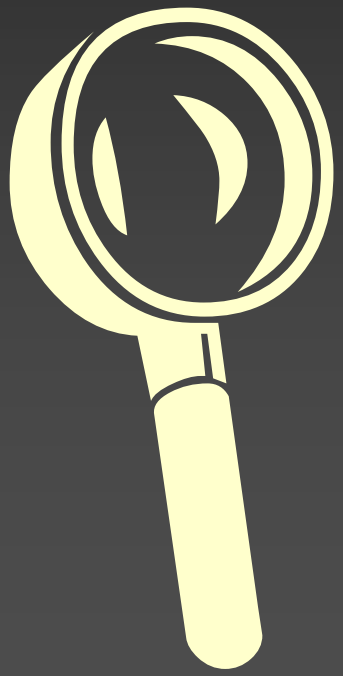
Institute of Microbiology shows:



TRACING THE CRIMINAL

Part one: Straphylococcus

Overview

Clinical characteristics: *Staphylococcus aureus*

Clinical characteristics: CONS (coagulase-negative staphylococci)

Diagnostics of staphylococci

Differential diagnostics of staphylococci I

Differential diagnostics od staphylococci II

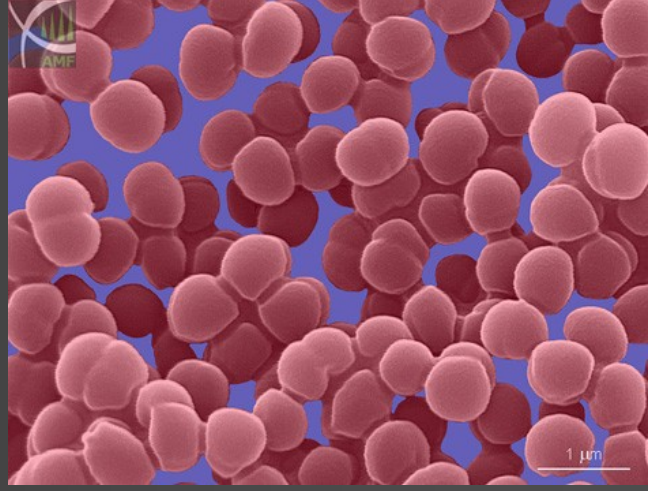
Antibiotic susceptibility testing and antibiotic treatment

Clinical characteristics: *Staphylococcus aureus*

Story One

- Mrs. J. K., cook in students canteen, has a blister on her hand, full of white-yellow pus. She is not aware. She takes dumplings by her hand, although the dumplings are already cooked (and now they ill be only slightly heated, not cooked).
- Student Rashid and his girl-friend eat the dumplings. In the afternoon, they should have a rendez-vous … BUT… half an hour before the rendez vous, Rashid started to have abdominal pain, vomitting and diarrhoea. The girl-friend, called, says she has the same problems… So, no romantic afternoon…

http://www.biology.ualberta.ca/facilities/microscopy/uploads/gallery/ESEM/10b-Staphylococcus-sp_mediu.jpg



Who is guilty?

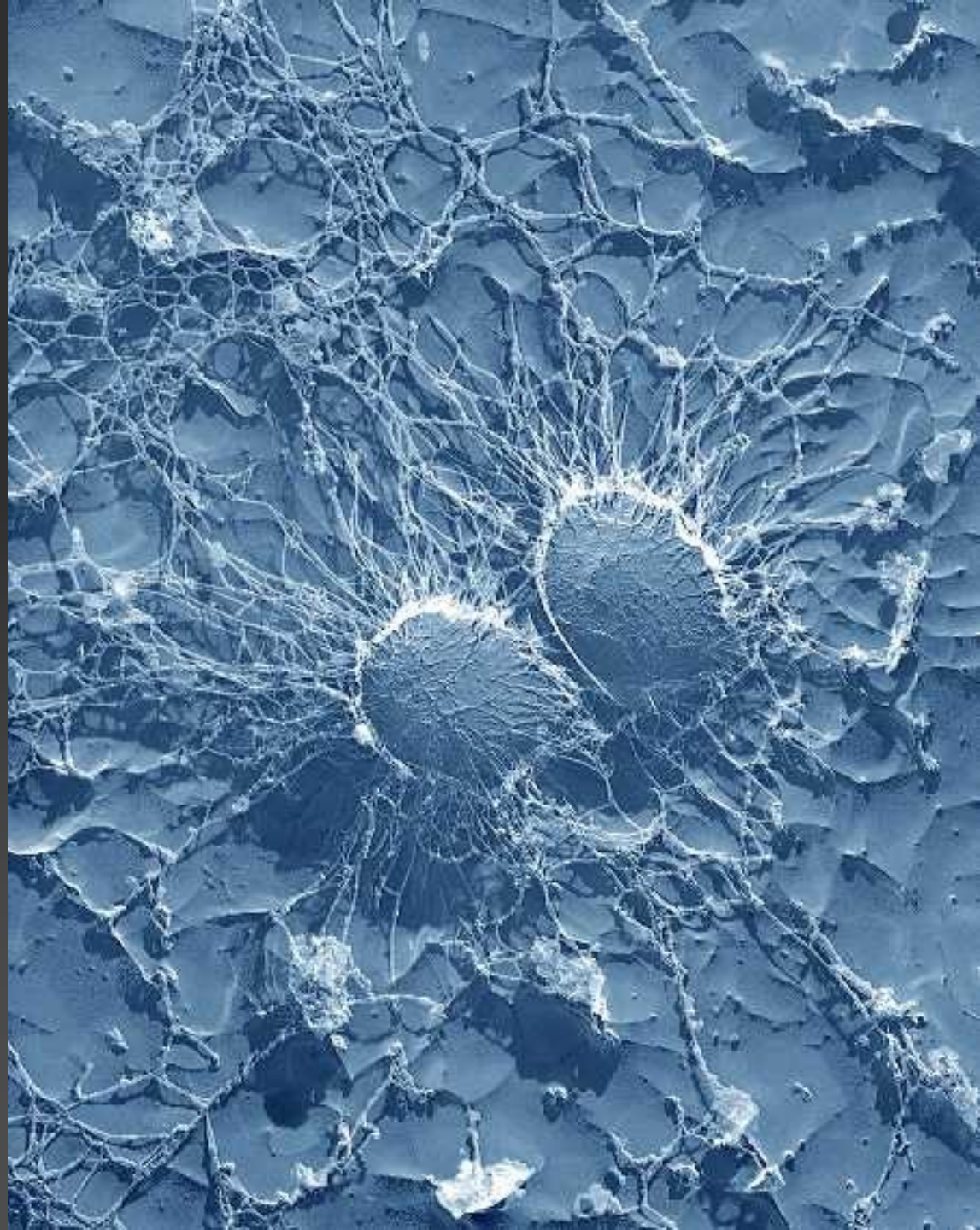
- It is *Staphylococcus aureus*

name from greek staphylé = grape

- This „golden staphylococcus“ often cases pyogene infections of skin, hairs, nails etc.
- Some strains produce enterotoxins, that act as so named superantigens
- Intoxication by a bacterial toxin usually starts quickly and finishes quickly, unlike a bacterial infection (e. g. salmonelosis)

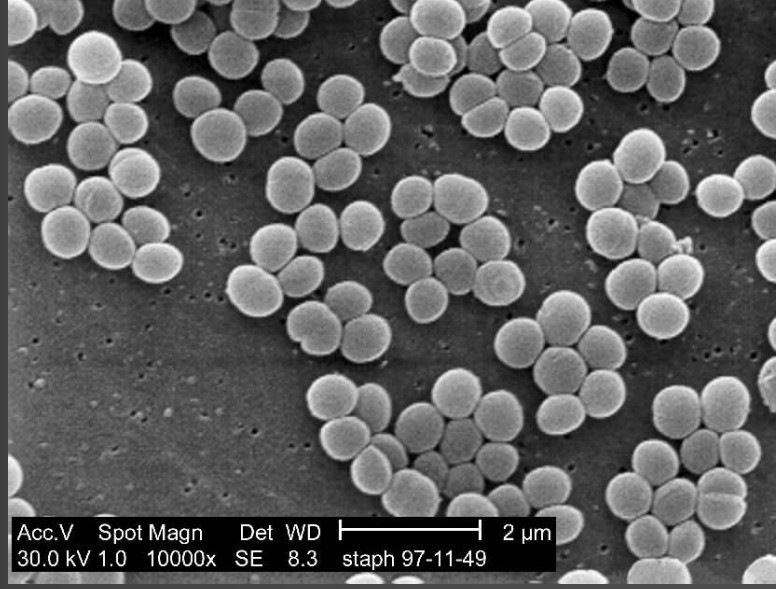
Of course, the cook, that prepared meals despite her blister, is guilty, too!

- Golden staphylococcus, 10 000 × magnified in an electron microscope



emu.arsusda.gov/typesof/pages/staph.htm

http://www.answers.com/topic/staphylococcus-aureus-01-jpg



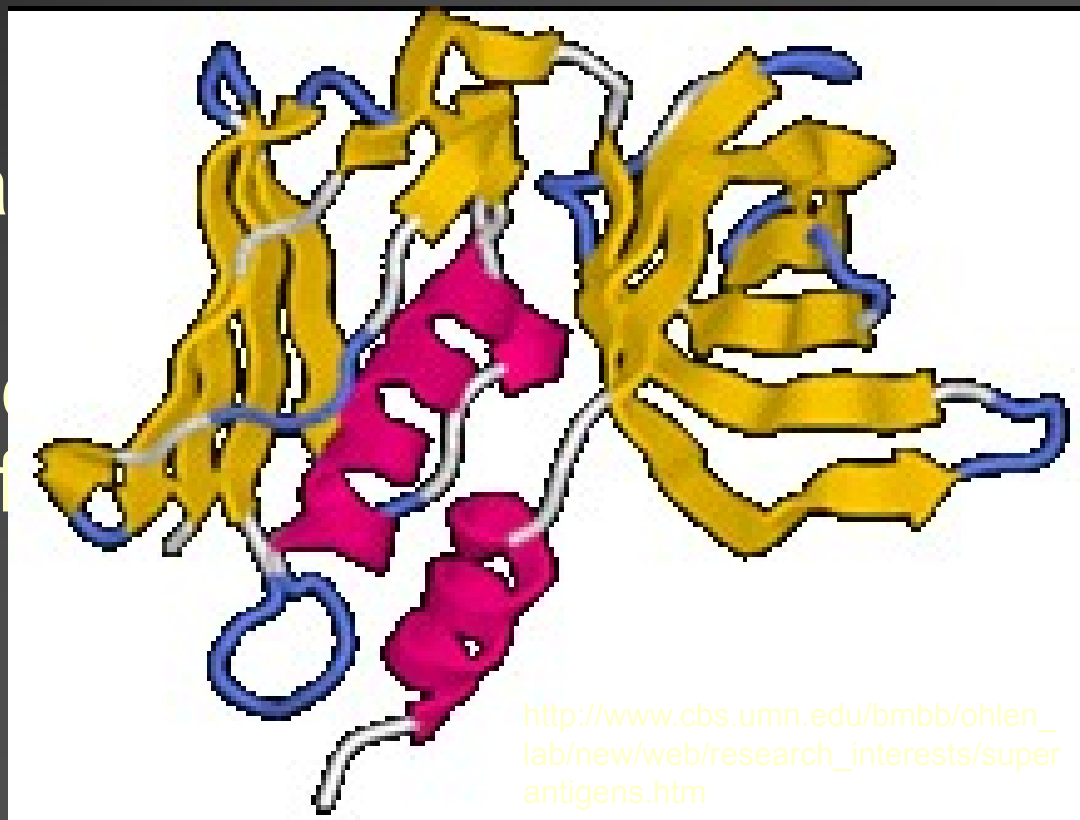
Story Two

- Student P. Z. is nervous: again, she has „her days“. Luckily, she has the cheap tampons, that she bought several days ago…
- Suddenly, she started to have shaking, faintness, fever. The room-mate found her lying on the floor, and called 155 (or maybe 112?). A rash emerged. The students is hospitalized on emergency unit of infectological clinic…

Who is guilty now?

