

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 of staphylococci II

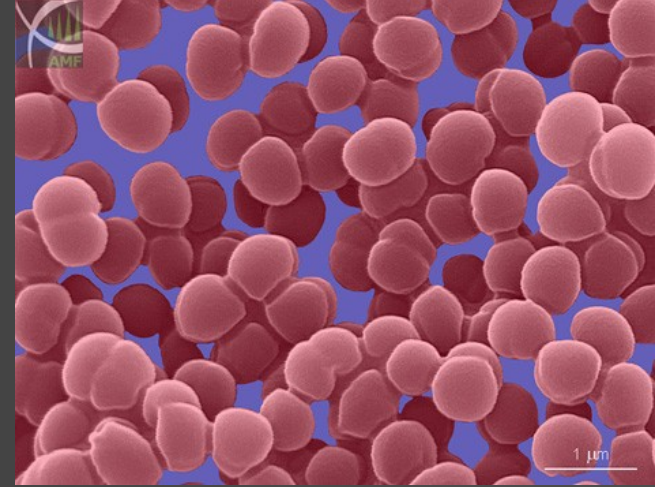
Antibiotic susceptibility testing and antibiotic treatment

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 will 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, vomiting and diarrhoea. The girl-friend, called, says she has the same problems... So, no romantic afternoon...



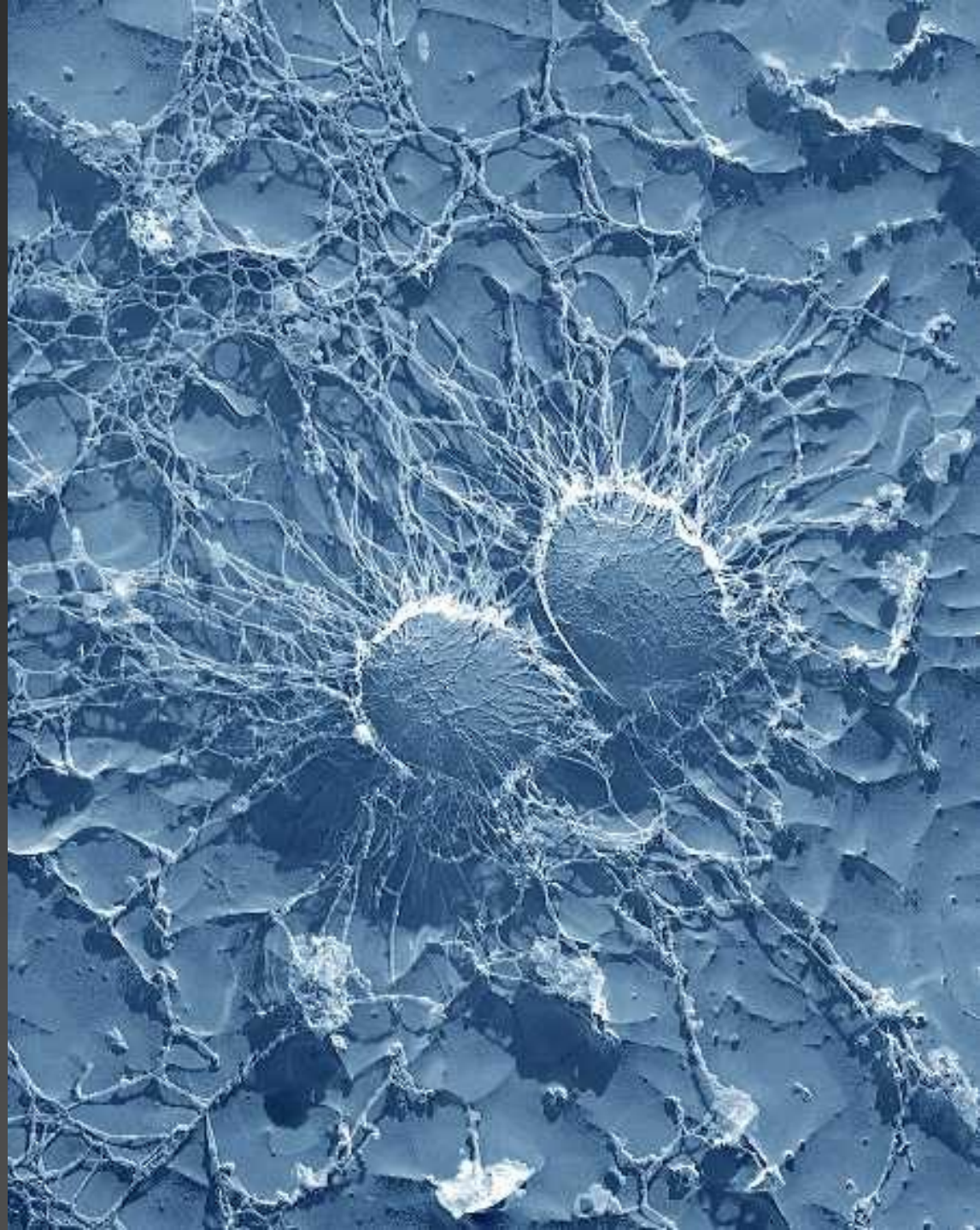
# Who is guilty?

- It is *Staphylococcus aureus*  
*name from greek staphylé = grape*
- This „golden staphylococcus“ often causes pyogenic 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. salmonellosis)

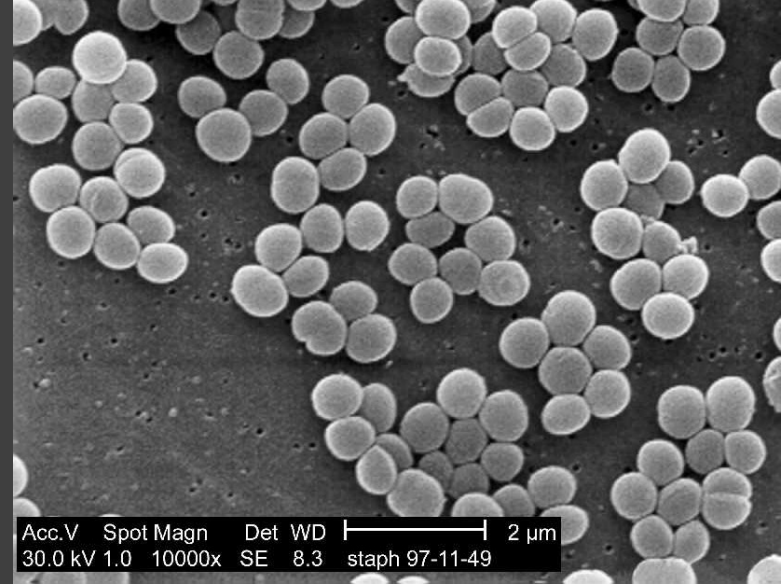
*Of course, the cook, that prepared meals despite her blister, is guilty, too!*



