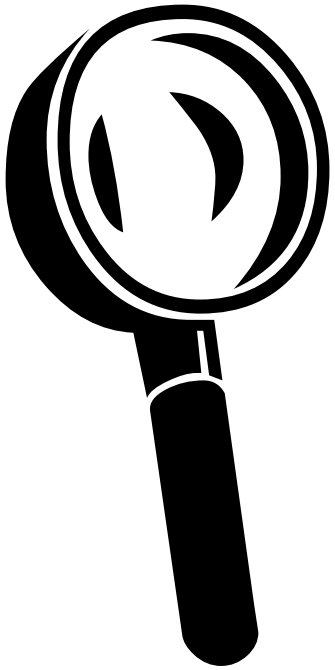
Institute of Microbiology shows:



TRACING THE CULPRIT
Part two: Streptococcus

Most important streptococci

Story	On BA	Name of the culprit
4.	viri-dation (alpha)	Streptococcus pneumoniae
5.		Group of „oral streptococci“
1.	(beta) hemo-lysis*	Streptococcus pyogenes
2.		Streptococcus agalactiae
3.		Group of „non-A-non-B“ streptococci
–	none	Ahemolytical streptococci

**in S. agalactiae partial haemolysis only*

Notes

- Classification of streptococci can be done by many ways. One of them is based on Lancefield antigens, (in this slideshow only used for a group on „non-A-non-B“ streps). Recent studies also mention similarity of genome, etc. Nevertheless, the classification based on type of haemolysis is traditional and still commonly used in practice, although it has some troubles: some strains may be quite variable in having complete, partial or no haemolysis, and some of them (e. g. *S. anginosus*) may even have sometimes viridation and sometimes (beta)haemolysis. Despite these exceptions, majority of streps can be identified using methods described later.
- Streptococci share coccoid shape with staphylococci, but taxonomically they are rather close to lactobacilli and other bacteria making lactic acid from lactose – all of them belong to the order Lactobacillales.

Survey of topics

Clinical characteristics: Haemolytic streptococci

Clinical characteristics: Viridating streptococci

Therapy of streptococcal diseases

Diagnostics of streptococci

Differential diagnostics of streptococci

Late sequels of streptococcal diseases

Clinical characteristics: (β-)haemolytic streptococci

(with partial or total haemolysis)

Story One

- Mr Hobby likes to work with wood. He worked at his workshop, when a large wood has fallen on his foot. A large lacerated wound emerged, and even dirty. Mr Hobby was taken to a hospital. The wound was sewed by a surgeon, but high fever and signs of sepsis were found. At reoperation, necrotizing inflammation of fascia with necrosis was found. Unfortunately, the care did not help: the leg had to be taken away.

Who is guilty?

- It is *Streptococcus pyogenes*

strepto = in chains, pyo-genes = making pus

- *Streptococcus pyogenes* is known as causative agent of acute tonsillitis. Nevertheless, it causes pyogene tissue inflammations, too. Unlike staphylococci, causing abscesses, here phlegmonas are rather common.
- Besides tonsillitis, it causes also scarlet fever and erysipelas. There are strains producing erythrogenous toxin (erythros = red)
- When the bacterium itself is infected by a bakteriophage, it is even more virulent and becomes a „meat eating bug“ – our case.

Typical diseases

- Streptococcal pharyngitis and tonsillitis
- Scarlet fever
- Erysipelas

Only rarely also diseases like:

- Necrotising fasciitis

Story Two

- Young lady Erika was not too often present at preventive controls during pregnancy. Few days before delivery she found herself in a birth house. Delivery itself did not bring any complications. Soon the child started to have signs of sepsis and respiratory failure. Quick treatment saved the child‘s life, and also prevented progression to meningitis that is, unfortunately, quite common here.
- Later Mrs. Erika was shown to be a carrier of a bacteria, that was shown to be guilty.

Who is guilty now?

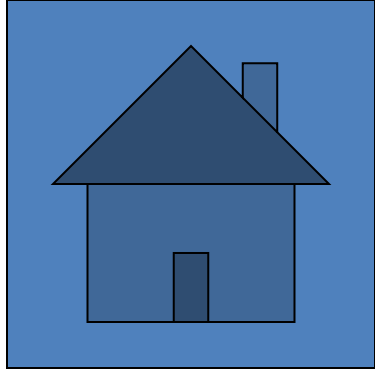
Bacterium ***Streptococcus agalactiae*** is a *Streptococcus*, too. In humans, it rather infects lower parts of body (**urogenital infections**) with risk of new-born infection

*Clever students should mention species name **a-galactiae**, i. e. „milk-less“. This bacterium really causes also milk gland inflammation with damaged milk production; these features, nevertheless, are seen in cattle, not humans*

Story Three

- Harry the boy has a sore throat. It looks like tonsillitis, but he already subdued both adenectomia and tonsillectomy.
- Parents went with Harry to see a doctor, to prescribe him some antibiotics. But the doctor said – first throat swab, and then maybe antibiotics. She invited Harry in three days. After that, she prescribed penicillin, and it started to have effect very soon.

Who caused Harry‘s problems?



- So named „non-A-non-B“ streptococci are called so as they do not belong neither to A group (in which *Streptococcus pyogenes* is the only one) nor to B group (where *S. agalactiae* is the most important one).
- They do not cause so often tonsillitis, but rather pharyngitis – inflammations of pharynx. Nevertheless, they are often present in healthy persons‘ throats.
- The same as in tonsillitis, in susceptible strains the first antibiotic to be used is penicillin; macrolids in allergic persons only.