- Again, it is *Staphylococcus aureus*, now a strain called TSST-1 (toxic shoc syndrome toxin)

- This toxin, too, is a superantigen
- It causes toxic sho typically in users of menstruation tampones

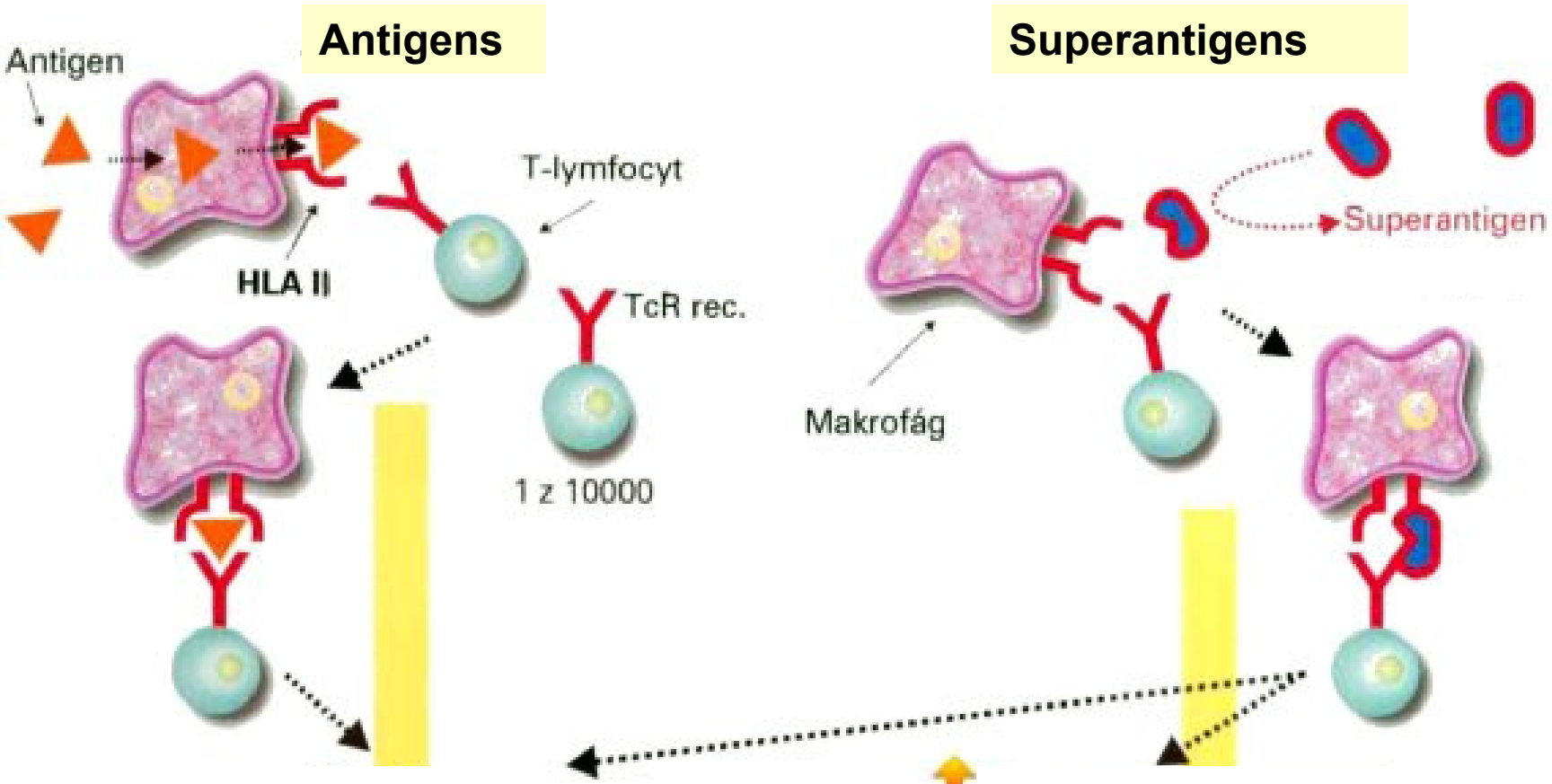


http://www.cbs.umn.edu/bmbb/ohlen_lab/new/web/research_interests/superantigens.htm

Superantigens

www.zuova.cz/informace/nrlpab16.php

Immunity response



T and B lymphocytes proliferation

PRODUCTION OF ANTIBODIES

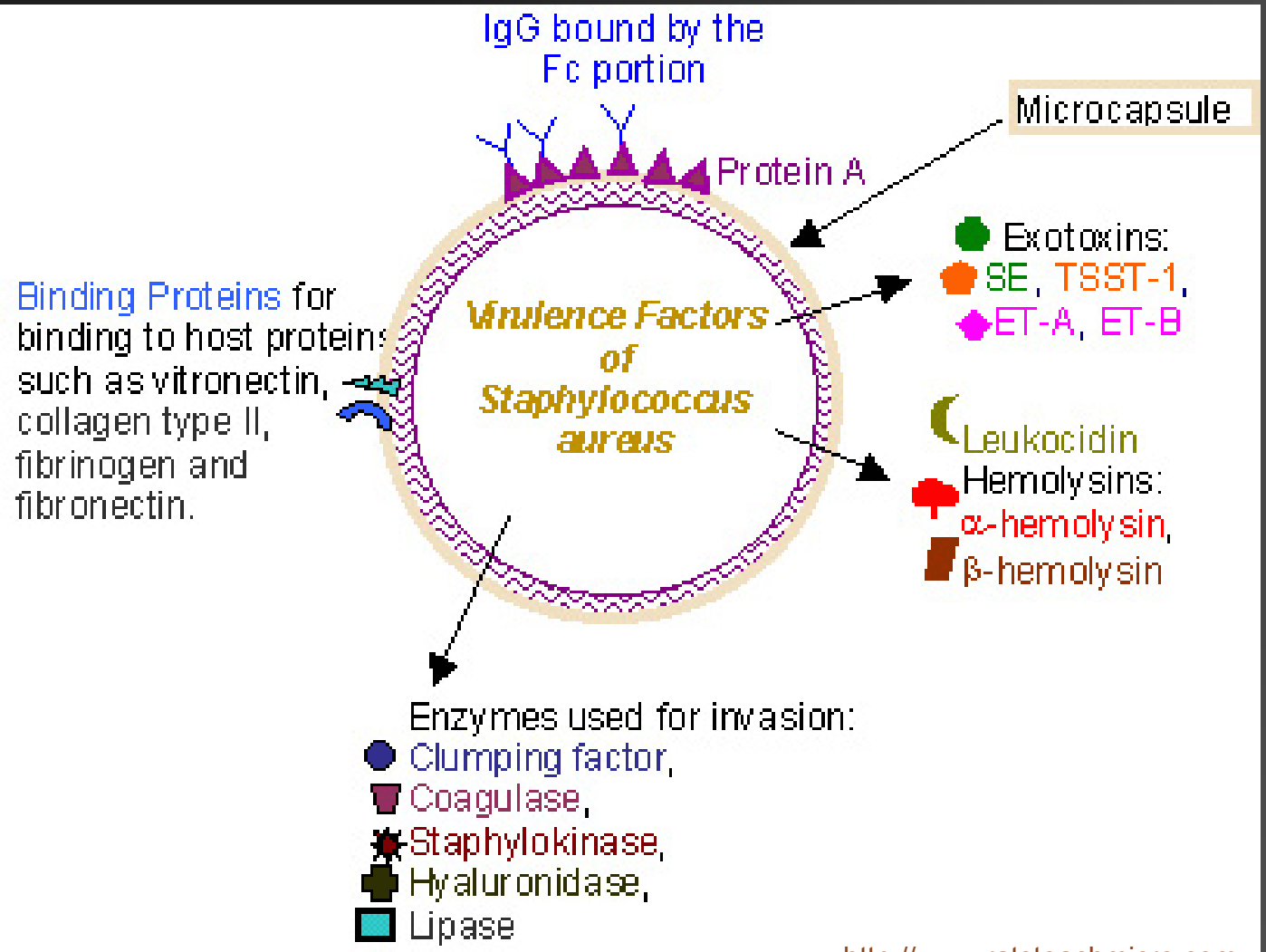
TNF production

SEPTICAL SHOCK

Staphylococcus aureus (golden staphylococcus)

- The only one routinely important for humans among so named **coagulase positive staphylococci**
- Causes skin, hair, nail infections, otitis externa, conjunctivitis, respiratory infections
- Sometimes also causes abscessi in tissues
- Some strains with **uncommon** virulence factors cause serious, but rare, diseases
- On the other hand, the microbe may be often found even on skin of healthy persons

