an 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 finally stopped.

# 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 have reservoir in **water installation, air condition**, etc.
- During building new hospital departments (but also senior houses, hotels, spa...) legionellosis prevention should be taken, mostly at planning water pipe system (namely it is necessary to avoid blind branches, which cannot be run through by hot water or a disinfectant when necessary).



# „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)

# Genus *Brucella*

- It is a causative agent of zoonoses
- *Brucella abortus* is a bovine pathogen. It commonly infects 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

# 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**



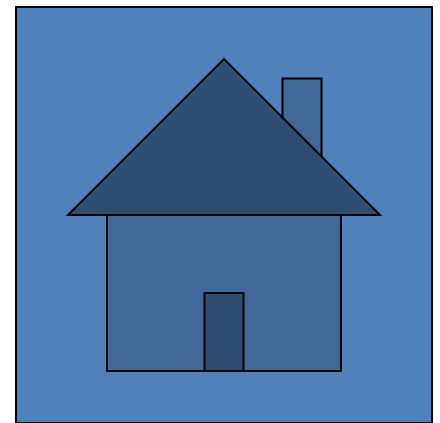
# From report about epidemiological situation (IX/2008) – I (abbreviated)

- **Tularemia – A21:** Woman, born 1970 from Valtice, gardener. 20<sup>th</sup> June GP visit for T 38°C, sore throat, enlarged lymph nodes next to right clavicle. The lymph node 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



# Diagnositics 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

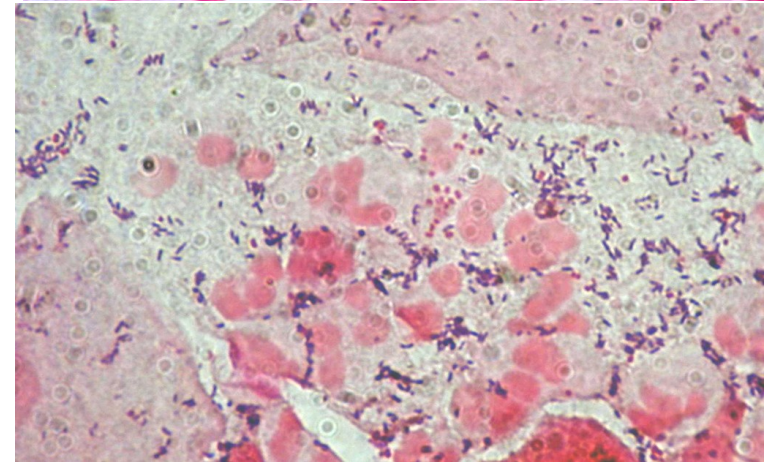
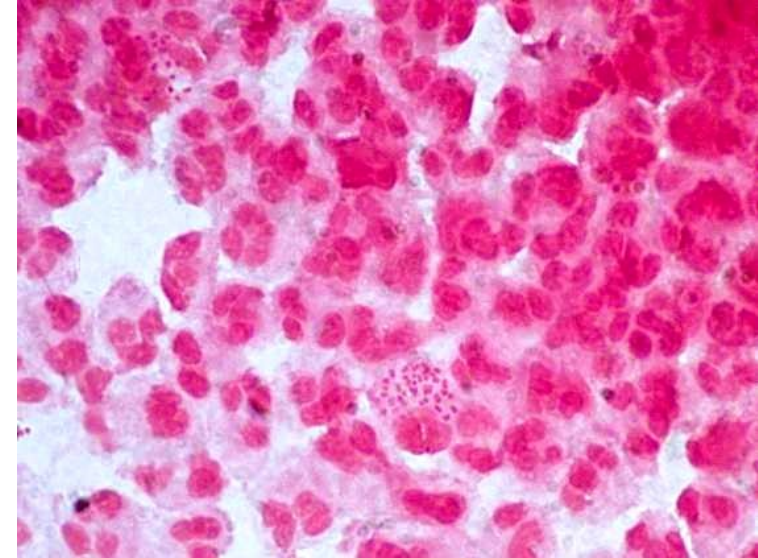
*Not regarding the diagnostics and its results, the most important is instant start of patient treatment!*