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



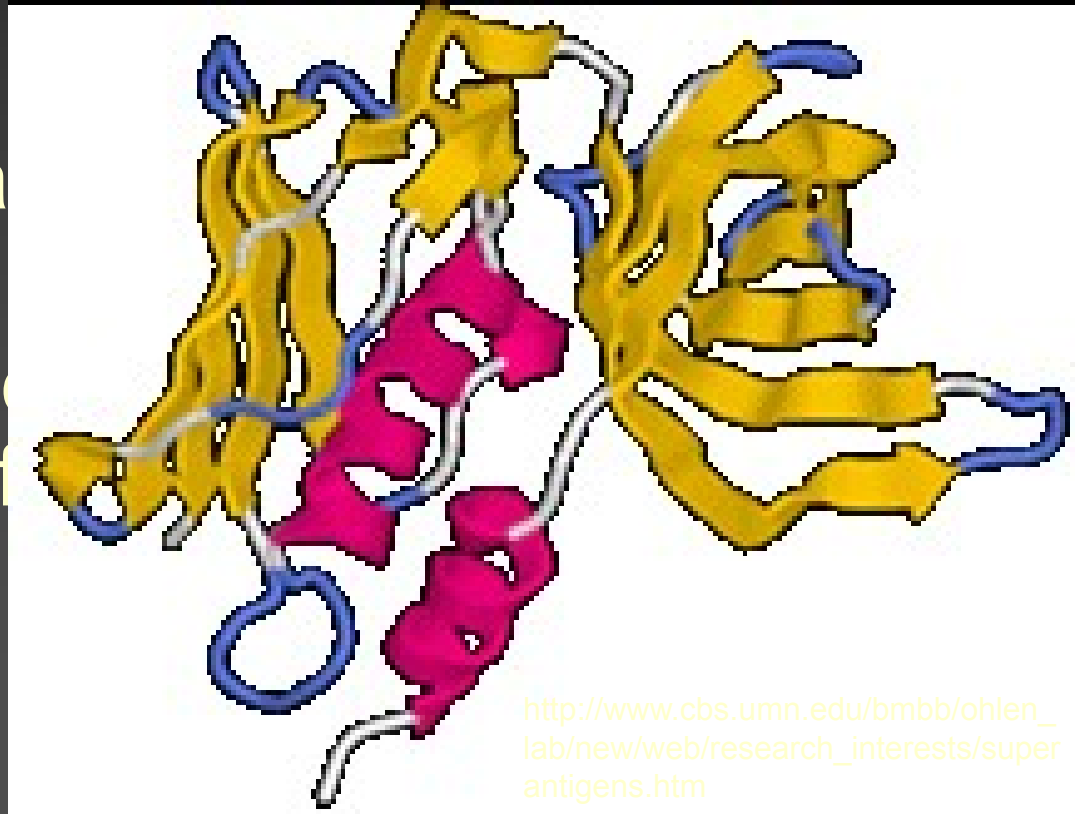
# Story Two



- **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 student is hospitalized on emergency unit of infectological clinic...

# Who is guilty now?

- Again, it is *Staphylococcus aureus*, now a strain called **TSST-1** (toxic shock syndrome toxin)
- This toxin, too, is a **superantigen**
- It causes toxic shock typically in users of **menstruation tampones**

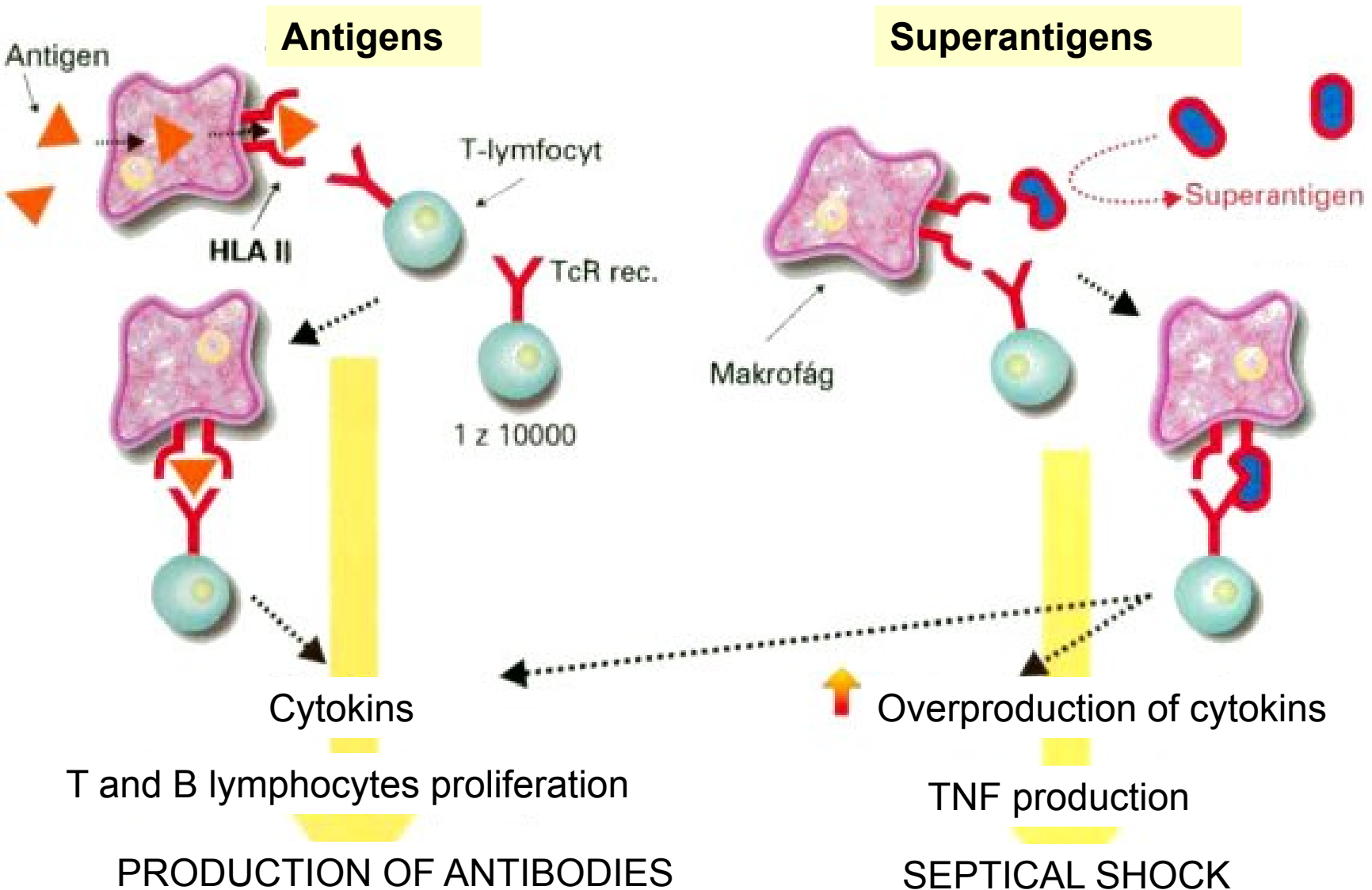


[http://www.cbs.umn.edu/bmbb/ohlen\\_lab/new/web/research\\_interests/superantigens.htm](http://www.cbs.umn.edu/bmbb/ohlen_lab/new/web/research_interests/superantigens.htm)