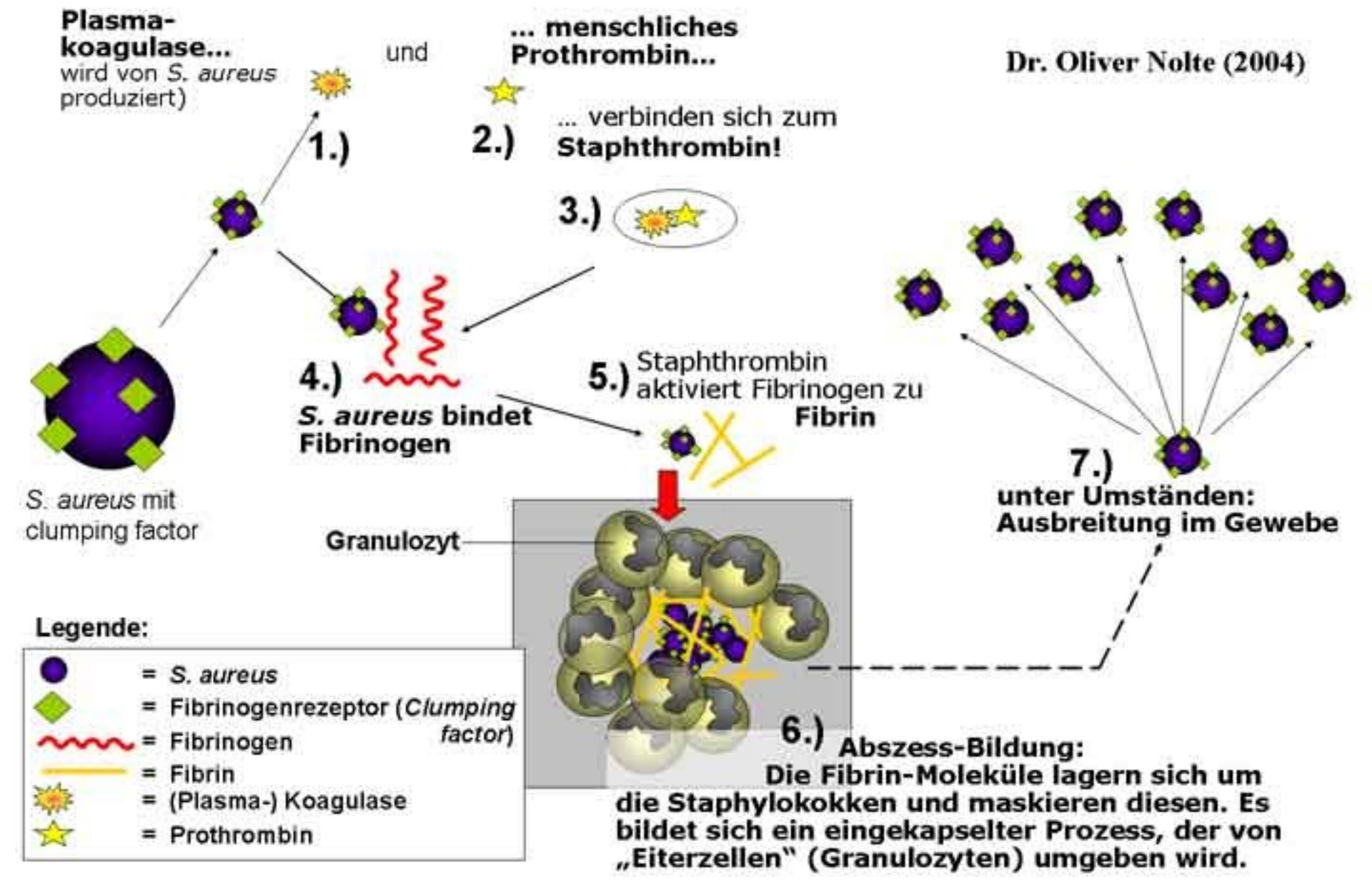
There exist plenty of virulence factors found in *S. aureus*…



…but only some of them are present in nearly 100 % strains; other are produced just by one strain among one thousand!

Abscessi

- Unlike streptococci, producing in tissues mostly uncoated **phlegmonae,** staphylococci form mostly coated **abscessi.** Formation of an abscessus (using so named clumping factor and plasmacoagulase – see further!) is in the next scheme, from a german website



http://www.autovaccine.de/abscess_formation/Abszess_b.jpg

Examples of infections caused by staphylococci: Impetigo…

http://pathmicro.med.sc.edu/fox/staph-impetigo.jpg

…bulous impetigo…

http://www.adhb.govt.nz/newborn/TeachingResources/Dermatology/BullousImpetigo/BullousImpetigo3.jpg

…otitis externa with a furuncle…

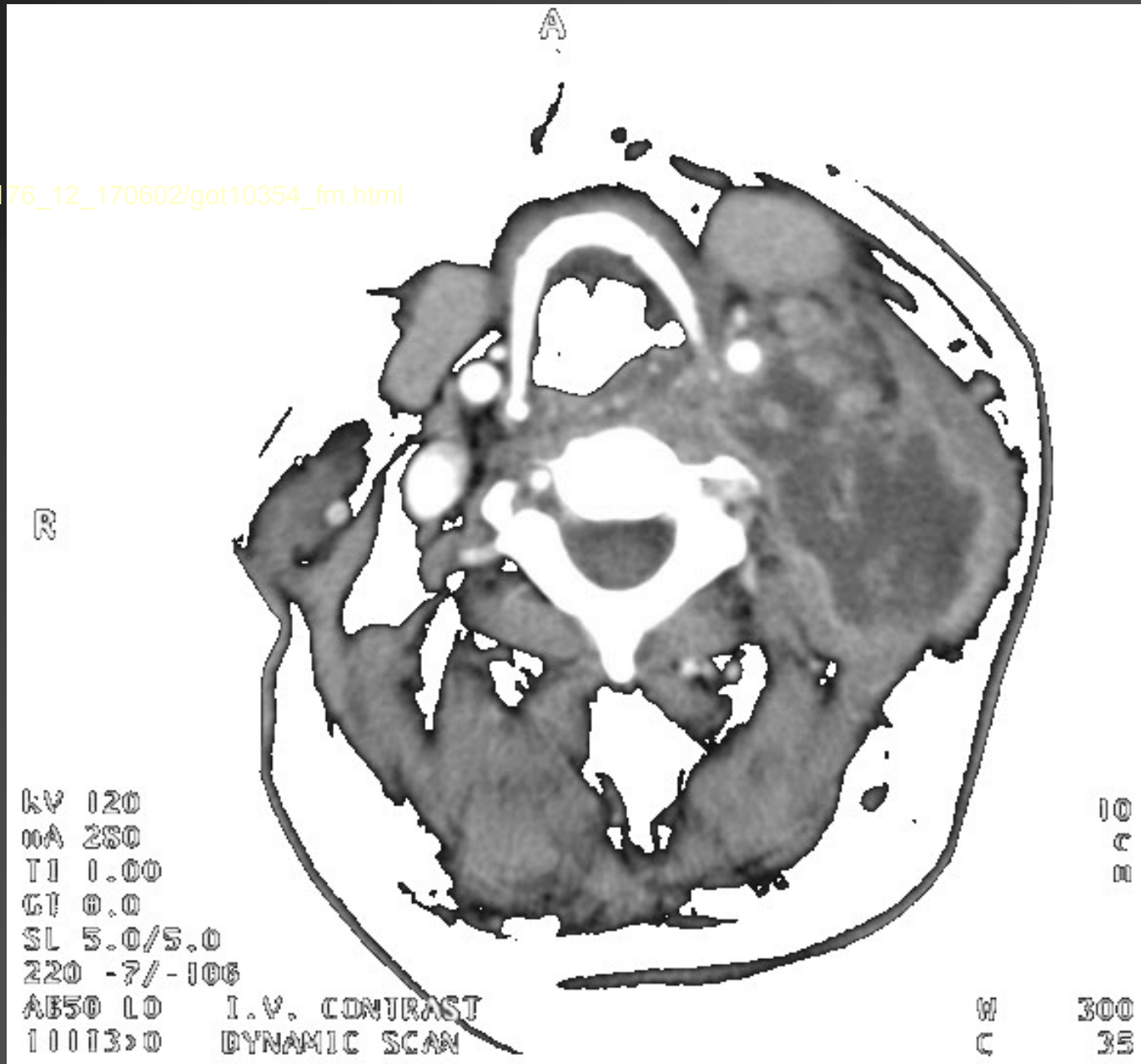
www.merck.com/mmpe/print/sec08/ch088/ch088c.html.

…or skin infection with crusts…

http://www.dermatology.co.uk/media/images/Infection_staphylococcus_crusting_chin_closeup.jpg

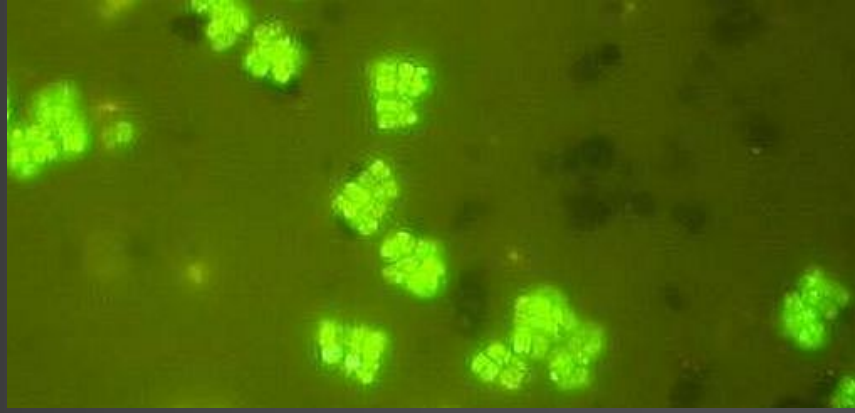
…but also brain abscessi

http://www.mja.com.au/public/issues/176_12_170602/got10354_fm.html



Clinical characteristics: *Staphylococcus* sp. (coag.-neg.)

Story Three



http://www.zuova.cz/informace/pic/ompovabac20b.jpg

- Young man F. B. recovers after a severe traffic accident. He has two venous cateters for infusion nutrition and blood taking.
- Suddenly, his status worsened acutally, high and quickly changing fevers – the ward doctor has suspicion for septicaemia and takes blood for blood culture
- After catether change and antibiotic treatment the status improved again

And who is guilty now?

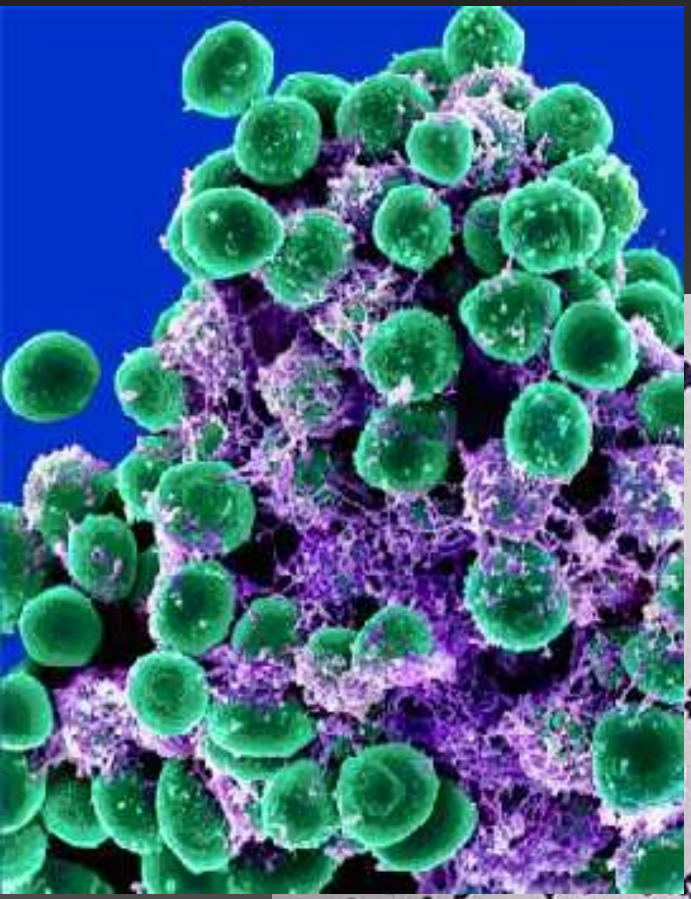
- The guilty is *Staphylococcus epidermidis*, the most common among coagulase negative staphylococci
- Coagulase negative staphylococci beong to the same genus as „golden staphylococcus“
- The are much less pathogenous
- In last decades, they started to be very important causative agents of infections in weekened persons, mostly as hospital infections
- Often forms biofilm on venous catheters