Clinical characteristics: viridating (α-haemolytic) streptococci

Story Four

- Missis Evelyn, retreated, has her spleen let extracted long ago after a car accident.
- Several days ago, she caught a „common cold“, she did not pay attention to this, but later her status worsened, so her daughter drove her to a hospital, where she was hospitalized on infectious diseases department with suspicious meningitis
- Grace to soon antibiotic treatment her status became better and she got back her health.

This time culprit is:

- ***Streptococcus pneumoniae***, or „pneumococcus“. It was also called *Diplococcus pneumoniae*, as it does not form chains, but couples. Its shape is not perfectly spherical, but rather lancet shaped.

(Remember this, examiners might ask you this the examination. ☺)

- In small amount, it is present in healthy persons‘ pharynx. On the other hand, it causes pneumoniae, sinusitis, otitis media and even sepsis and meningitis.

Story Five

- Mr. Hearty has long durating heart problems. Even the artificial heart valvula had to be installed into his body.
- One month ago, he ad an awful dental carries, and it continued long time before he came to see a dental doctor.
- Now his heart problems worsened so that he had to be hospitalised. Diagnosis endocarditis lenta was set down.

Who is the culprit in this crime?

- **Oral streptococci, viridans streptococci, alpha streptococci**, all these names describe streptococci viridating on blood agar; usually we mean „viridans streptococci, but not pneumococcus“
- They are part of normal oral and pharyngeal flora. Even at physiological conditions, all the time some streptococci penetrate in small amounts into the bloodstream The problem starts, when they come there too many together, and when they meet a suitable terrain.

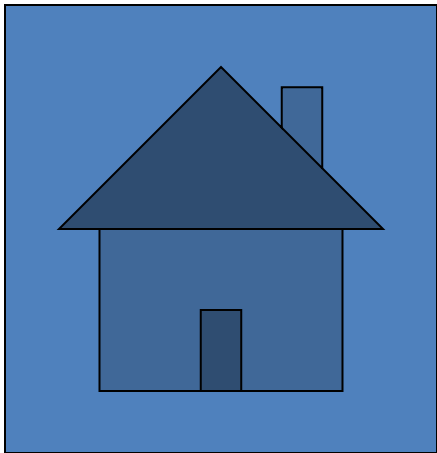
Some possible culprits

S. salivarius is present in human saliva

S. sanguis (synonym: *S. sanguinis*) related with endocarditis

Among „oral“ streptococci, *S. mutans* has probably the highest relation to dental caries.

More about oral streps, including their groups, on Wikipedia



Therapy of streptococcal diseases

Treatment: the culprit should be punished

- Guilty Streptococci will be punished by a suitable antibiotic. In Streptococci the No. 1 drug is the classical Fleming‘s penicillin (either G-penicillin for parenteral use of V-penicillin for oral use). Macrolids should be used in PNC-allergic persons only. Doxycyklin, co-trimoxazol, ampicilin and others might be used. Vancomycin is a reserve, 100% effective antibiotic (no zone = a mistake, it is not a streptococcus)

Susceptibility testing

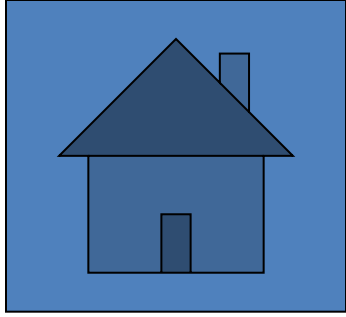
- Usually we read the diffusion disk test by measuring the zones and comparing with the reference zones
- Again: the worse pathogen (pyogene streptococcus) is more susceptible than milder pathogens
- The tests are performed on MH agar with blood or on blood agar. On the MH agar without blood streptococci grow poorly, or do not grow at all.

Nevertheless, we cannot utilise this fact in diagnostic – some streptococci are able to grow there!

Reference zones for the most common antibiotics

Antibiotic	Abb.	„S“ if ≥ than (mm)	„I“ if between (mm)	„R“ if < than (mm)
Penicillin (penicillin)	P	≥ 18		< 18
Erythromycin (macrolid)	E	≥ 21	18–20	< 18
Clindamycin (lincosamid)	DA	≥ 17		< 17
Chloramphenicole	C	≥ 19		< 19
Tetracycline (tetracycline)	TE	≥ 23	20–22	< 20
Vancomycin (glycopeptide)	VA	≥ 13		< 13

Reference zones for the most common antibiotics – UTI



Antibiotic	Abb.	„S“ if ≥ than (mm)	„I“ if between (mm)	„R“ if < than (mm)
Penicillin (penicillin) (interpreted as ampicillin)	P	≥ 18		< 18
Tetracycline (tetracycline)	TE	≥ 23	20–22	< 20
Vancomycin (glycopeptide)	VA	≥ 13		< 13
Nitrofurantoin (nitrofuran)	F	≥ 17	15–16	< 15