# Superantigens

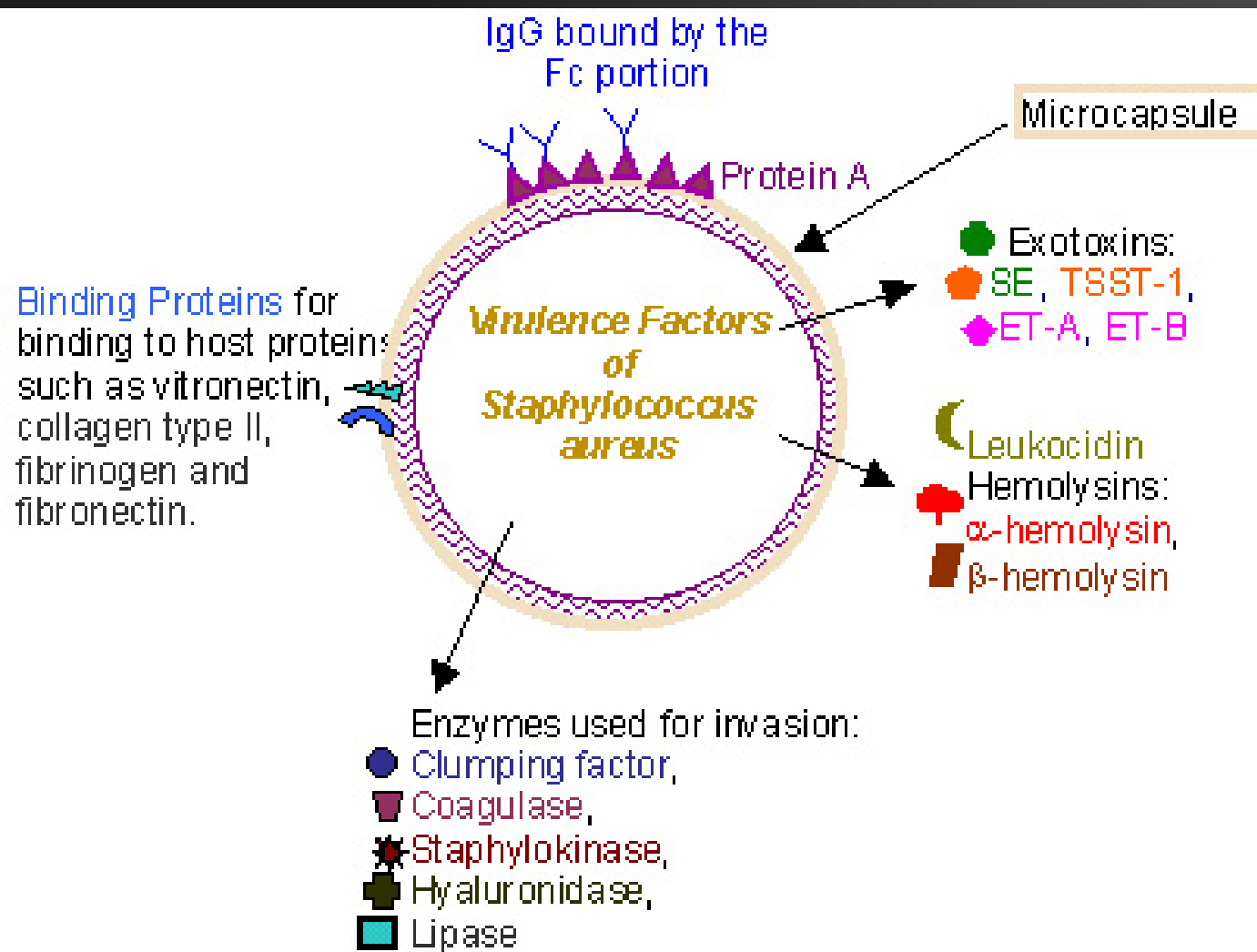
## Immunity response



# *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

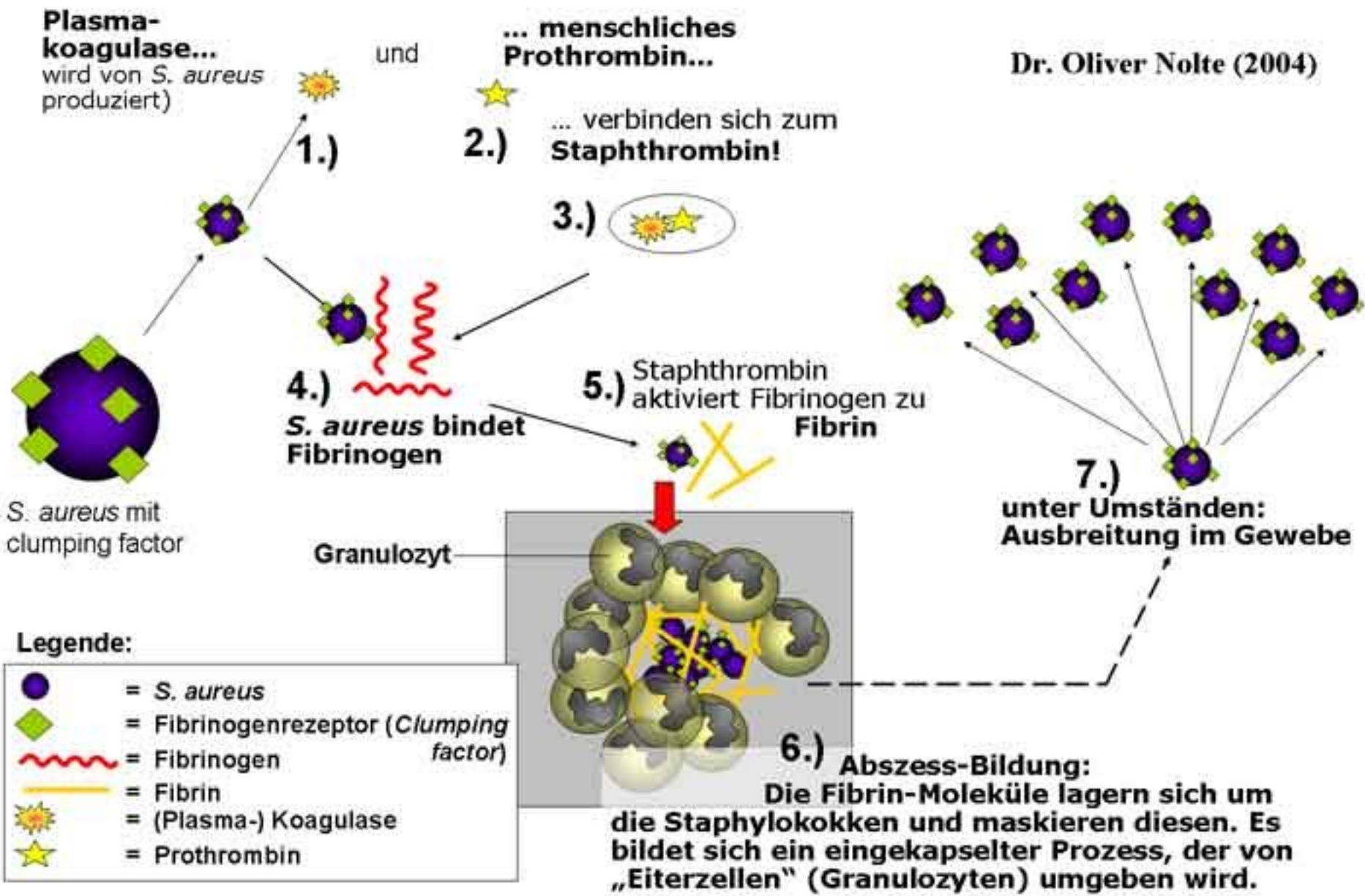
# There exist plenty of virulence factors found in *S. aureus*...



...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



# Examples of infections caused by staphylococci: Impetigo...





...bullous impetigo...



...otitis externa  
with a  
furuncle...



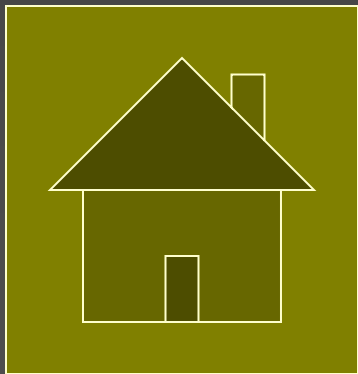
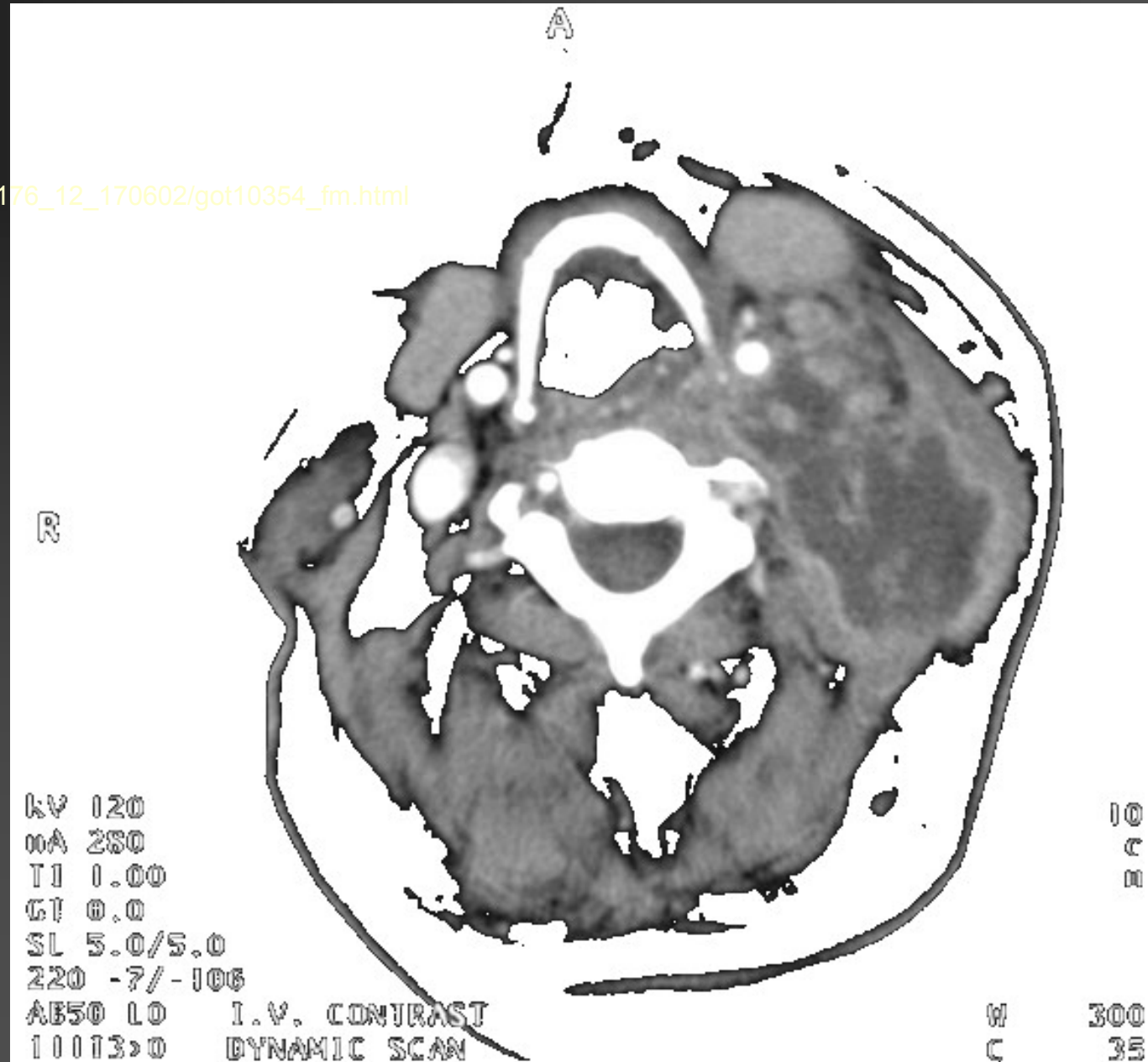
# ...or skin infection with crusts...