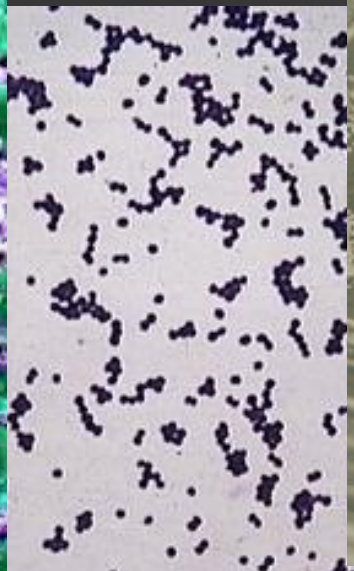
Why „coagulase negative staphylococci“? See later…

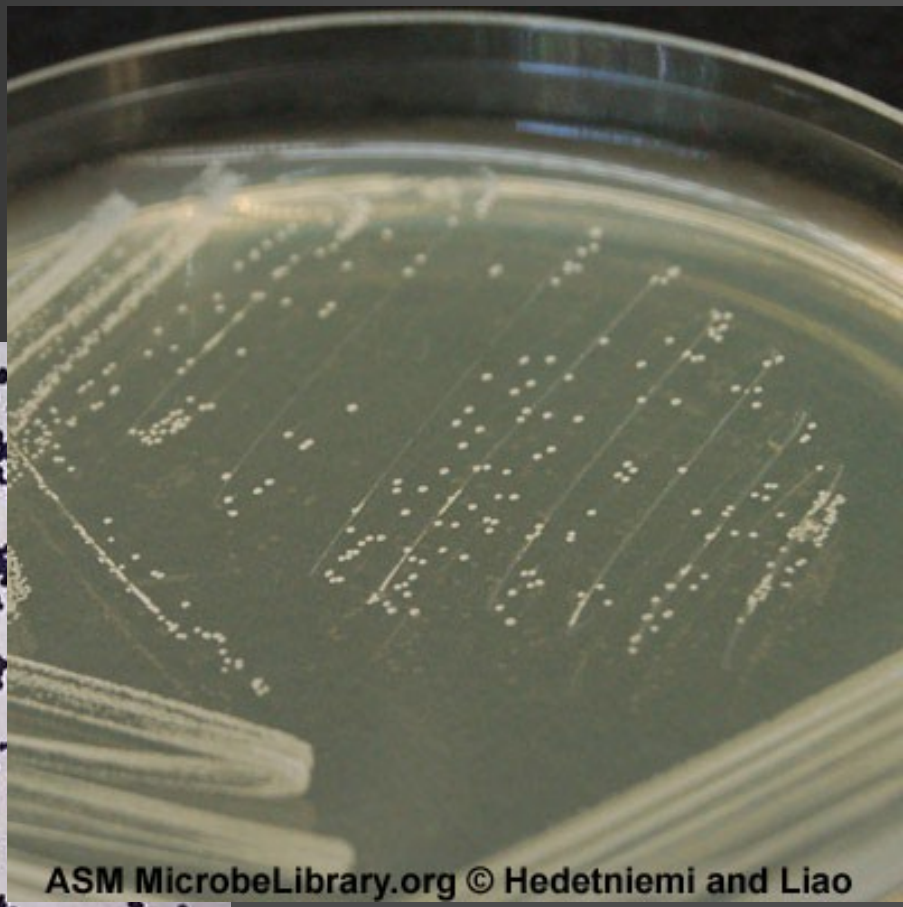
Staphylococcus epidermidis

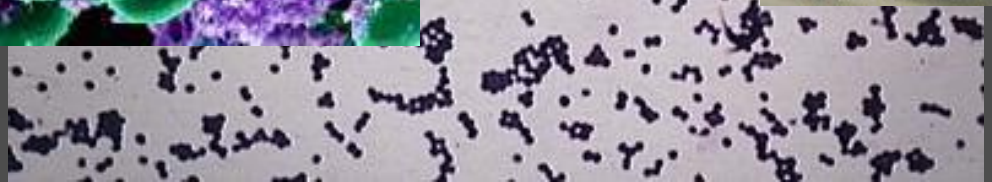
http://www.microbelibrary.org

http://www3.niaid.nih.gov









http://www.difossombrone.it

Coagulase negative staphylococci

- Coagulase negative staphylococci (*Staphylococcus epidermidis, S. hominis, S. haemolyticus* and about fourty other species and subspecies) are the main parts of the common skin microflora.
- Nevertheless, they may cause UTI (mostly *S. saprophyticus*), wound infections, catheter septicaemiae etc.
- So, the finding has a different meaning e. g. in nasal cavity (or in stool), in the urine, and of course, in blood culture.

http://www.szu.cz/cem/zpravy/zpr0905/s

There are many species of staphylo-cocci today

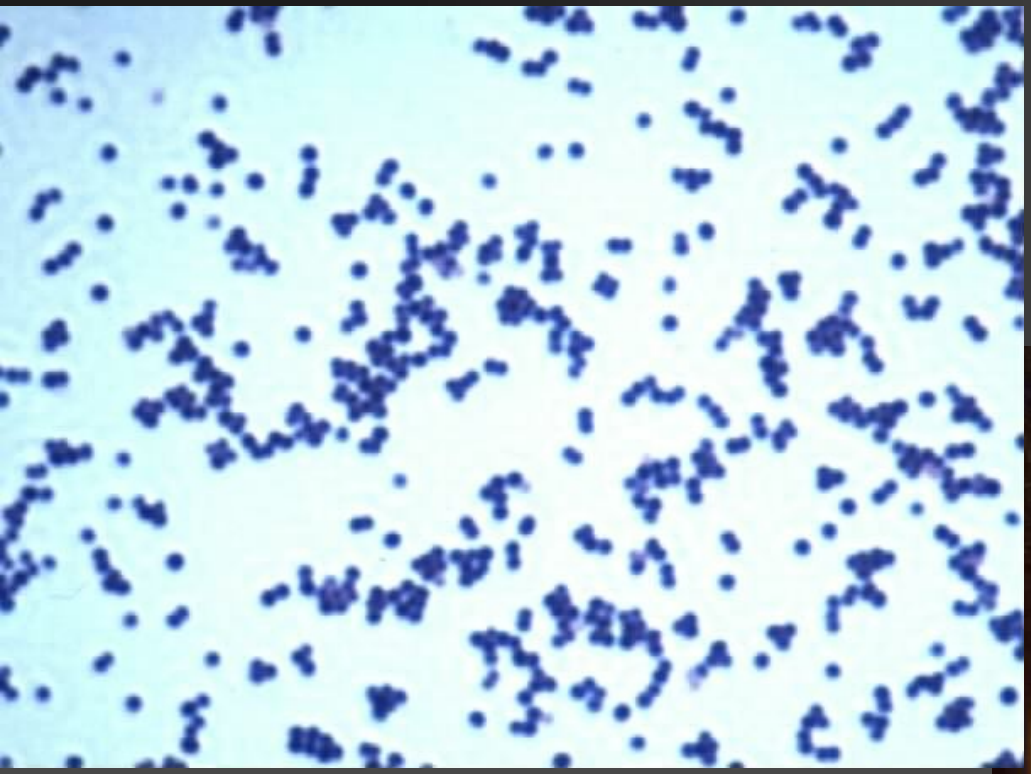
- E. g. *S. simiae* was found by Moravian scientists in rectal swabs of *Saimiri sciureus* monkey in a ZOO on Saint Hill at Olomouc

Diagnostics of staphylococci

Description of criminals (diagnostics) 1

- Microscopy: grampositive cocci
- Cultivation: on BA colonies 1–2 mm, slightly convex, butter consistence, white, or (mostly in golden staphylococcus) goldish
- Biochemical tests: catalase positive, oxidase negative, it is possible to differenciate individual species biochemically
- Antigen analysis and special tests maybe very helpful at the diagnostics

Photos from Criminal Database



www.medmicro.info (our webpage)

Differential diagnostics of staphylococci I: from „unknown bacterium“ to „*Staphylococcus*“

Survey of microbiological diagnostics of a staph infection

- *(Microscopy of SPECIMEN (e. g. sputum)*
- Microscopy of isolated STRAINS

Now, we are able to distinguish G+ cocci from others

- (Description of colonies on blood agar)
- Catalase test (Staphylococci × other G+ cocci)
- Growth on BA with 10 % NaCl

Now, we have differenciated staphylococci from the other G+ cocci

- Differenciation of "golden" *Staphylococcus* from coagulase negative species
- Species diagnostics of *Staphylococcus*
- Atb susceptibility testing *(when Staph is a pathogen)*

Searching for criminal microscopically in the specimen

- We observe a Gram stained microscopic sputum preparation
- We search for Gram-positive cocci in clusters, but also for leucocytes (polymorphonuclears mainly), typical for bacterial inflamation)

Photo: O. Z.

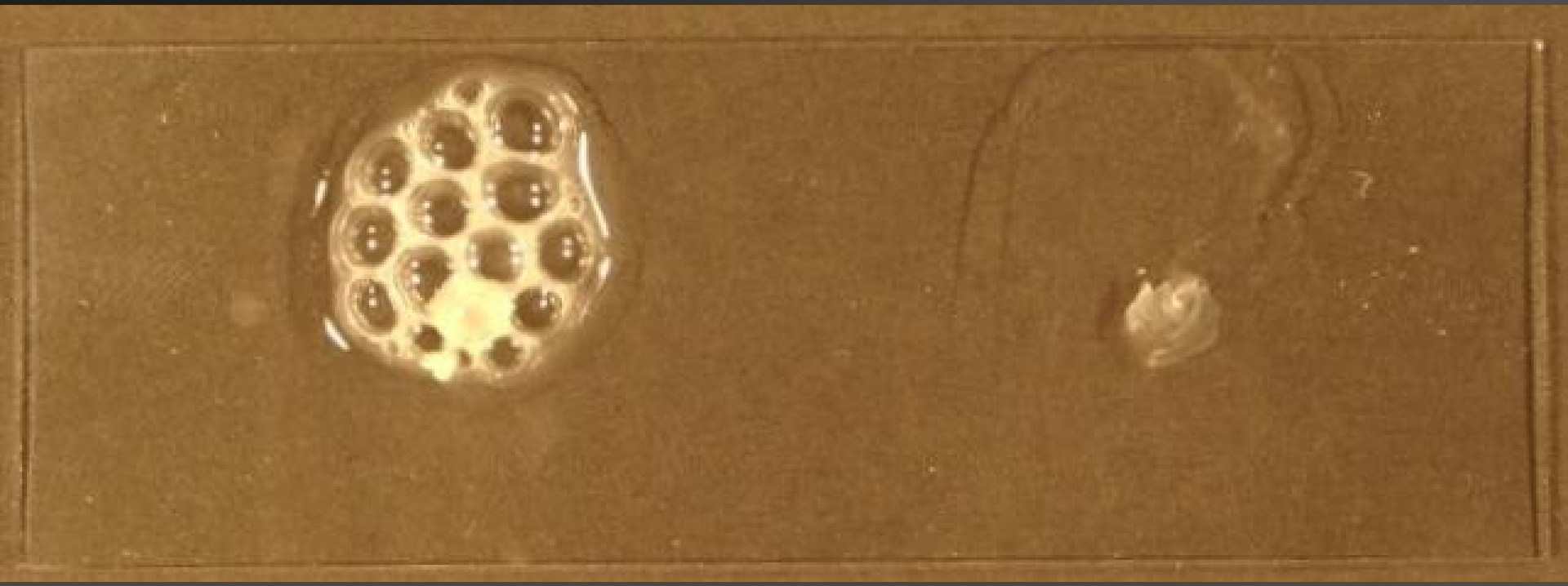
Singling out of other suspects (differencial diagnostics 1)