# *Neisserias* and *Moraxellas* – characteristics 1

- **Microscopy:** G – diplococci, coffee bean shaped, often intraleucocytar (see next slide)
- *Some Neisserias a Moraxellas may be prolonged and so they might be coccobacilli or bacilli (e. g. Neisseria elongata)*

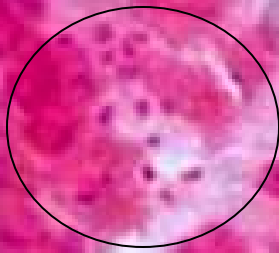
# Observation of gonorrhoea smear

- 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





## *Neisserias* and *Moraxellas* – characteristics 2

- **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**

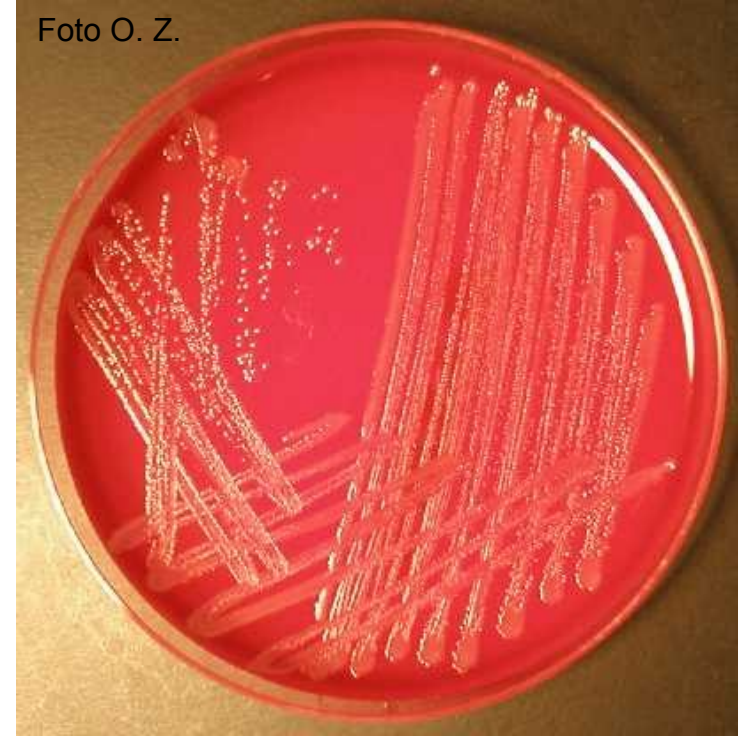
# Culture of *Neisseria* and *Moraxella*

There exist differences between G- cocci in culture properties:

**Oral Neisserias and Moraxellas (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 chocolate agar.



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

# Gonococcus atb testing



# *Neisserias* and *Moraxellas* – characteristics 3

- **Biochemical diagnostics:** 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 latex agglutination, very important in meningococci for differentiation between serogroups (for finding a proper vaccine)

# Basic biochemical tests

- Quick tests with diagnostic strips simplify the diagnostics
- *Neisserias* are oxidase positive, *Moraxellas* 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 Neisserias and Moraxellas

- For detailed identification of Neisserias and Moraxellas, biochemical tests are used, in Czechia mostly NEISSERIAtest, in other countries other tests (below or on the next slide)
- Both pathogenic Neisserias have little biochemical activity: *Gonococcus* splits glucose only, *meningococcus* glucose and maltose.

# *Neisserias* and *Moraxellas* – 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 *Neisserias*** (growth on both poor and rich blood agar and chocolate agar)
  - ***M. catarrhalis*** (growth like oral *Neisserias*)

# *Neisserias* and *Moraxellas* – 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 *Neisserias*
- **Antigen analysis** (determination of meningococcal serogroup in invasive infections)