[http://www.dermatology.co.uk/media/images/Infection\\_staphylococcus\\_crusting\\_chin\\_closeup.jpg](http://www.dermatology.co.uk/media/images/Infection_staphylococcus_crusting_chin_closeup.jpg)



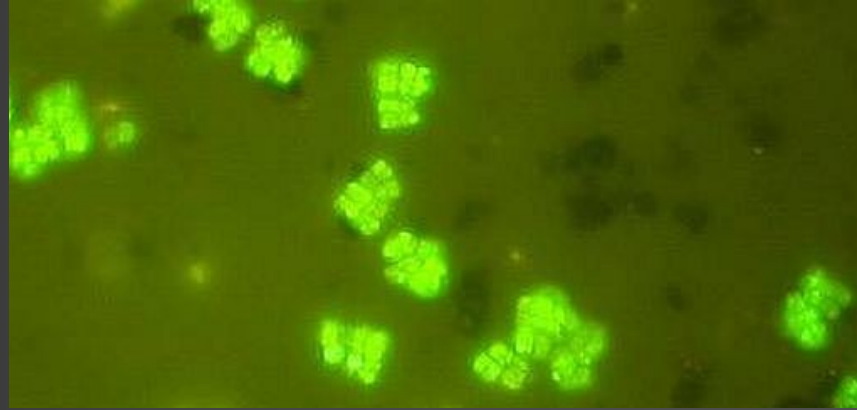
# ...but also brain abscessi

[http://www.mja.com.au/public/issues/176\\_12\\_170602/got10354\\_fm.html](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 catheters for infusion nutrition and blood taking.
- Suddenly, his status **worsened acutely**, high and quickly changing fevers – the ward doctor has suspicion for septicaemia and takes blood for blood culture
- After **catheter change** and **antibiotic treatment** the status improved again



# And who is guilty now?

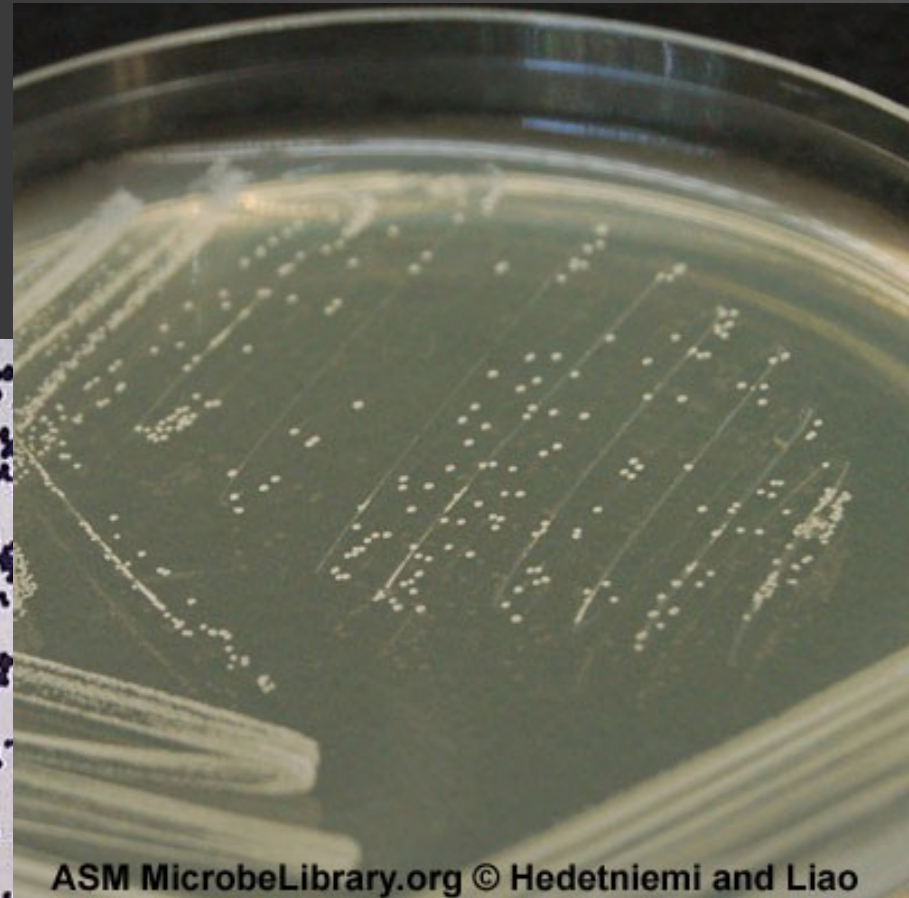
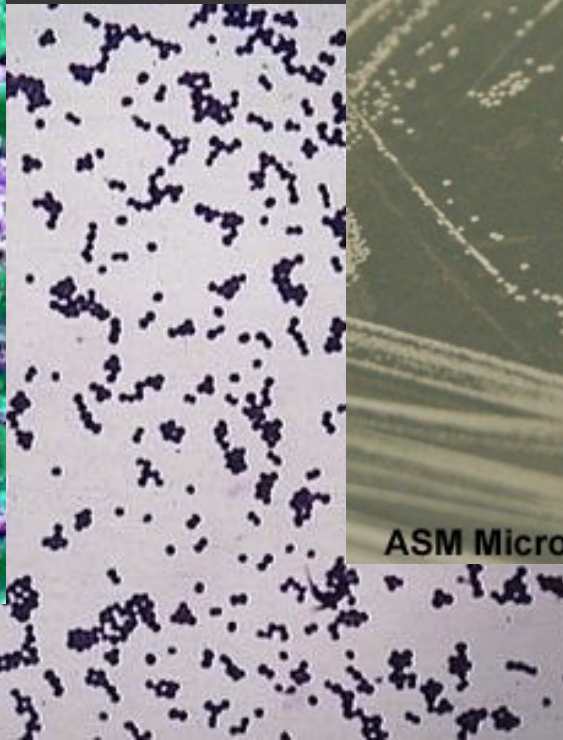
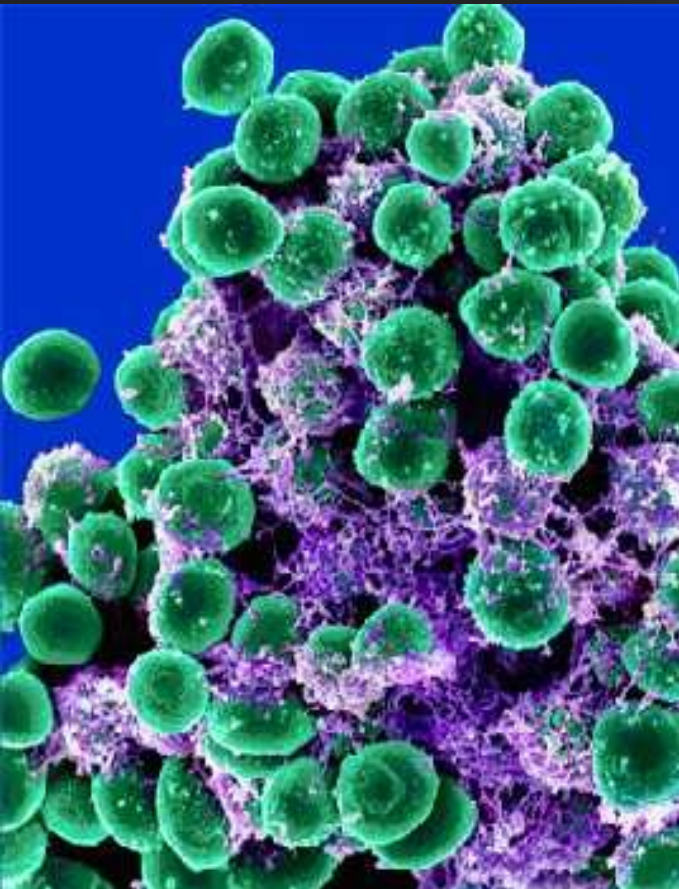
- The guilty is *Staphylococcus epidermidis*, the most common among coagulase negative staphylococci
- Coagulase negative staphylococci belong to the same genus as „golden staphylococcus“
- They are much less pathogenic
- In last decades, they started to be very important causative agents of infections in weakened 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>