- Gram stain differenciates gram-positive cocci from other shape/cell wall type bacteria
- Positive catalase differenciates staphylococci from streptococci and enterococci
- The same (and even better in a mixture) is cultivation on BA with 10 % NaCl
- *For orientation we can also use the fact that colonies of other G+ cocci are neither white nor goldish, and in microscopy, there do not have clusters*

Gram stain (repeating)

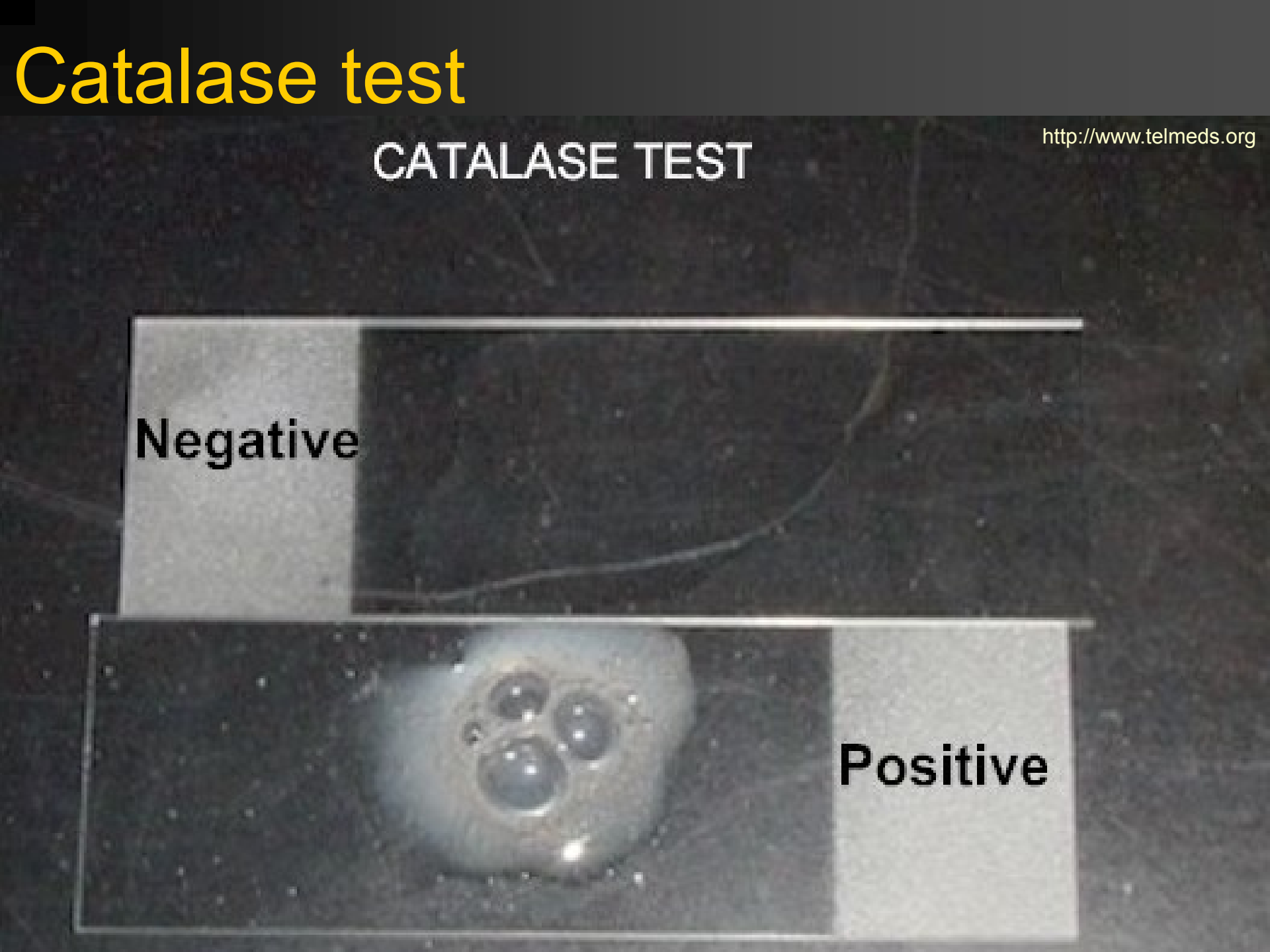
- Gram stain: we make a smear (using a small drop of saline), we let it dry, we fixate by a flame, then we stain: Gram 30 s, Lugol 30 s, alcohol 15 s, water, safranin 60 s, water, dry, imersion obj. 100× magnifying)
- Now, we can exclude all object that are gram negative and/or rods, eg. that do not belong into group of „G+ cocci“

Catalase test (for remembering)



http://memiserf.medmikro.ruhr-uni-bochum.de

Catalase test



Survey of diagnostics (simplified)

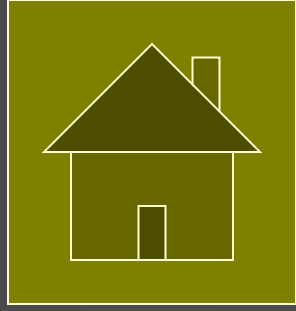
(or other tests)

Enterococcus or

Description of colonies on BA

- Description of colonies on blood agar does not have a specific place in differential diagnostics of staphylococci. Nevertheless, it is usefull, as it can lead us to certain suspicion (e. g. stapyhlococci have rather whitish/yellowish collonies, unlike grey/colourless streptococci)

Discrimination between *Staphylococcus* and *Streptococcus/Enterococcus*



- In a bacterial mixture, a *Staphylococcus* may be selected using growth on BA with 10 % NaCl; other G+ cocci do not grow.
- If a pure strain is available and we require a quick diagnostics, catalase test catalase test may be used (a colony is mixed with a drop of hydrogen peroxyde, bubbles = positivity).

Attention! By jumping over the previous steps, we would do a mistake. Positive catalase test is common in many bacteria. Only in a known G+ coccus it is possible to use it for diagnostics!

Differential diagnostics of staphylococci II: steps inside genus *Staphylococcus*

Singling out of other suspects (differencial diagnostics 2)

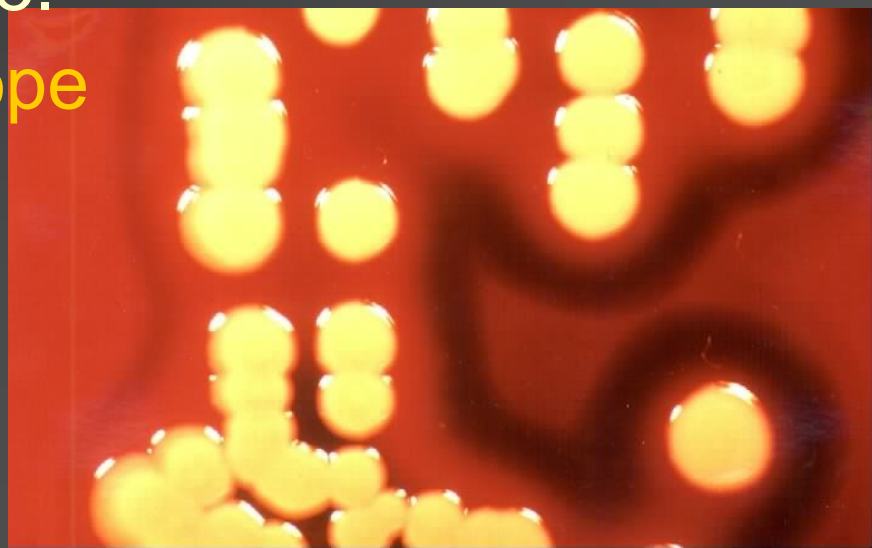
- Free plasmacoagulase is positive in „golden staphylococcus“, negative in coagulase negative ones (here the origin of their name)
- Clumping factor or bound plasmacoagulase is used in the same situations, but is worse
- Comercial tests based on antigen analysis are very good on the other hand (but expensive)
- Hyaluronidase is not only good, but cheap, too

Less sure tests: useful in searching, but cannot be used as a proof for court!

- Hemolysis: Coagulase negative staphlococci may produce delta haemolysin, „Golden“ staphylococci may produce alfa, beta and delta haemolysin, so their haemolysis uses to be stronger.
- Goldish colour of colonies and their larger diameter may be useful, too.
- Larger clusters in microscope

  are also typical

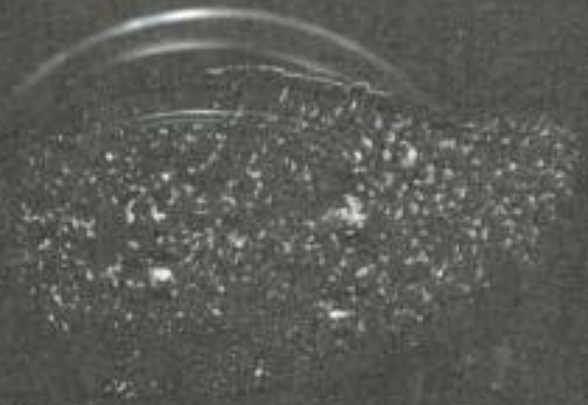
  for „golden“ staphylococci



Clumping factor or bound plasmacoagulase – quick

- Colonies are mixed with a drop of rabbit plasma on a slide
- Positivity is formation of „clusters“ in plasma drop (see next screen)
- In fact, it is not a COAGULATION, but AGLUTINATION of plasma
- The test is not very sure