Diagnostics of streptococci

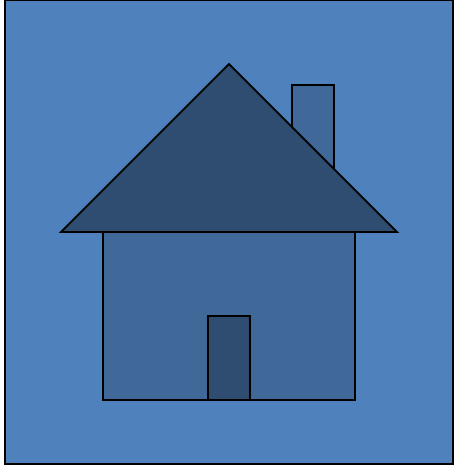
Description of culprits (diagnostics) 1

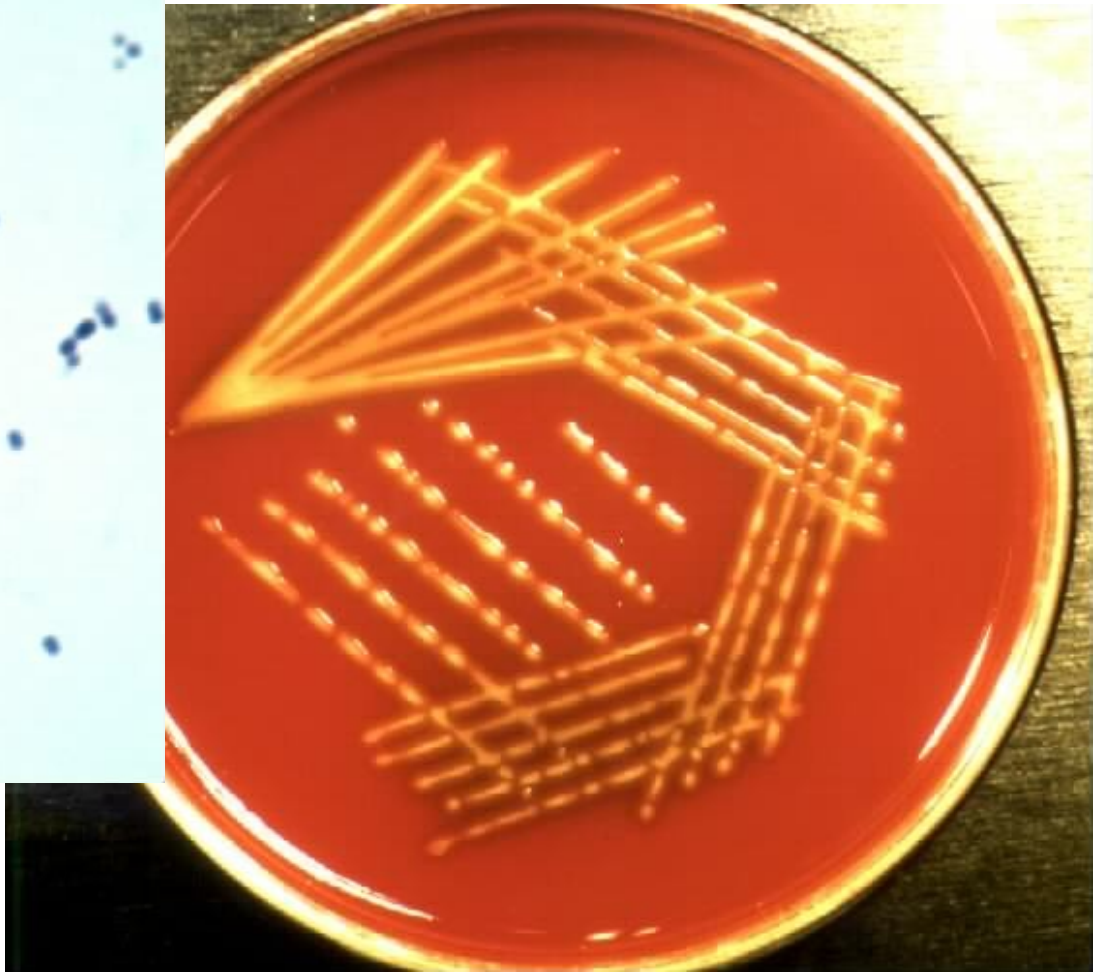
- Microscopy: gram-positive cocci
- Cultivation: on BA grey to colourless colonies, usually small, larger colonies has only *Streptococcus agalactiae*
- Haemolytic properties: some viridate, some partially or totally hemolyze
- They do not grow neither on BA with 10 % NaCl, nor on Slanetz-Bartley or Bile aesculin medium.
- Together with enterococci, they are resistant to aminoglykosides, so medium with amikacin is used as a selective medium.

Description of culprits (diagnostics) 2

- Biochemical tests: both catalase and oxidase negative, biochemical differentiation of individual species possible especially in viridating streptococci
- Antigen analysis helps rather in haemolytic streptococci. Lancefield system is used – theoretically all streptococci are involved, but many viridans streptococci have no antigen in this system. Groups are labelled by letters A, B, C, E, F, G etc.