ASM MicrobeLibrary.org © Hedetniemi and Liao

<http://www.difossombrone.it>

# Coagulase negative staphylococci

- Coagulase negative staphylococci (*Staphylococcus epidermidis*, *S. hominis*, *S. haemolyticus* and about forty 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.

There are many species of staphylococci today

*S. simiae* (AY727531)

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



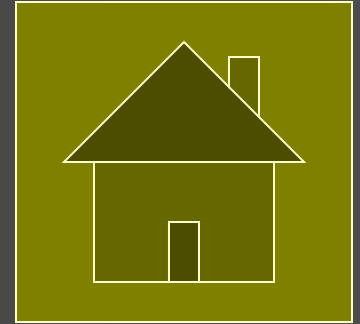
# Diagnosatics of staphylococci

# 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 differentiate individual species biochemically
- **Antigen analysis and special tests** maybe very helpful at the diagnostics



# Photos from Criminal Database



[www.medmicro.info](http://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

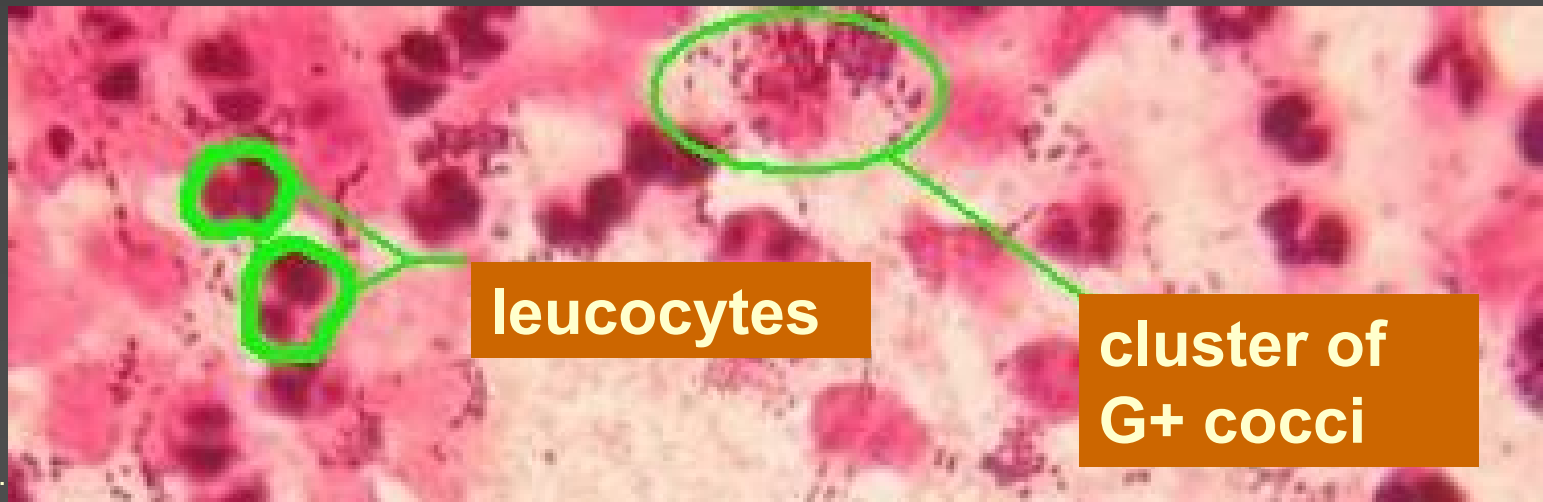
- (Description of colonies on blood agar)
- Catalase test (Staphylococci × other G+ cocci)
- Growth on BA with 10 % NaCl

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

- Differentiation 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 inflammation)



# Singling out of other suspects (differential diagnostics 1)

- Gram stain differentiates gram-positive cocci from other shape/cell wall type bacteria
- Positive catalase differentiates 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, immersion 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)

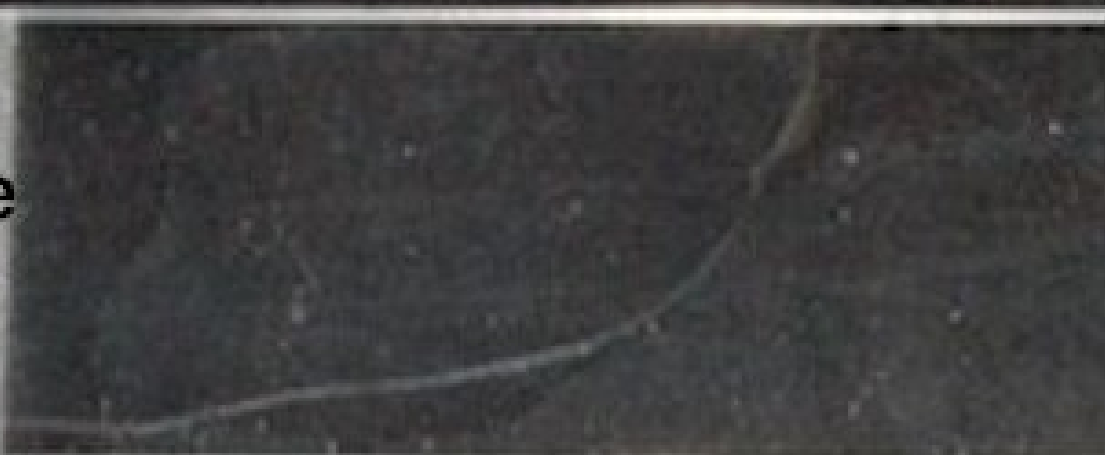


# Catalase test

## CATALASE TEST

<http://www.telmeds.org>

**Negative**



**Positive**



# 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 useful, as it can lead us to certain suspicion (e. g. staphylococci have rather whitish/yellowish colonies, 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*