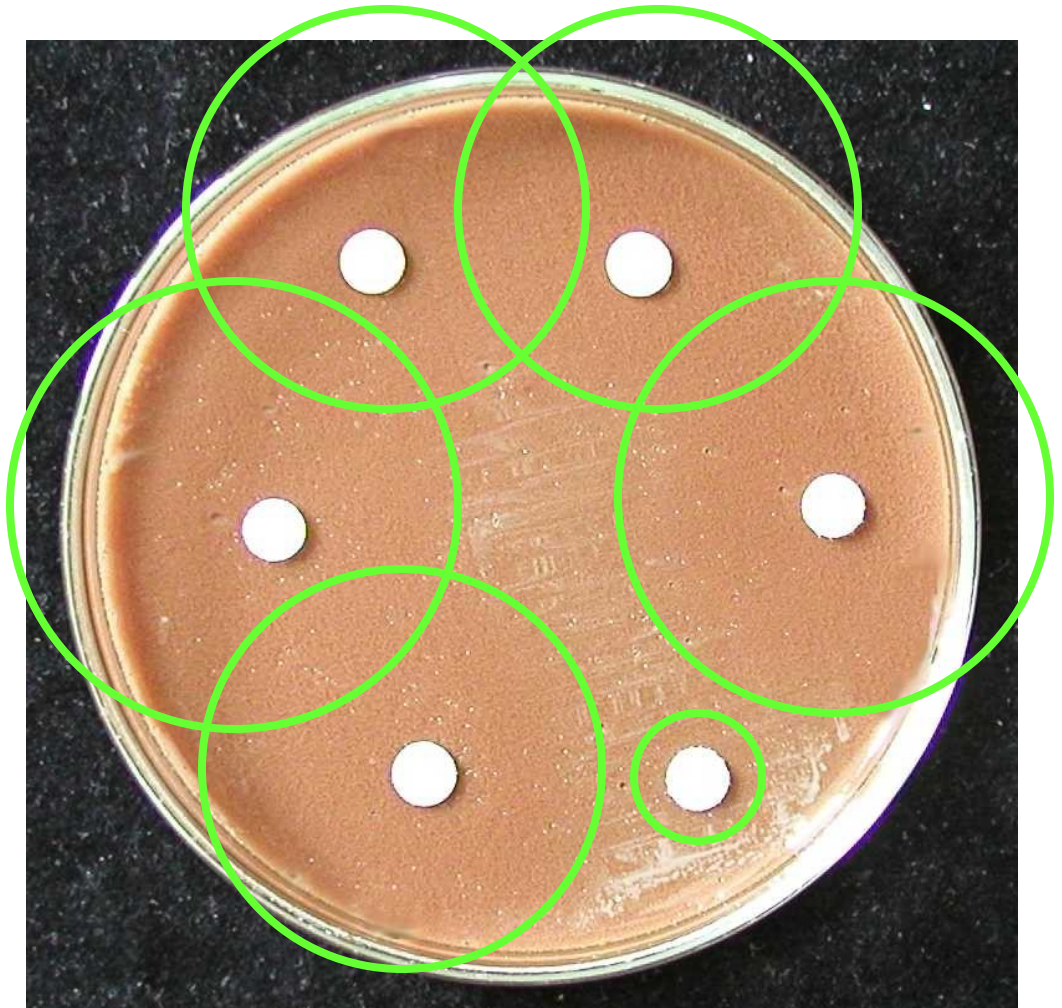


# Antibiotic susceptibility testing in Neisserias and related bacteria

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

# In Neisserias, zones are often large and confluent.

- If the zones are so large that it is not possible to measure the diameter, we measure the radius and multiply by two.
- *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 *Neisseria gonorrhoeae*

Antibiotic	Abbr.	Reference zone / concentration*
Penicillin (penicillin) – E-test	P	$S \leq 0,06$ $R > 0,25$
Cefotaxime (3G cephalosporin)	CTX	$S \geq 34$ $R < 34$
Meropenem (karbapenem)	MEM	$S \geq 30$ $R < 30$
Azithromycin (azalide)	AZM	$S \geq 20$ $R < 20$
Ciprofloxacin (fluoroquinolone)	CIP	$S \geq 35$ $R < 33$

\*In diffusion disc tests reference zone in mm, in e-tests breakpoint in mg/l

# A set of antibiotics against *Neisseria gonorrhoeae*

Antibiotic	Abbr.	Reference zone / concentration*
Penicillin (penicillin) – E-test	P	$S \leq 0,06$ R > 1
Cefuroxime (2G cephalosporin)	CXM	$S \geq 31$ R < 26
Cefotaxim (3G CS) – E-test	CTX	$S \leq 0,12$ R > 0,12
Azithromycin (azalide)	AZM	$S \geq 25$ R < 25
Tetracycline (tetracycline)	TE	$S \geq 38$ R < 30
Ciprofloxacin (fluoroquinolone)	CIP	$S \geq 41$ R < 28