Clumping–Factor positiv

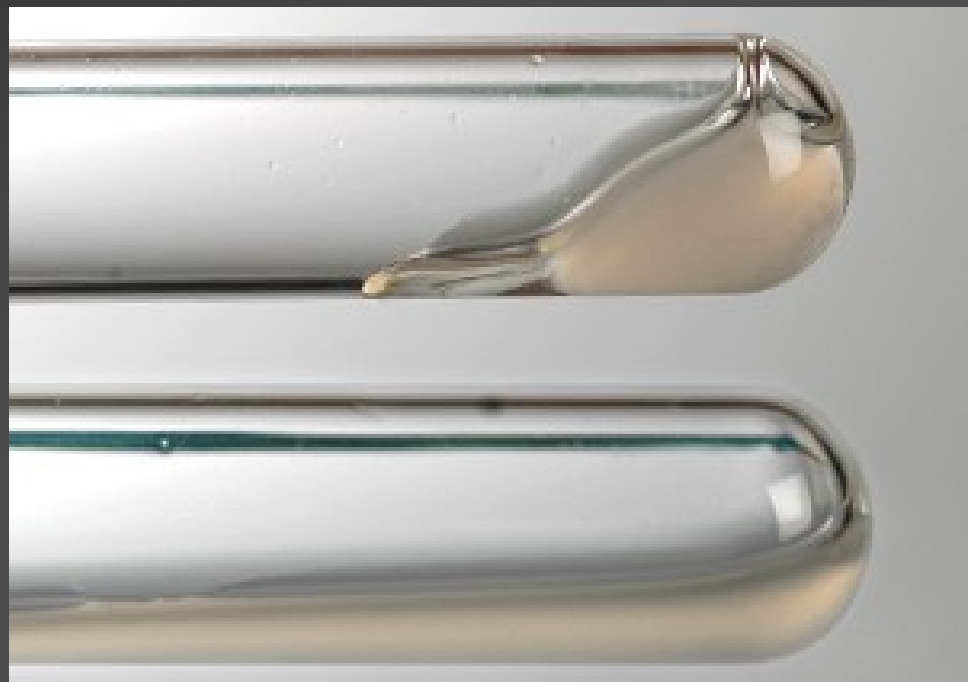


http://memiserf.medmikro.ruhr-uni-bochum.de

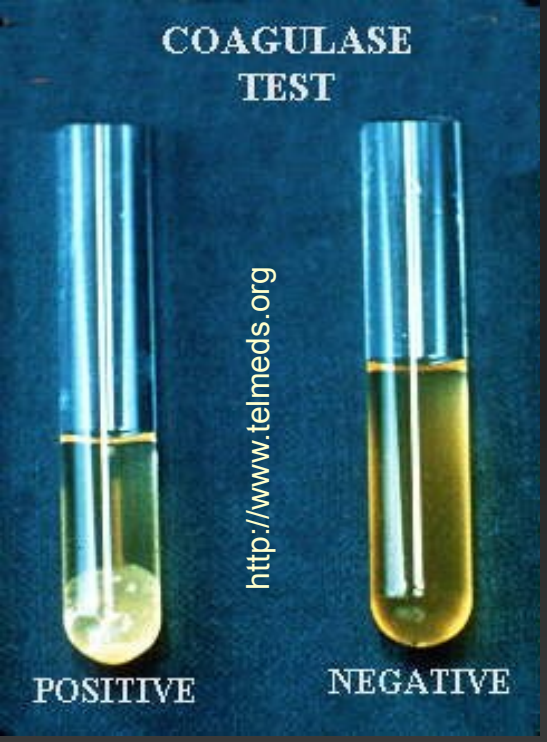
Clumping–Factor negativ

Free coagulase – classic

- The most classical among diferenciation test for "golden" *Staphylococcus* (the coagulase positive *Staphylococcus*)
- Colonies, taken by a loop, are mixed with rabbit plasma in a test-tube
- When the plasma coagulates (gel consistence), the strain is coagulase positive

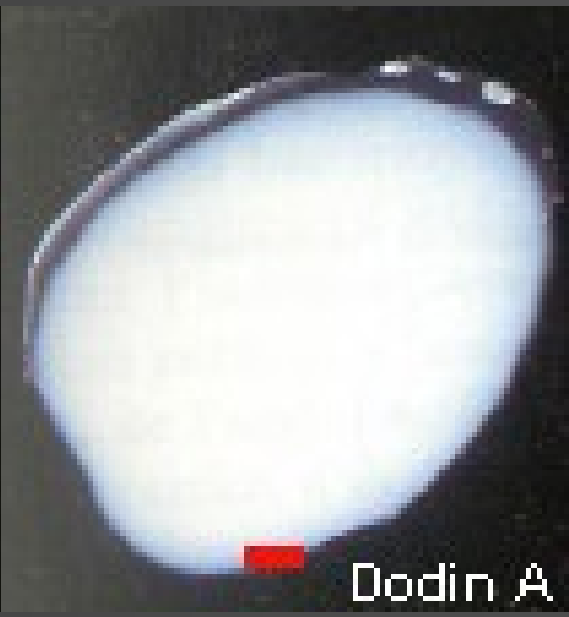


More pictures of plasmacoagulase



Comercial tests, e. g. Staphaurex (not in the practical)

- The way of using them is the same as in the clumping factor test, but they are even more sure than free coagulase
- Unfortunatelly, they are quite expensive

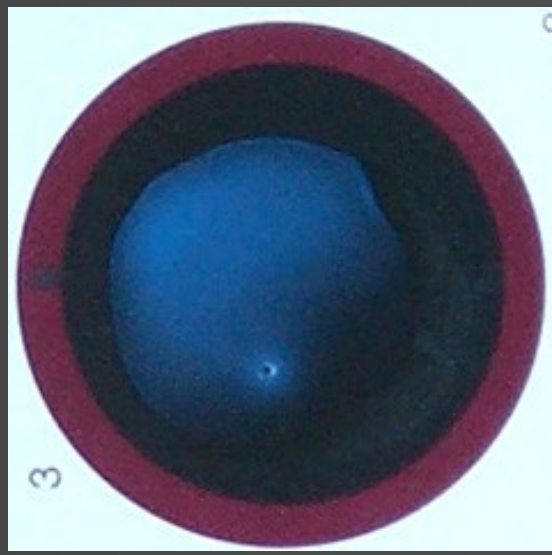


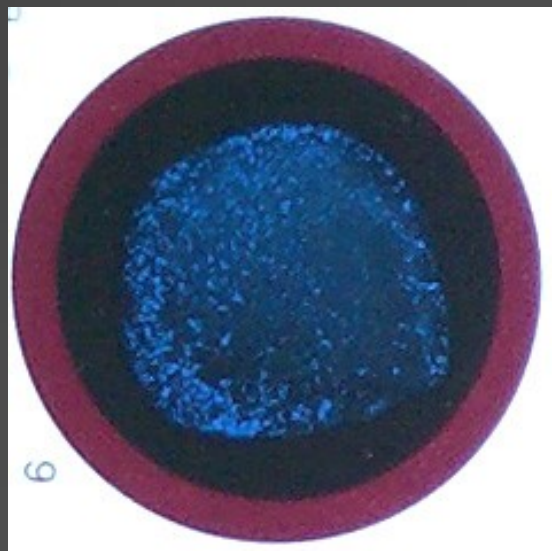
www.microbes-edu.org

Staphaurex kit and results

http://www.pathologyinpractice.com

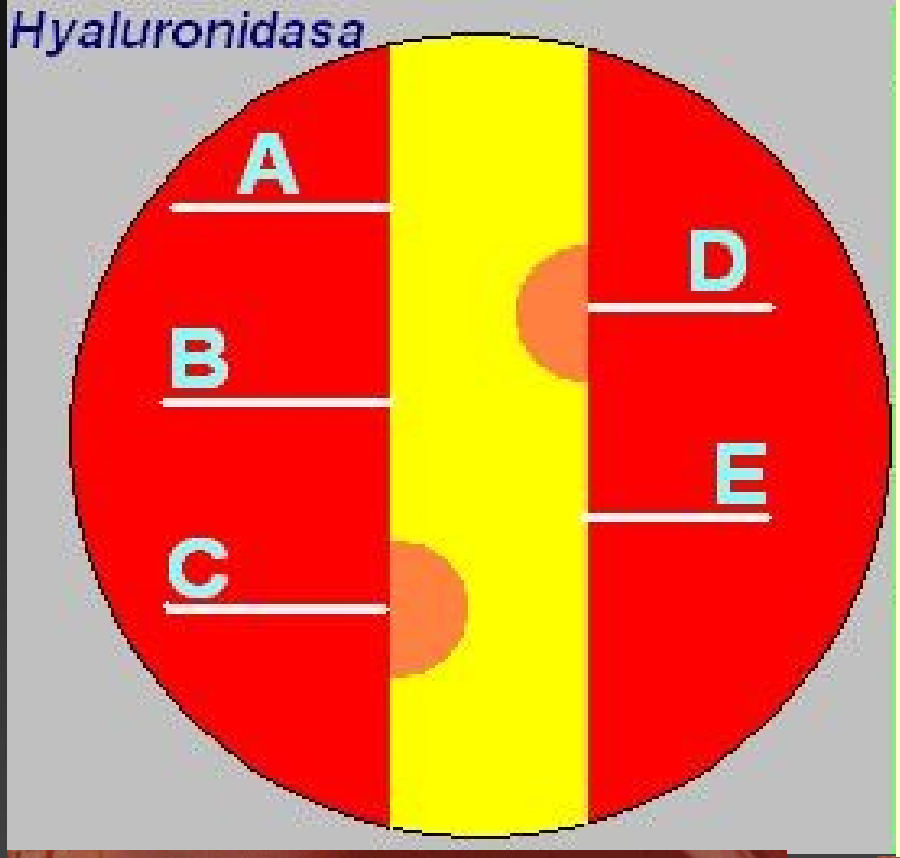
www.microbes-edu.org





Hyaluronidase (decapsulation)

- An elegant test, its principle is the fact, that the hyaluronidase, produced by S. aureus (but not coagulase negative staphylococci) breaks the capsula of encapsulted bacteria. We use *Streptococcus equii*, a streptococcus that is not pathogenous for humans
- Lack of a capsulla is seen as change of feature of streptococcus (no „mucosity“)



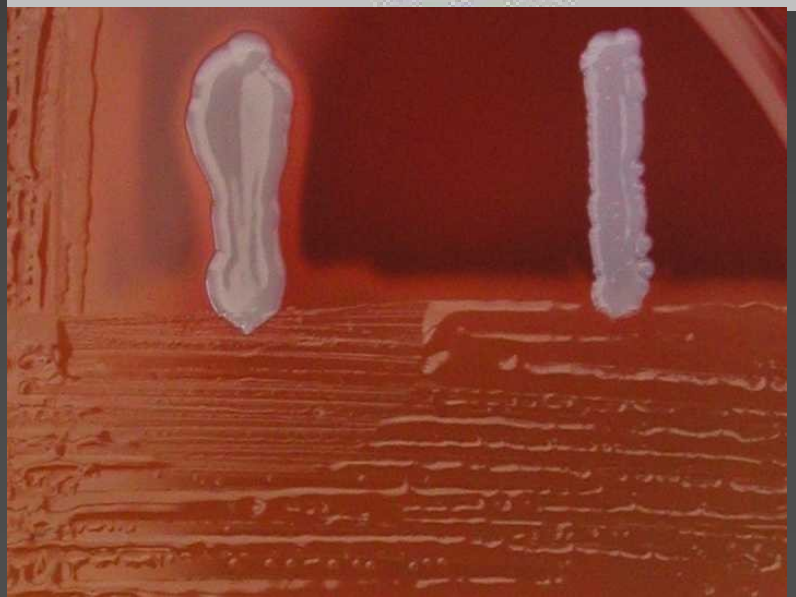
Yellow – *Streptococcus equi* (mucous)

White – tested staphylococci

Results for this example:

C and D are positive (*S. aureus*)

A, B and E are negative (coagulase negative staphyl.)