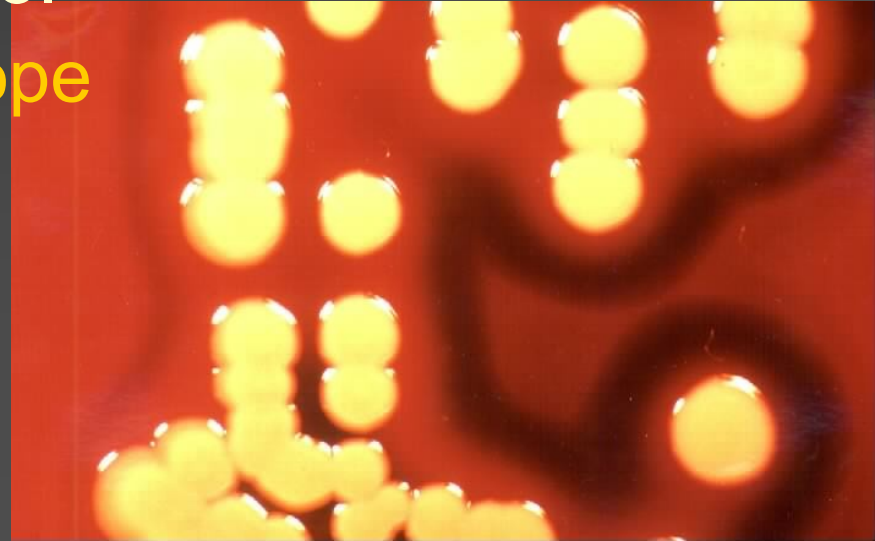


# Singling out of other suspects (differential diagnostics 2)

- **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 staphylococci 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



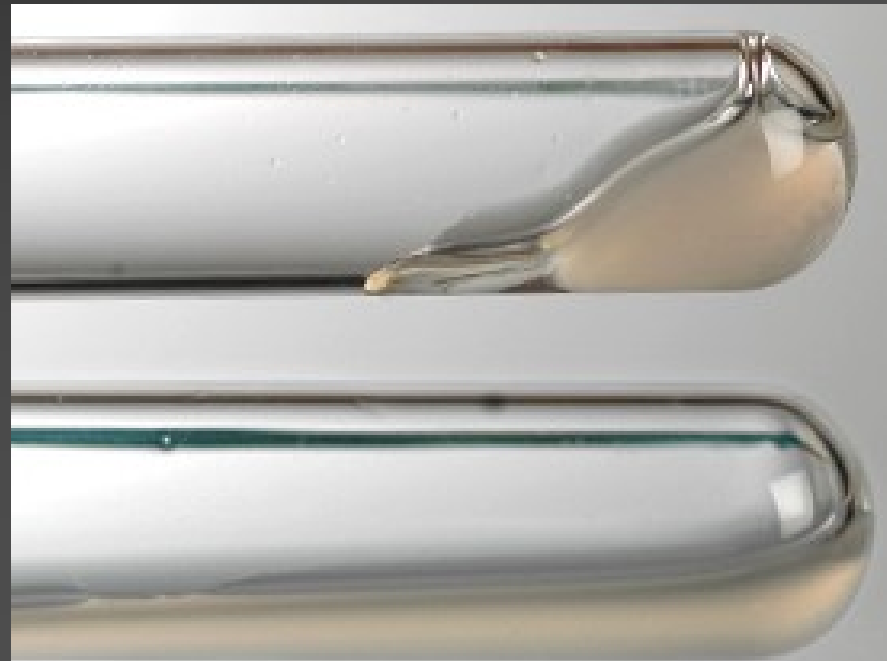
<http://memiserf.medmikro.ruhr-uni-bochum.de>

Clumping-Factor negativ



# Free coagulase – classic

- The most classical among differentiation 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





COAGULASE  
TEST



POSITIVE

<http://www.telmeds.org>



NEGATIVE

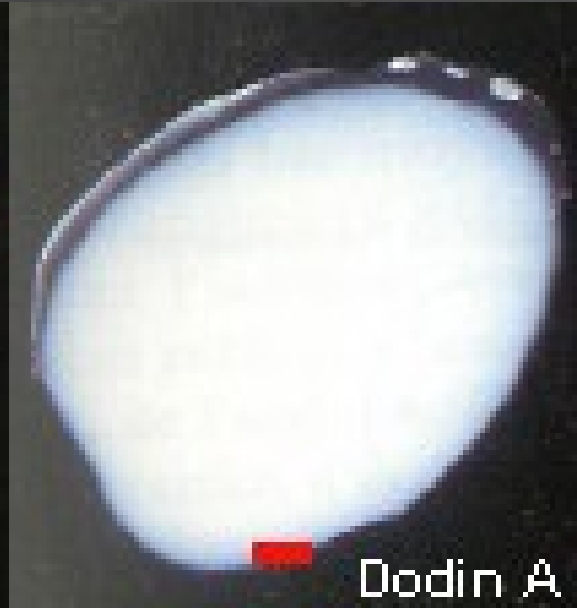
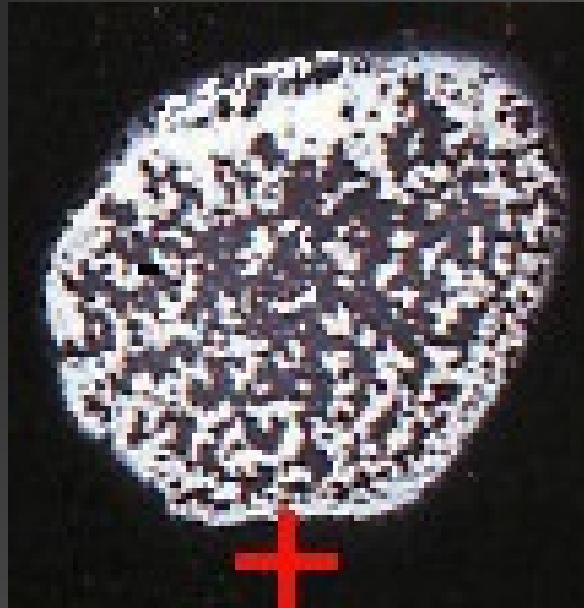
# More pictures of plasmacoagulase





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

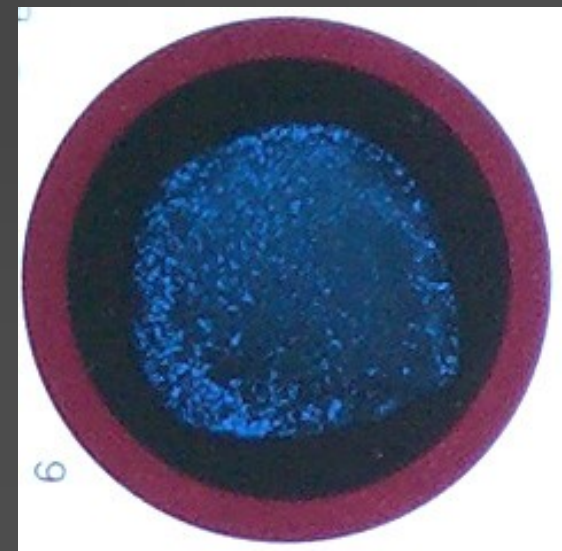
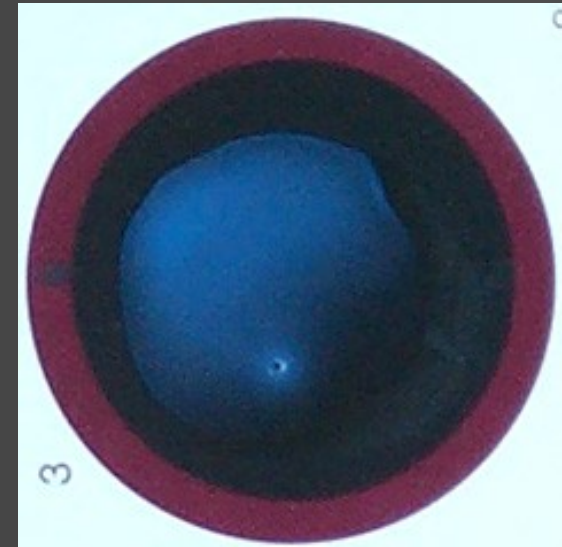
- The way of using them is the same as in the clumping factor test, but they are even more sure than free coagulase
- Unfortunately, they are quite expensive