\*In diffusion disc tests reference zone in mm, in e-tests breakpoint in mg/l

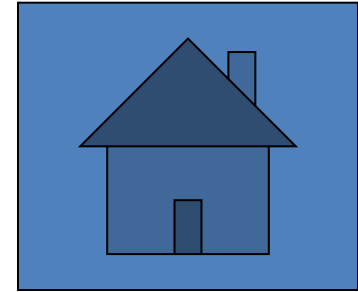
# Antigen detection / antigen analysis

- Agglutination set for CSF agglutination is used for identification of pathogens.
- 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 **children**
- *Neisseria meningitidis* C
- *N. meningitidis* Y/W135
- *Haemophilus influenzae* b **toddlers** (prior to vaccination)
- *Streptococcus pneumoniae* **seniors**
- *Streptococcus agalactiae* **newborns**
- **In green colour there is the age group, where the infection is the most typical**

**teens, young adults,**

# Diagnosics of „other G– bacilli“

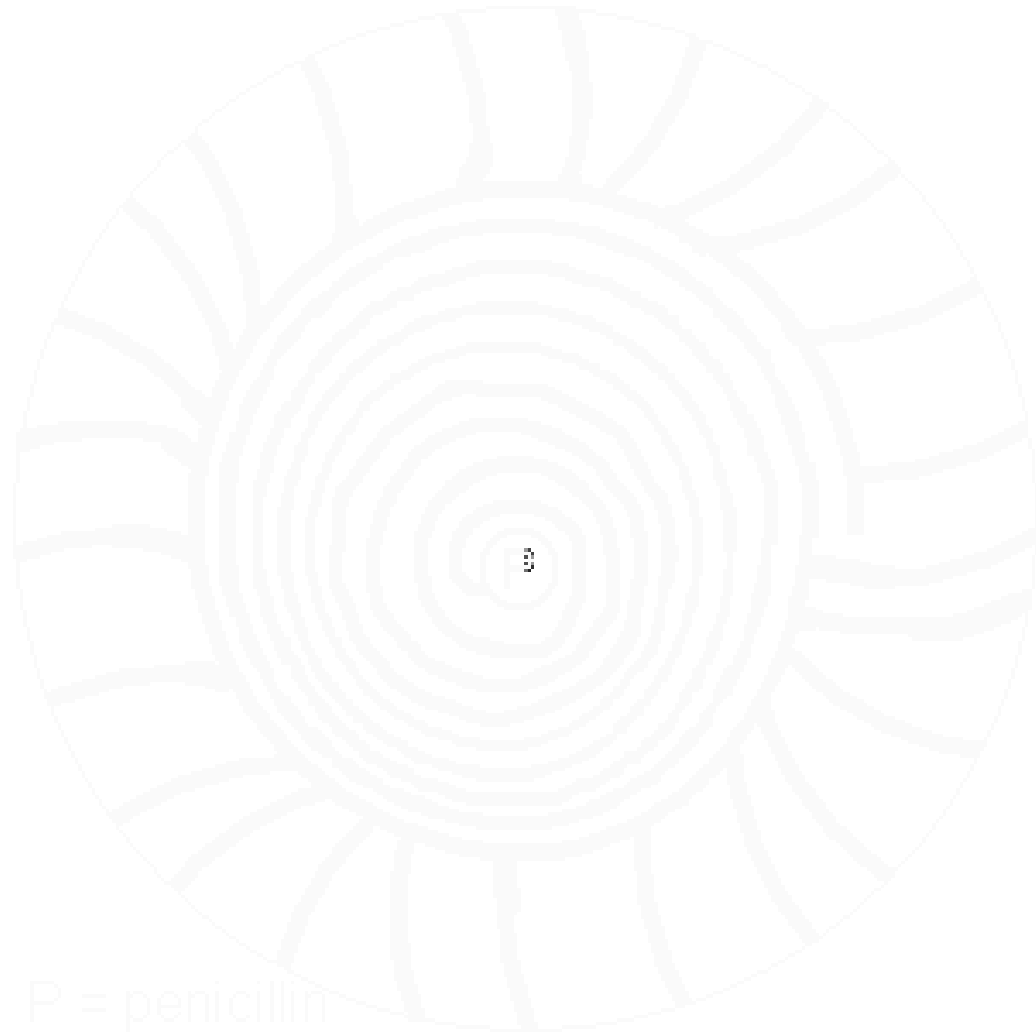
# „Other gram negative“ characteristic

- **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 tularaemia
- **Differential diagnostics** is not algorithmic here. Usually specimens are sent to the laboratory with suspicion for legionellosis, whooping cough, Bang disease etc.