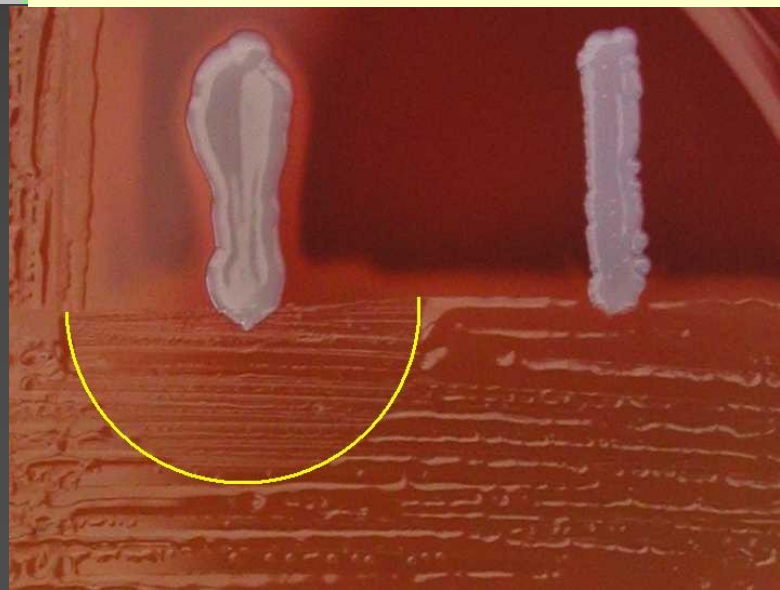


Foto: O. Z.

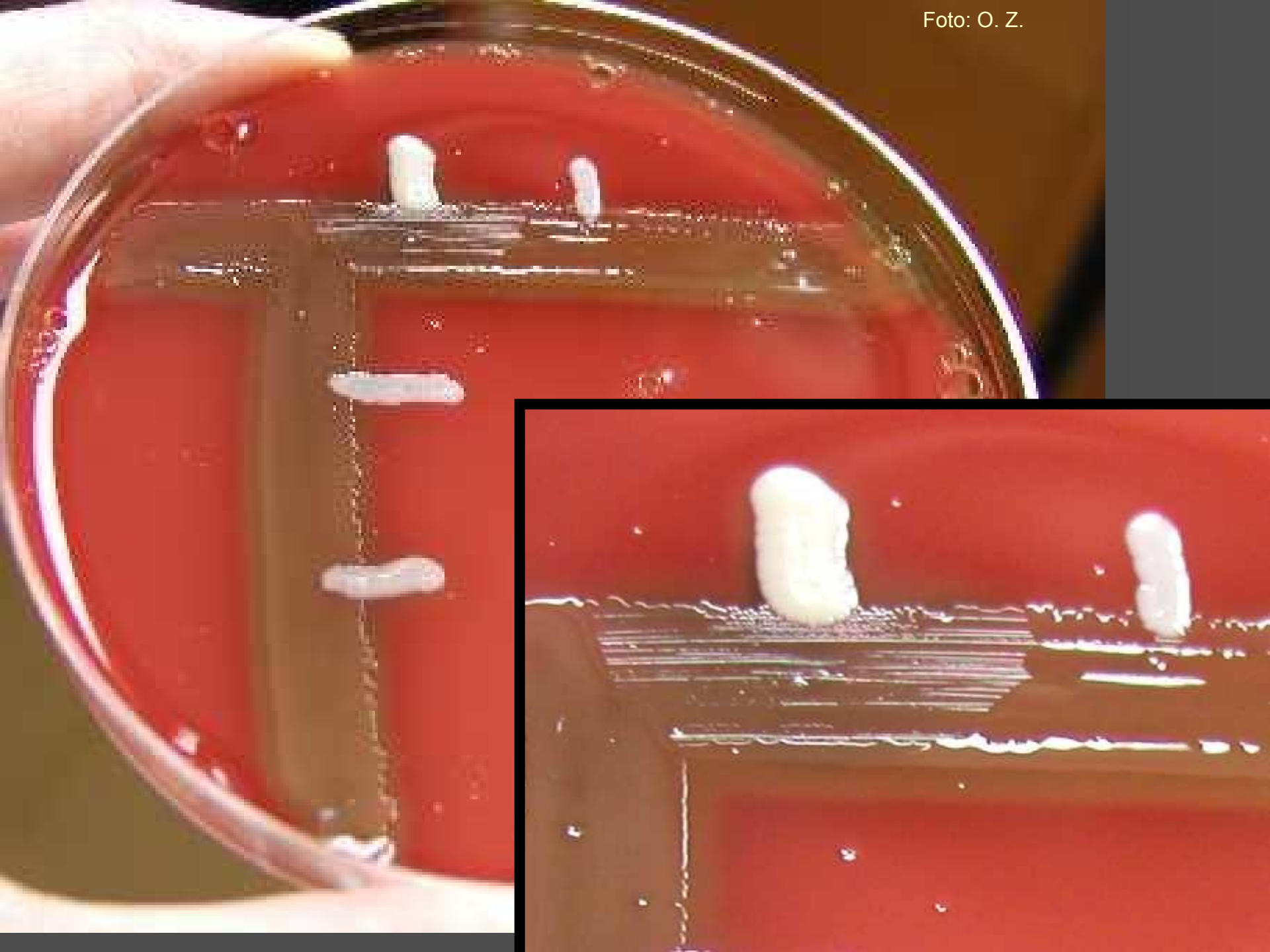


Foto: O. Z.

Survey of methods distinguishing S. aureus from CONS (coagulase negative staphylococci)

- Clumping factor test: a drop of plasma is mixed with a tested strain on a slide
- Plasmacoagulase test: strain is mixed with rabbit plasma in a test tube. Prelimnary reading is done after 4 h and deffinitive reading after 24 h. Coagulated liquid = positive
- Hyaluronidase test: Positive strain dissolves the mucosity of an encapsulated strain (a horse streptococcus *Streptococcus equi* is used mostly for this test)

It is not „The Golden“. What now?

- Usually we simply say „it is a coagulase negative one“ and we do not insist on species diagnostics
- When species would be important (e. g. in blood cultures), it can be performed biochemically
- In Czech conditions, mostly STAPHYtest 16 (Erba-Lachema) is used

Mutual differenciation of staphylococci

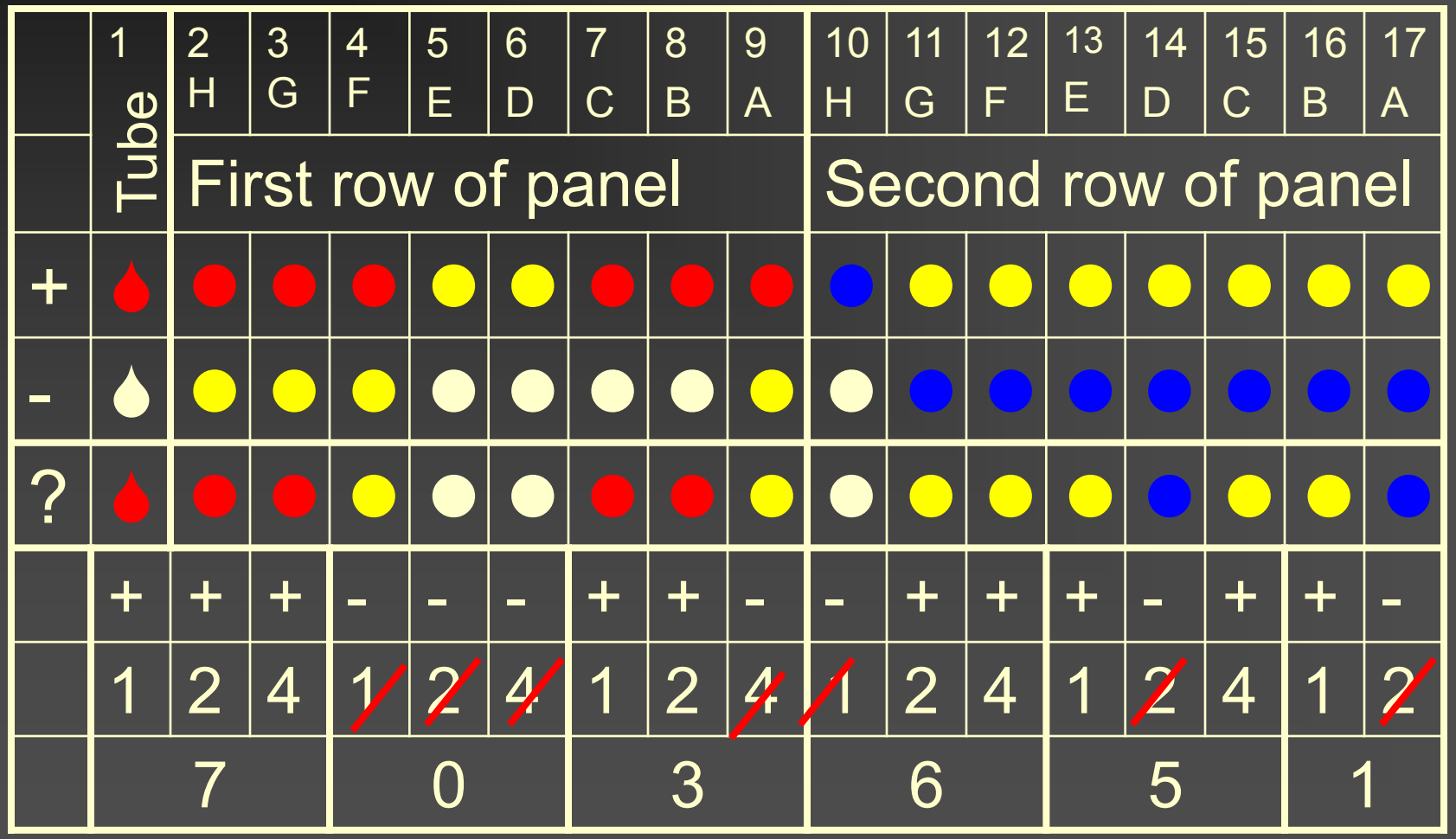
- STAPHYtest 16 is the most typical Czech variant of a biochemical testing systém of staphylococci. It should be done according to guidelines. It certifies the identity of "golden" Staphylococcus and it identifies the other.
- Normally it is useless to diagnose „golden staphylococcus“ by STAPHYtest 16 , tests of tasks 6a, b and c, or comercial tests are rather used for this
- So the test is used for diagnostics of coagulase negative staphylococci