# Staphaurex kit and results

<http://www.pathologyinpractice.com>

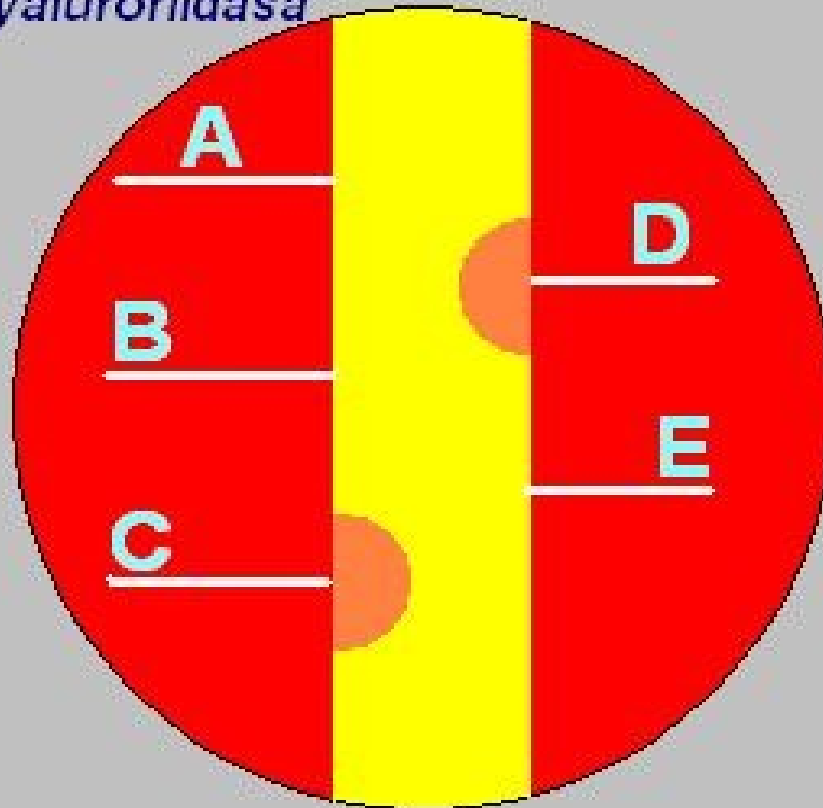
[www.microbes-edu.org](http://www.microbes-edu.org)



# 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 encapsulated 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“)

Hyaluronidasa



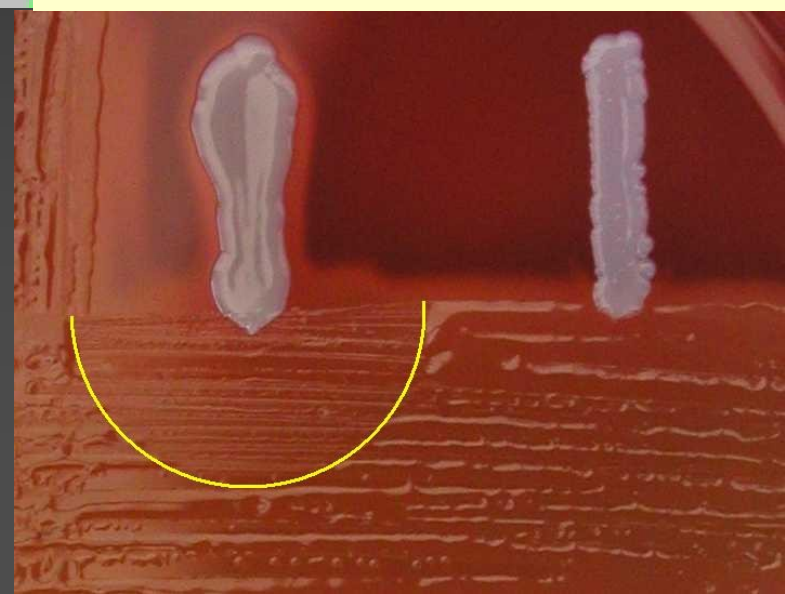
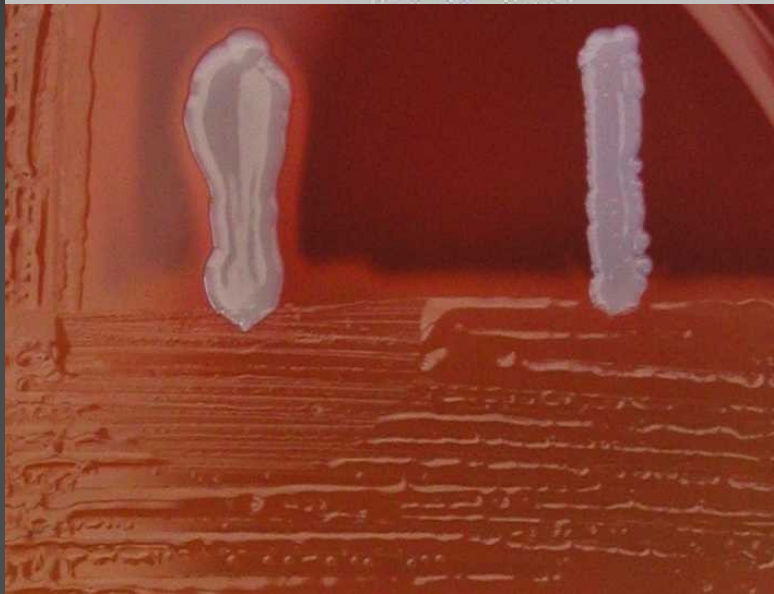
Yellow – *Streptococcus equi*  
(mucous)