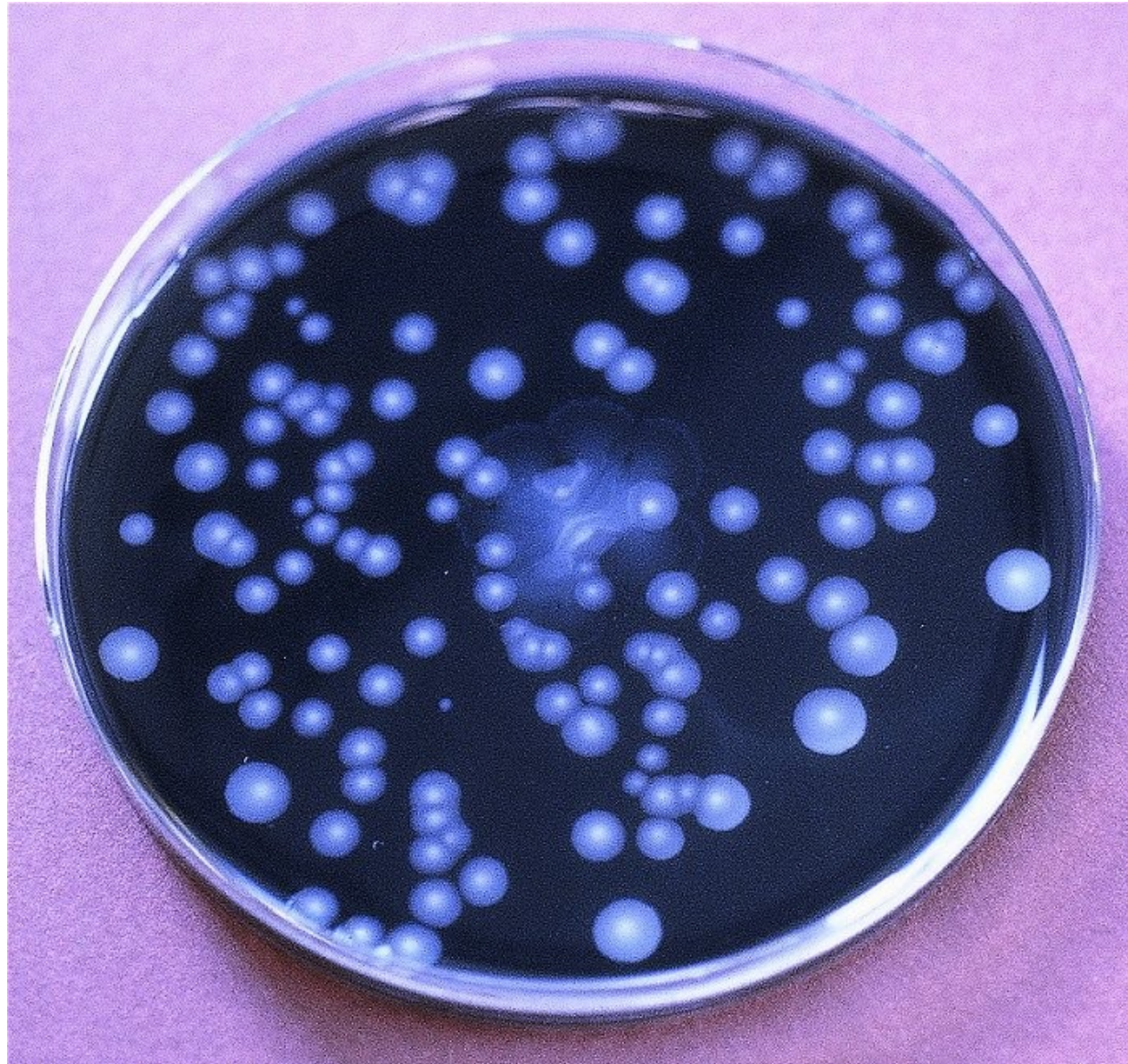
# *Bordetella*: Inoculation on Bordet-Gengou (BG) agar