STAPHYtest 16 – how to read it

- Attention – despite its name, there are 17 reactions in it. We start by reading VPT test in a test tube. Red fluid in the test tube = positive VPT, colorless fluid = negative
- First row of the STAPHYtest = 2nd–9th reaction
- Second row of the STAPHYtest = 10th– 17th r.
- Count the code and compare with the codebook
- The code consistis of six numbers. Five of them are based on triplets of test, the sixth is based on the last two tests (16 + 17)

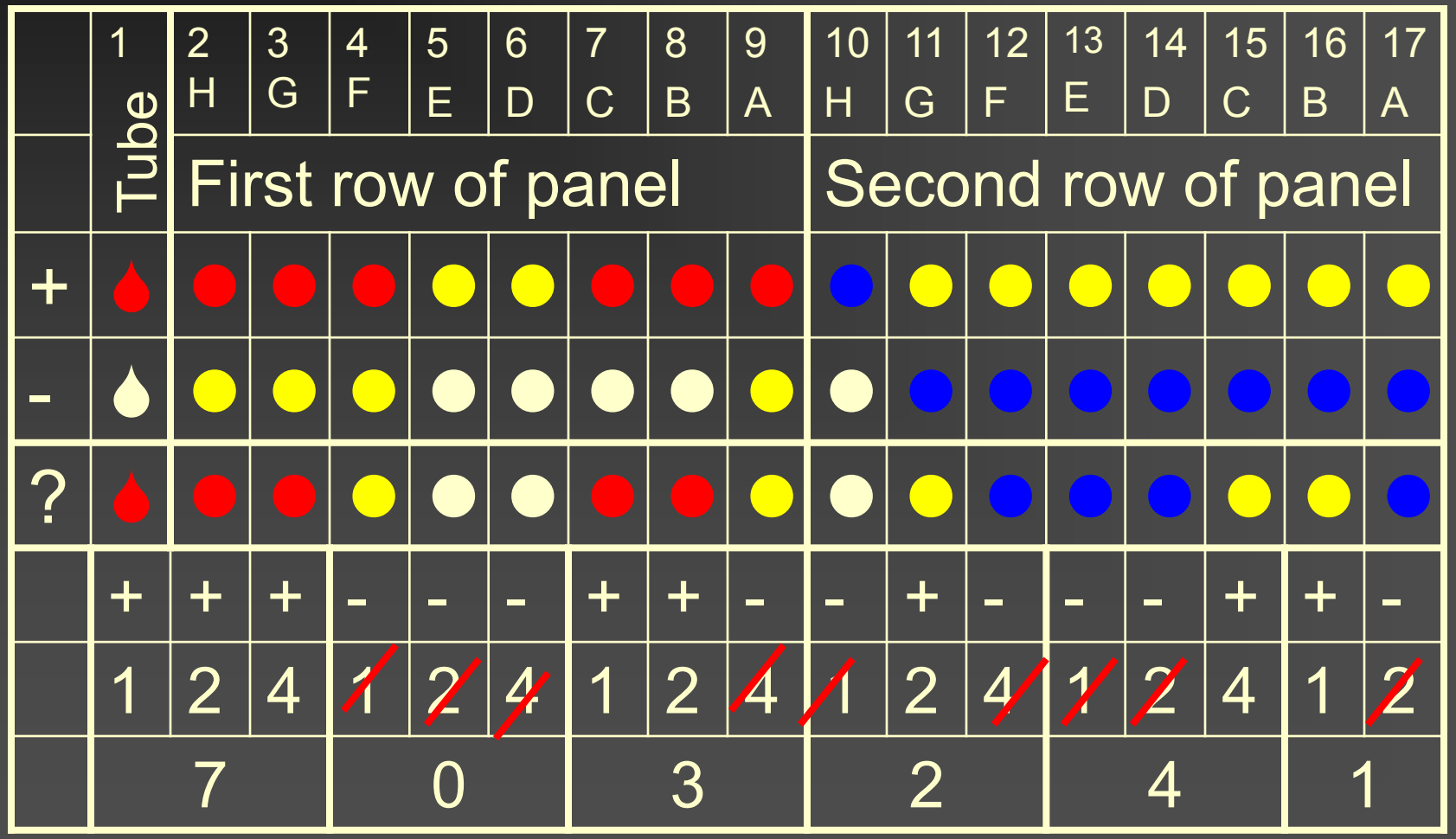
An example of a result

(703 651 = *S. aureus*, 99.8 %, T_{in}=1,00)



Another example of a result

(703 241 = *S. epidermidis*, 97.95 %, T_{in}=1,00)



Api Staph – in some countries used equivalent of STAPHYtest 16

- Not regarding the producer, the principle is the same – combination of many enzymatic reactions, that can be seen as colour change

http://www.microbes-edu.org

Another variant of a API-Staph

- The previous one was an API-Staph for automatic reading in a photometer. This one is for „ocular“ reading

Antibiotic susceptibility testing and antibiotic treatment of staphylococcal infection

Susceptibility testing

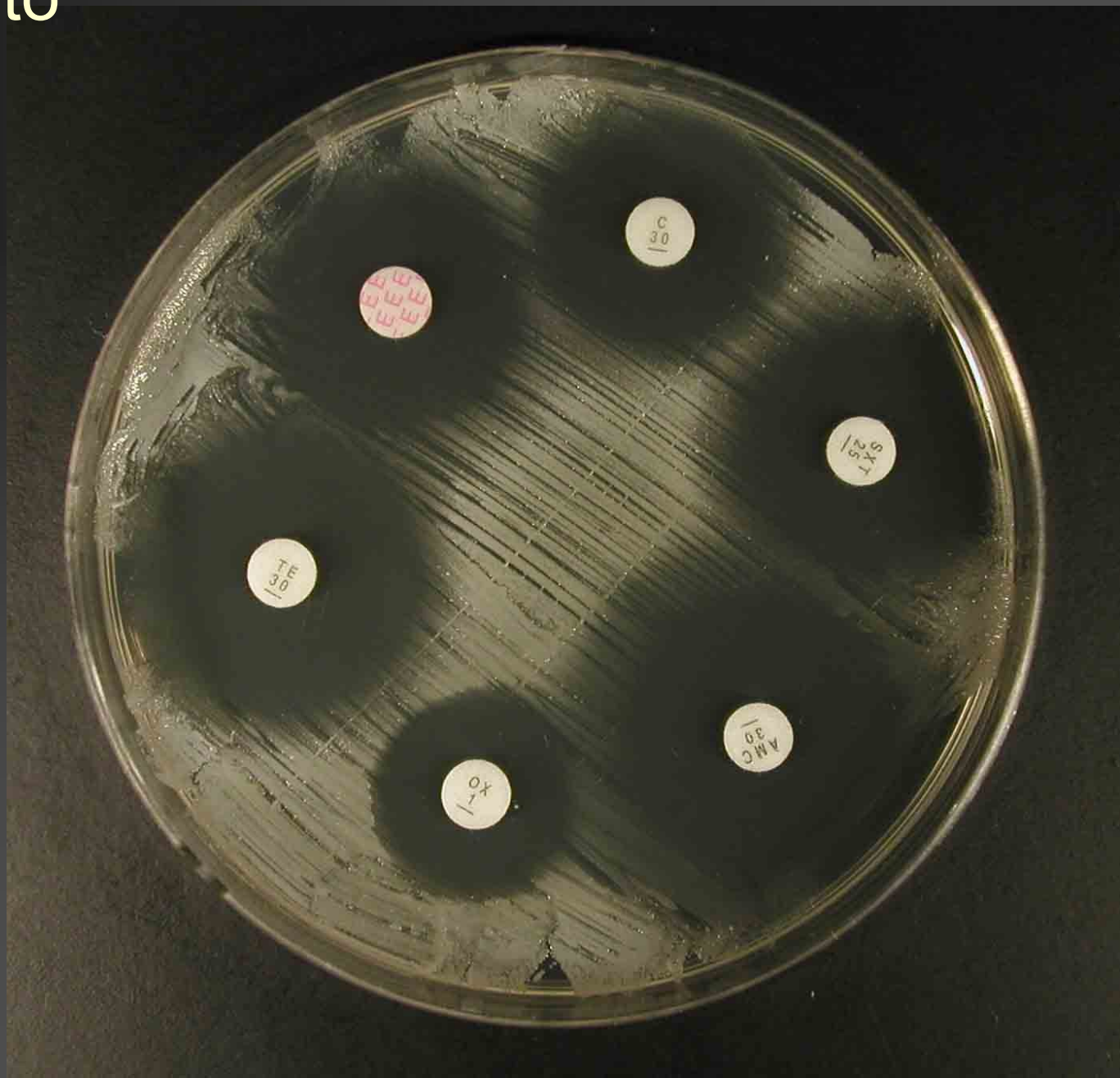
- To check secondary resistances, we mostly use a diffusion disk test – we measure the inhibition zones and compare with reference zones
- Among used antibiotics we use commonly e. g. OX = oxacilin, VA = vankomycin, RD = rifampicin, KF = cefalotin, MY – linkomycin CN = gentamicin
- Of course, antibiotic testing is only performed for pathogens (= not for staphylococci belonging to common microflora)

Reference zones for the most common antibiotics

Antibiotic	Abbr.	Refer. zone
Oxacilin (protistaf. penic.)	OX	13/18 mm
Vankomycin (glycopept.)	VA	12 mm
Rifampicin (rifamycine)	RD	20 mm
Cefalotin (cephalo. 1. g.)	KF	18 mm
Linkomycin (lincosamid)	MY	21 mm
Gentamicin (aminoglyk.)	CN	15 mm

OX: 13 mm *S. aureus*, 18 mm coagulase negative st.

Ilustration photo



Quantitative and qualitative tests

- As mentioned, usually we use a qualitative test (diffusion disc test). Nevertheless, it is also possible to use quantitative tests (microdilution test. E-test)

According to situation, we use either

←qualitative, or

quantitative tests →

http://www.microbes-edu.org

Usual law: worse pathogen – better susceptibility

- You will probably see, that a worse patogen (*S. aureus*) uses to be more susceptible than the milder pathogen (coagulase negative stafylococcus). It is logical: milder pathogenicity shows better adaptation, ability of a microbe to coexist without causing a disease → being used to common antibiotics
- It is not absolute! There are nicely susceptible *S. epidermidis* strains, and MRSA.

Anti-styphylococcal drugs

- In staphylococci, the drug of choice is oxacilin, in UTI cefalosporins of first generation. Often used macrolids are good in allergic persons only, lincosamids are good in locomotor system infections and aminoglycosides in combination only. Glykopeptidic antibiotics (vankomycin and teikoplanin) are a reserve. They are used in strains resistant to oxacilin, so named MRSA and MRSKN. In strains resistant even to glycopeptices, or in patients that has contraindications, newer antibiotic linezolid can be used.

MRSA and their detection

- Methicilin resistant staphylococci (MRSA) are epidemiologically important strains, often causing serious hospital infections
- They are caused by change of so named membrane penicillin binding proteins (PBP)
- Problem is seen by a small zone in oxacilin. But it is not a clear proof.
- The proof is, when the zone is small not only in oxacilin, but also cefoxitin

The End

A victim of a staphylococcal infection