Photos of culprit database



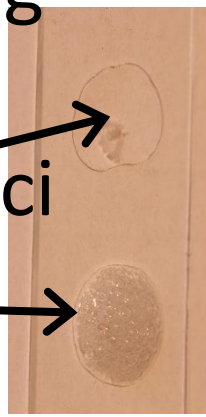


Source: Archive of Institute for microbiology

Differential diagnostics of streptococci

Differentiation from other suspects (differential diagnostics 1)

- Gram stain show all bacteria, that do not belong among gram-positive cocci.
- Negative catalase test differentiates streptococci from staphylococci
- Growth on SB and BE media differentiates enterococci. All of them are also positive in so named PYR-test, while among streptococci only one of them is positive, and that one is rarely confused because of its very strong haemolysis and other properties



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Differentiation from other suspects (differential diagnostics 2)

- Haemolysis should be observed now – it classifies streptococci into haemolytic, viridating and others
- Pneumococcus vs. other viridans streptococci: Pneumococcus has positive optochin test, test of solubility in powder bile etc.
- *S. pyogenes* vs. other haemolytic streptococci:

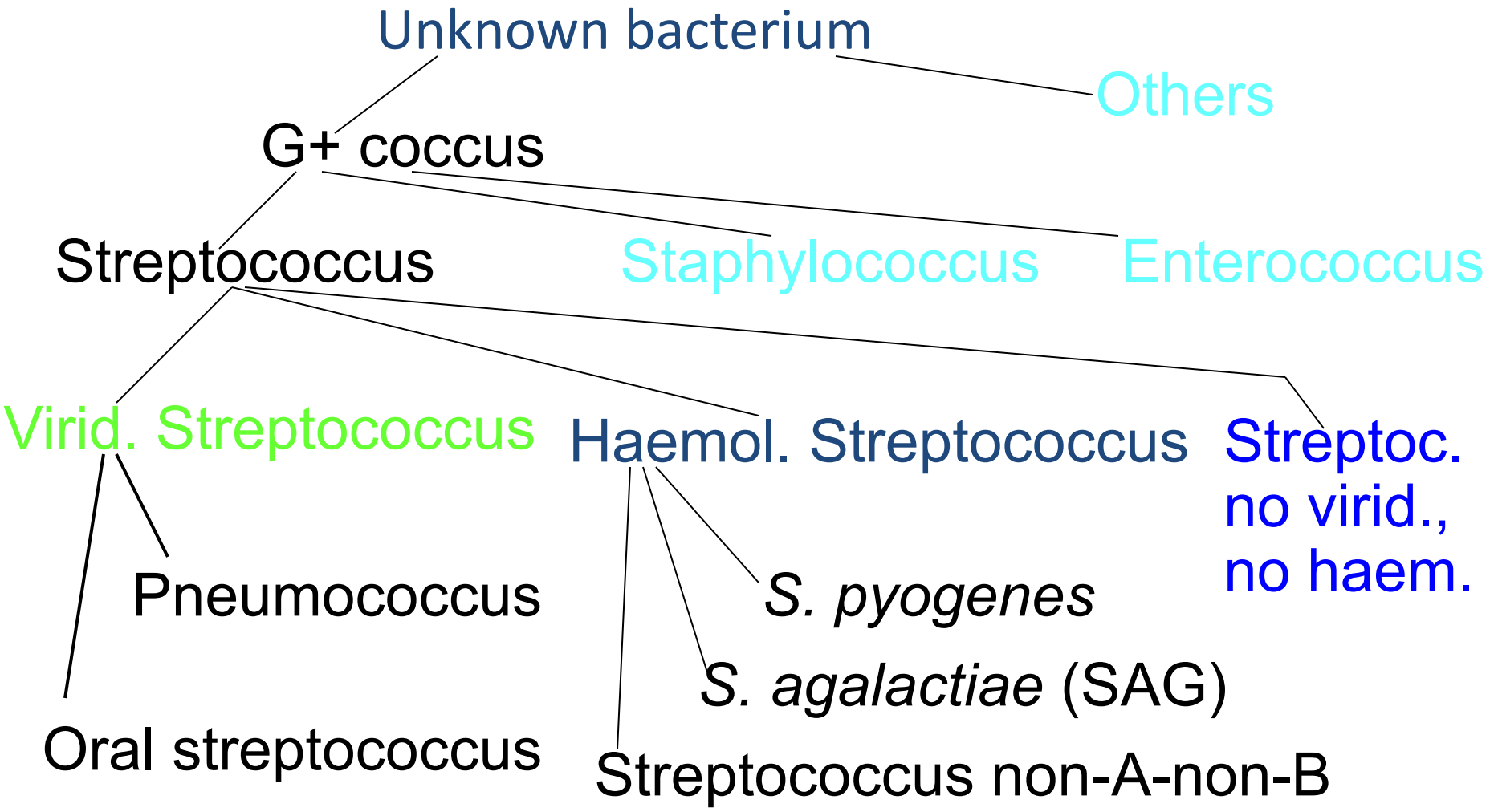
Both Bacitracin and PYR test are ⊕ in *S. pyogenes*

- *S. agalactiae vs*. other haemolytic streptococci:

CAMP test is ⊕ in *S. agalactiae*

About all these tests – more info later

Schematically:



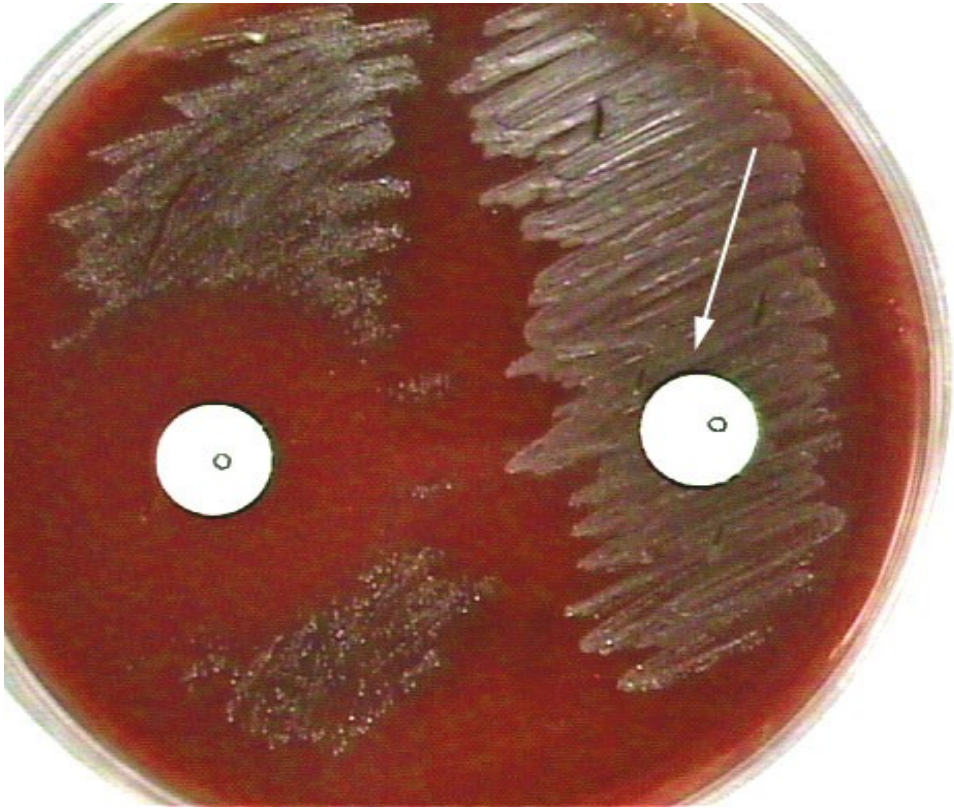
Pneumococcus: How to become suspicious

- Pneumococcus can be differentiated by the optochin test – see following slide.
- Suspicion maybe taken, when:
  - microscopically lancet-shaped diplococci can be seen
  - cultivation: colonies flat, coin-shaped to dish-shaped, sometimes with a central elevation
  - on the other hand, sometimes the colonies are large and mucous: those are strains with a strong capsule production (usually highly virulent)

Optochin test

- Classical test to differentiate pneumococcus from oral streptococci. Pneumococcus is susceptible to optochin (antibiotic), oral streptococci are resistant. (Optochin is not used therapeutically today, it remained in diagnostics only)

- Sometimes, the test of solubility in powder bile is used. Test of mouse pathogenicity is today considered to be historical



http://www.mc.maricopa.edu

Species determination of oral *Streptococcus*

- Only someone mad (or a researcher – sometimes it is the same) would differentiate an oral streptococcus to species level, when the strain is from oral cavity of pharynx. Why to do it, when we consider it to be a part of normal flora?
- On the other hand, in strains from blood cultures, differentiation is logical. In viridating streptococci, it has no sense to attempt the antigen analysis, but, as we know already, biochemical tests are very useful.
- In Czech conditions, it is mainly STREPTOtest 16