- 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

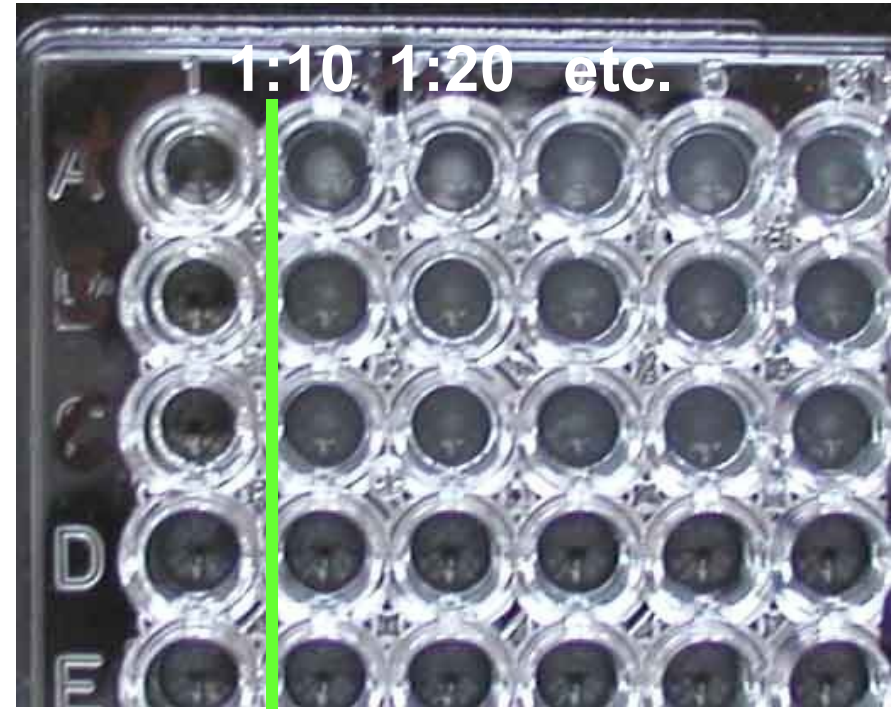


# *Francisella* diagnostics:

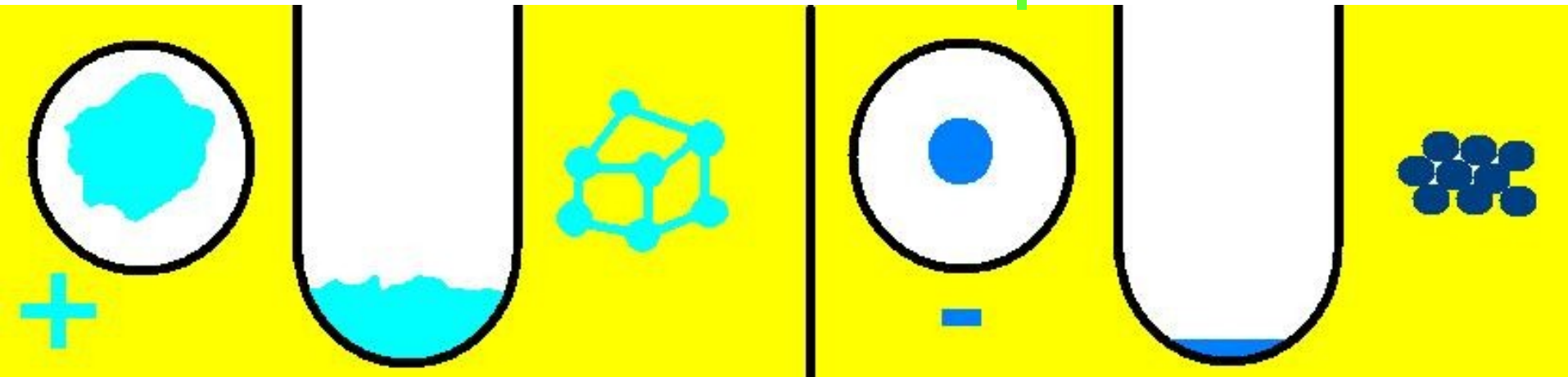
## Reading the agglutination set

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

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



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# *Brucella* diagnostics

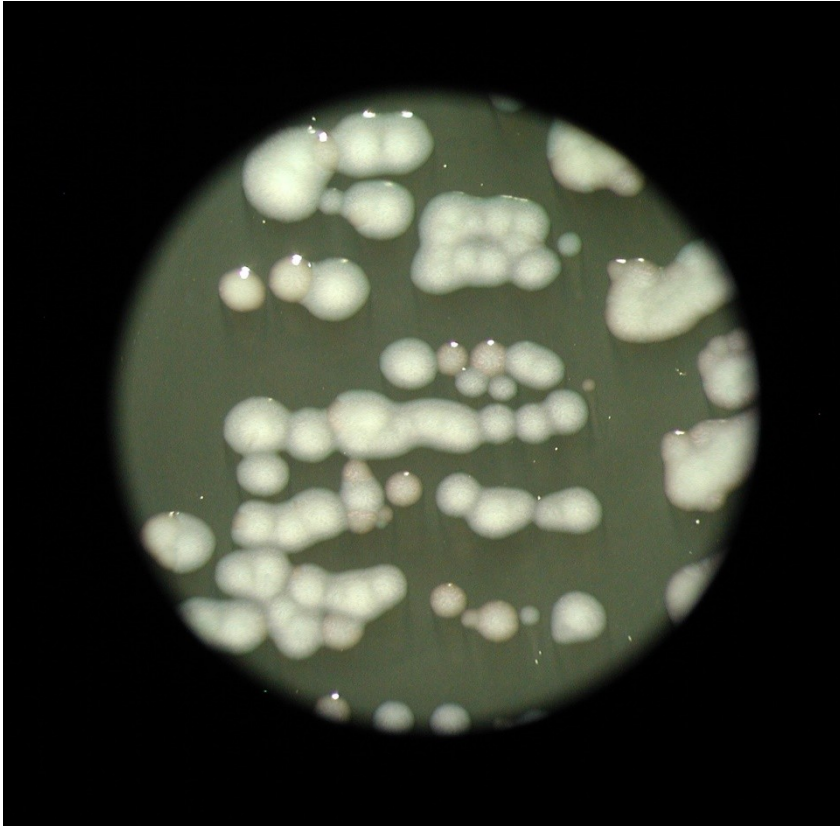
- Serology reactions, e. g. ELISA, are used, but the diagnostics is only performed by specialized laboratories