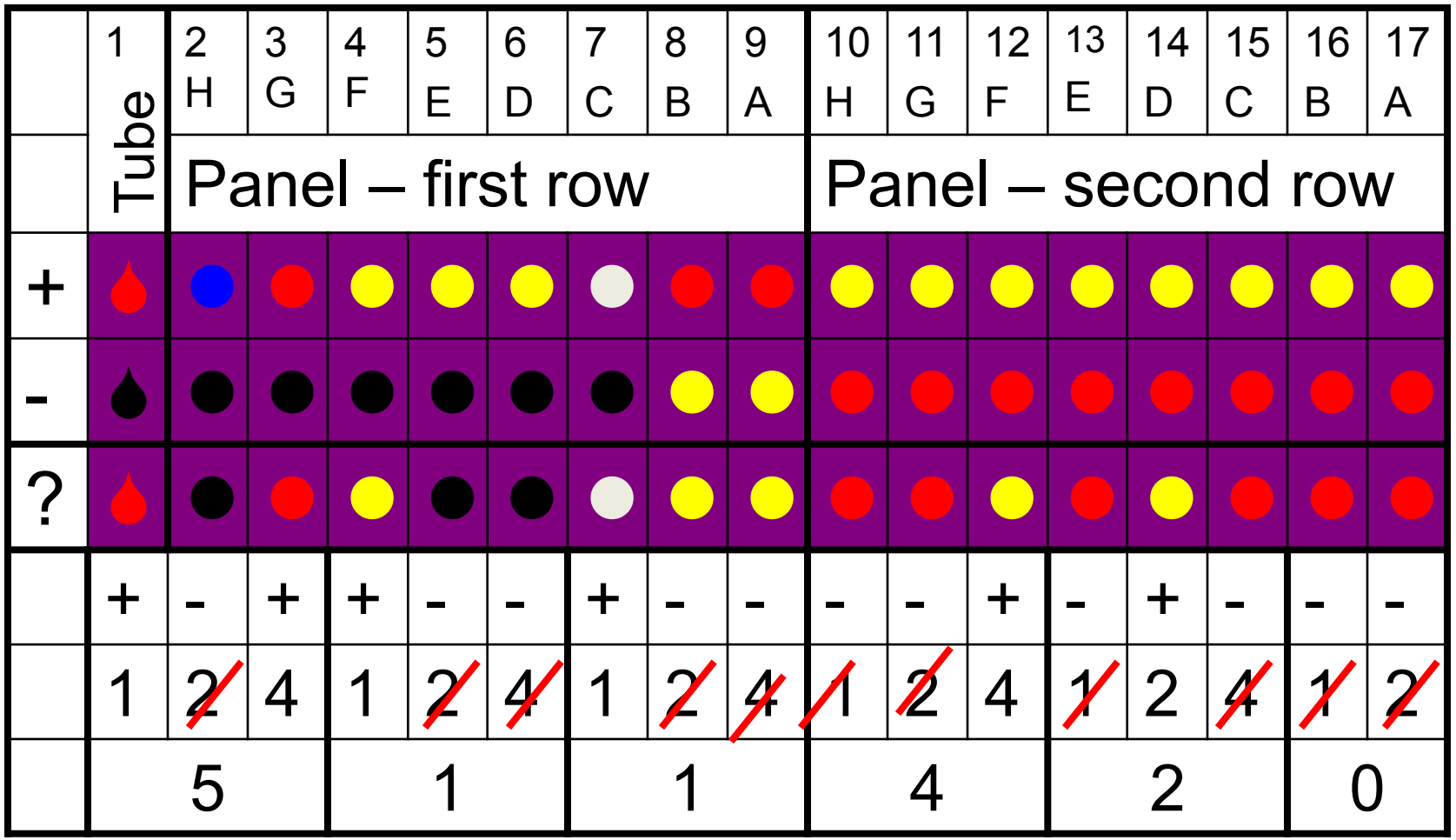
STREPTOtest 16 – how to read it

Three musketeers were four. STREPTOtest 16 (and STAPHYtest 16 and ENTEROtest 16) use 17 reactions.

- First reaction is again VPT (D'Artagnan!)
- 2nd to 9th reaction is again the first strip in the double-strip
- Similarly, 10th to 17th reaction is the second strip in the double strip

An example of result of Streptotest 16: Code 511 420 *Streptococcus salivarius*

% probab. 97.19 Typicity index 1.00



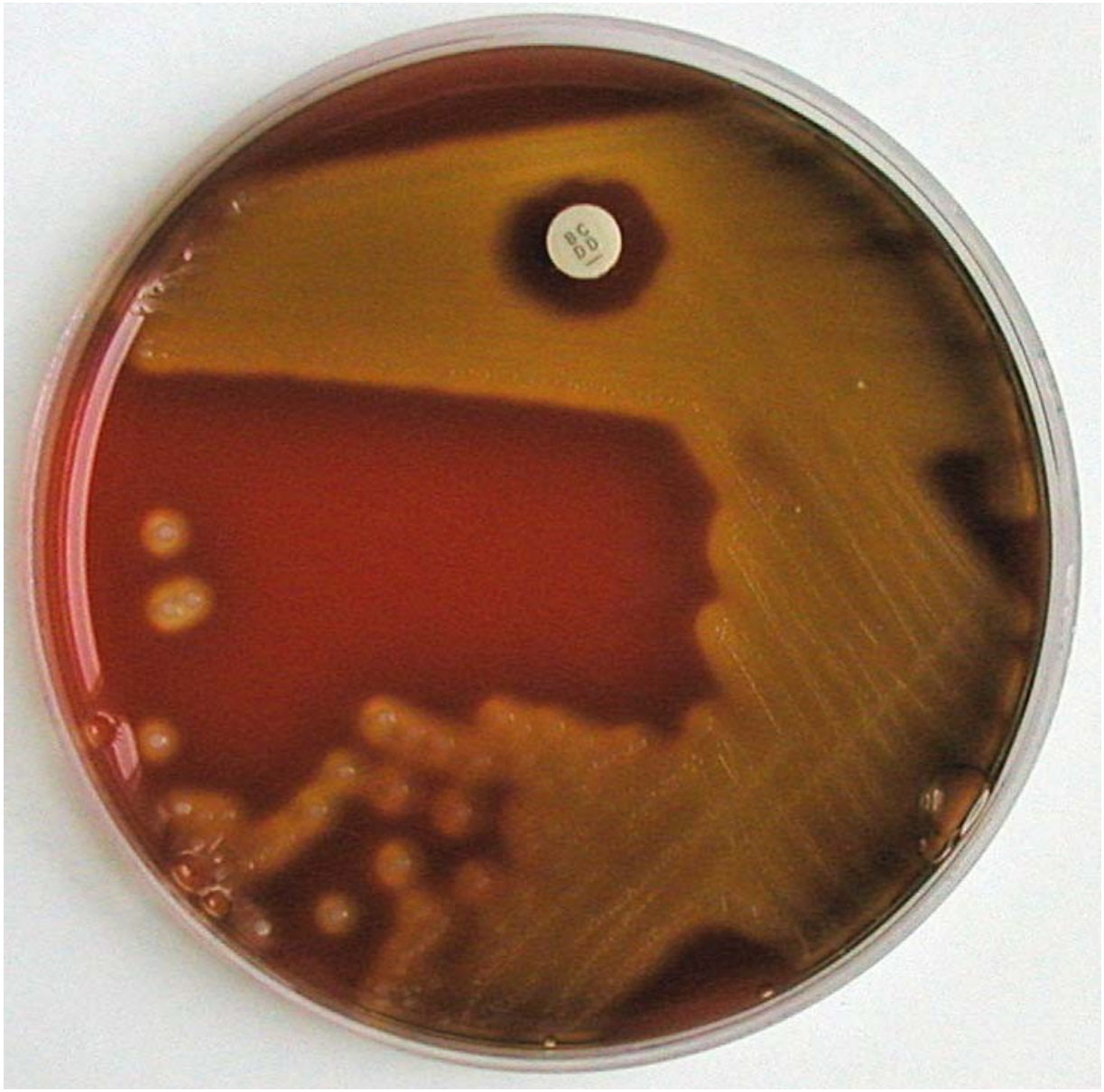
Especially dangerous culprit: the pyogene *Streptococcus*. What to do?

PYR test

- PYR test is performed similarly as oxidase test. We touch by the strip (its reaction square) the colonies. Then we wait ten minutes. A reagent is added, one more minute of waiting follows. Red = positive
- *Bacitracin test was used sooner. It had the same principle as the Optochin test, only an other antibiotic was used.*

Bacitracin test

Photo: Archive of Institute for Microbiology

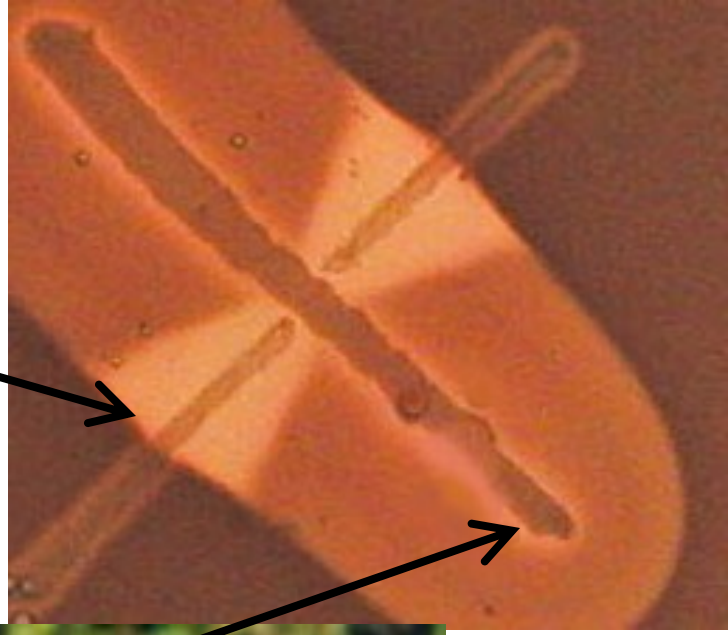


And now the second: *Streptococcus agalactiae* – 1

- Many bacteria produce haemolysins
- When two bacteria produce haemolysins, their co-operation may be either synergic or antagonistic.
- An example of a synergism is CAMP factor of *Str. agalactiae* and beta lyzin of *Staph. aureus*
- It is not possible to use it for *Staphylococcus* diagnostics – not all strains of Staphylococci produce the beta lyzin! So, the test is used in *Streptococcus* diagnostics only.

Streptococcus agalactiae – 2 CAMP test

- TESTED strain of a *Streptococcus* and TESTING strain of beta-lyzin producing *Staphylococcus* are inoculated on the blood agar
- In case of positivity, we see stronger haemolysis in shape of two triangles, or, more poetically, butterfly wings



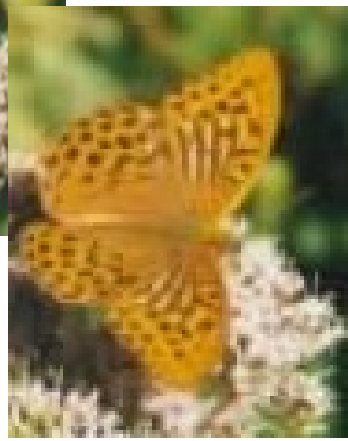


Photo O. Z.

CAMP test – another picture

Photo O. Z.

Haemolytic culprits – conclusion

Bacitracin and PYR test	CAMP test	*Streptococcus*
positive	negative*	*S. pyogenes*
negative	positive	*S. agalactiae*
negative	negative	non-A-non-B *Streptococcus***
positive	positive	a nonsens, a bad test, mix of two strains etc.

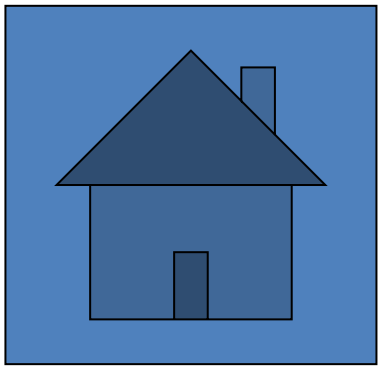
*sometimes week synergism, not having the proper size and shape
*eventually more detailed diagnostic using antigen analysis

Remember:

- **Streptococci with haemolysis** (total or partial), but also streptococci with no haemolysis at all can be usually determined using **latex agglutination** (if necessary). Their biochemical activity uses to be poor.

- **Streptococci with viridation** (alpha-streptococci) can be usually determined using **biochemical testing** (if necessary). Their antigen determinants use to be poor.