Major-general Sir David Bruce (1855-1931)

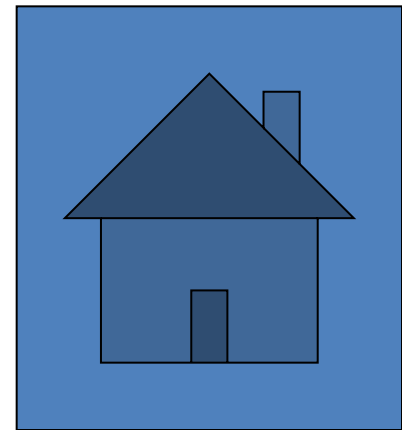


By see above - Dietmar Steverding. The history of African trypanosomiasis. Parasites & Vectors 2008, 1:3doi:10.1186/1756-3305-1-3, CC BY 2.0, <https://commons.wikimedia.org/w/index.php?curid=4306063>

# The End

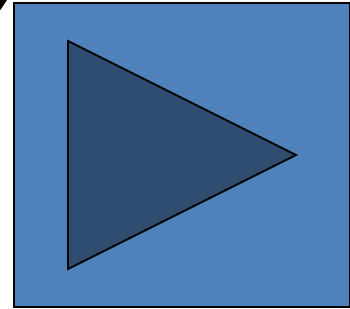


Legionella pneumophila – photo by Dr. V. Drašar



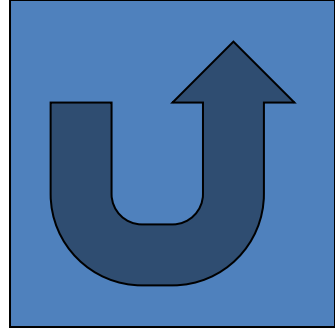
# How to build the buildings for health care to prevent legionellosis

- 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**