Latex agglutination



- Latex agglutination is used for detailed diagnostics of non-A-non-B streptococci, if necessary, according to Lancefield scheme. However, conclusion „it is a non-A-non-B strep“ is usually sufficient. The principle of latex agglutination is showed on the pictures. Agglutination of streptococci with the antibody is helped by latex particles

Photo: Inst. for Microbiology

Late sequels of streptococcal diseases

Streptococcus pyogenes is even worse than we already knew

- You know that *S. pyogenes* causes tonsillitis, scarlatina, erysipelas. But the worst still waits: Even after being flown out from the organism, a terrible sequels may occur! Antibodies circulate in the blood… and mistakenly, instead of being bound to streptococci, they bind to some structures of the organism. So, acute glomerulonephritis or rheumatic fever occurs.

You may mention, that we have had this already once in the spring semester…

ASO: how to see, if the risk exists

- Using ASO test you will see, if a normal antibody response is formed, or an autoimmunity over-response with risk of development of glomerulonefritis/rheum. fever
- ASO test is usually performed after a streptococcal infection. By the antibody detection, we do not try to detect the infection (we know about it), but to clarify, whether autoimmunity response is developed. So it is NOT an indirect diagnostic, although antibodies are measured.

ASO: principle (repeating)

- The antibody blocates the haemolytical effect of the toxin (streptolyzin O) on a RBC.
- In ASO, we do not use the geometrical row. The values of dilution are in a table.
- Titre over cca 250 means a risk of antibody response
- In Czech, abbreviation ASLO is used instead of ASO in English.

How to read an ASO panel

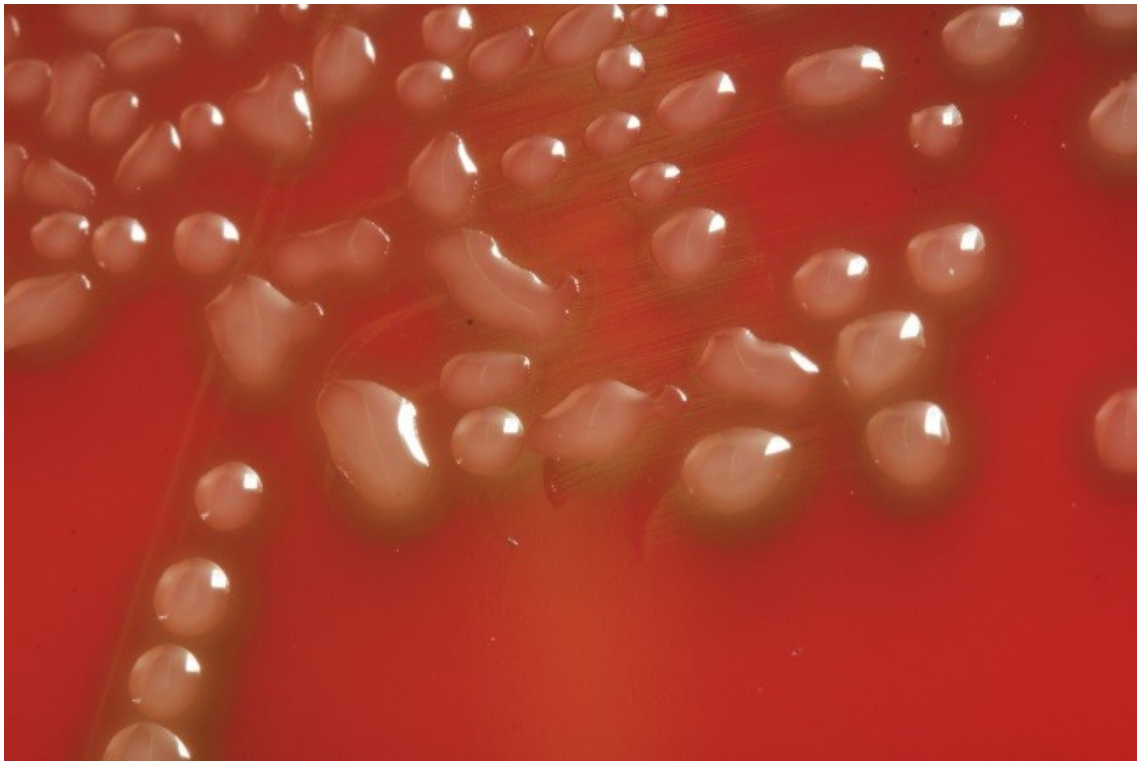
- Each patient has one row. The dilutions are here and in tables on your working tables.

Hodnocení výsledků ASLO

jam ka č.	1	2	3	4	5	6	7	8	9	10	11	12
hod nota mi.j.	100	120	150	180	225	270	337	405	506	607	759	911

- Panel has a positive control and five patients

Goodbye at the next part!



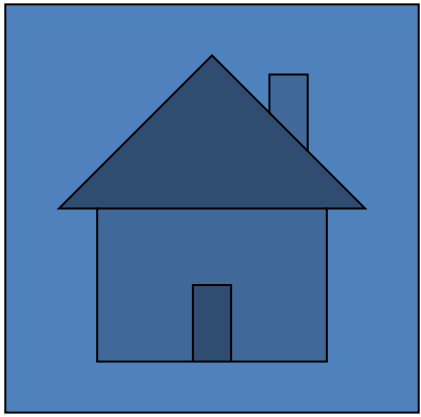


Photo: Inst. for Microbiology

S. pneumoniae –
detail of colonies