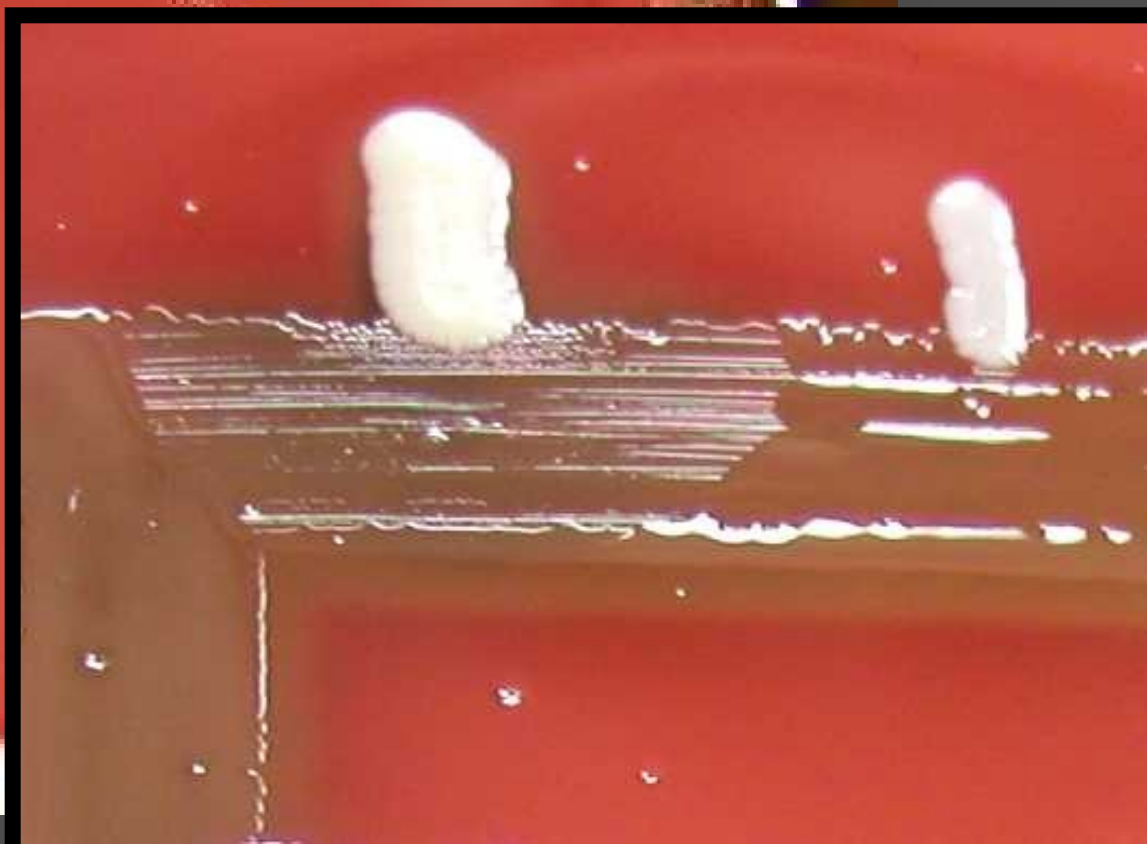
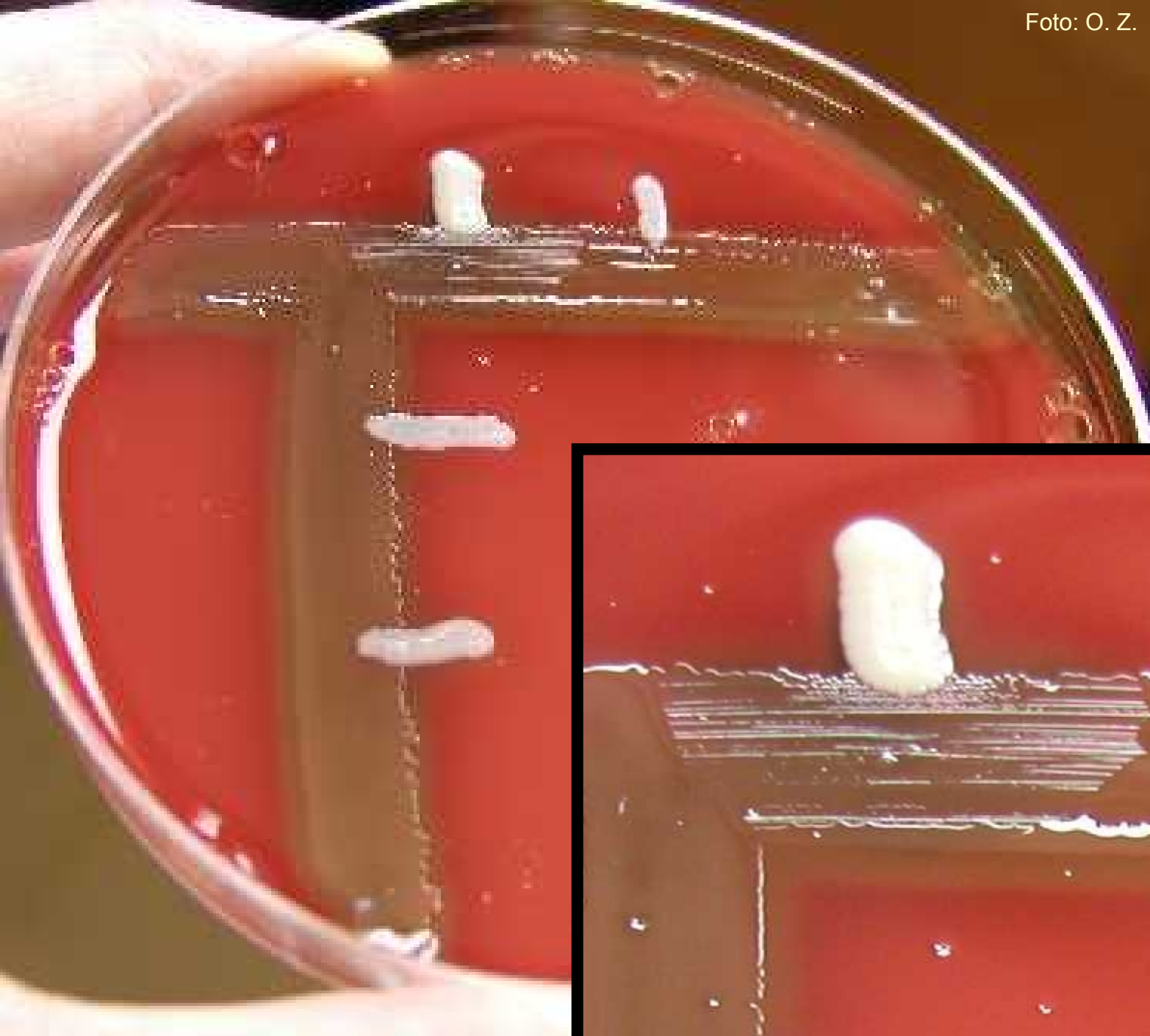
White – tested staphylococci

Results for this example:

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

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





# 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. Preliminary reading is done after 4 h and definitive 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 differentiation of staphylococci




















































- STAPHYtest 16 is the most typical Czech variant of a biochemical testing system 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 commercial 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 = 2<sup>nd</sup>–9<sup>th</sup> reaction
- Second row of the STAPHYtest = 10<sup>th</sup>– 17<sup>th</sup> r.
- Count the code and compare with the codebook
- The code consists 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 %,  $T_{in}=1,00$ )

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17
	Tube	H	G	F	E	D	C	B	A	H	G	F	E	D	C	B	A
	Tube	First row of panel								Second row of panel							
+																	
-																	
?																	
	+	+	+	-	-	-	+	+	-	-	+	+	+	-	+	+	-
	1	2	4	<del>1</del>	<del>2</del>	<del>4</del>	1	2	<del>4</del>	<del>1</del>	2	4	1	<del>2</del>	4	1	<del>2</del>
	7			0			3			6			5			1	

# Another example of a result

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

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17
	Tube	H	G	F	E	D	C	B	A	H	G	F	E	D	C	B	A
		First row of panel								Second row of panel							
+																	
-																	
?																	
	+	+	+	-	-	-	+	+	-	-	+	-	-	-	+	+	-
	1	2	4	<del>1</del>	<del>2</del>	<del>4</del>	1	2	<del>4</del>	<del>1</del>	2	<del>4</del>	<del>1</del>	<del>2</del>	4	1	<del>2</del>
	7			0			3			2			4			1	

# Api Staph – in some countries used equivalent of STAPHYtest 16

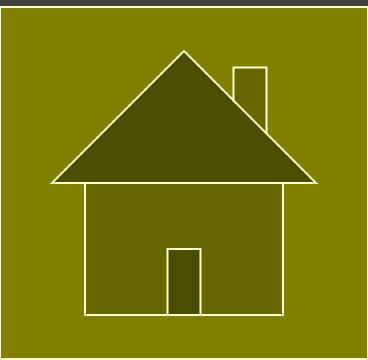
- Not regarding the producer, the principle is the same – combination of many enzymatic reactions, that can be seen as colour change

<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.

# Illustration photo



# Quantitative and qualitative tests

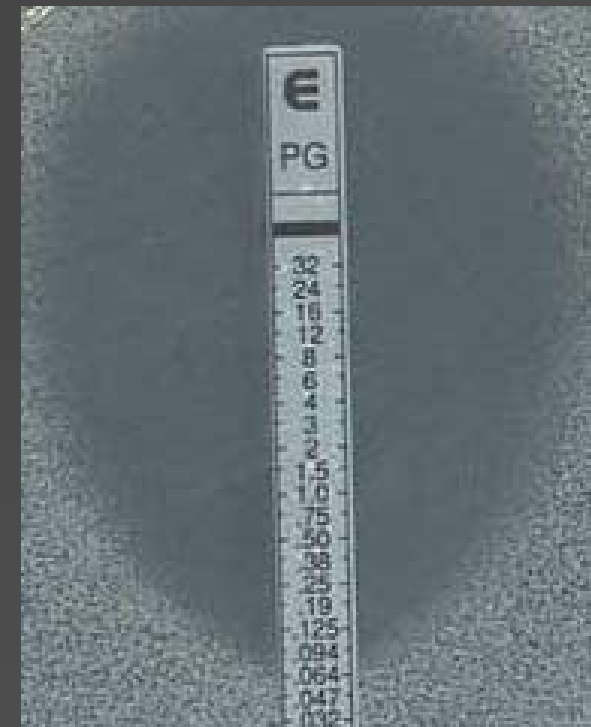
- As mentioned, usually we use a qualitative test (diffusion disc test). Nevertheless, it is also possible to use quantitative tests (microdilution test, E-test)



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 pathogen (*S. aureus*) uses to be more susceptible than the milder pathogen (coagulase negative staphylococcus). 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

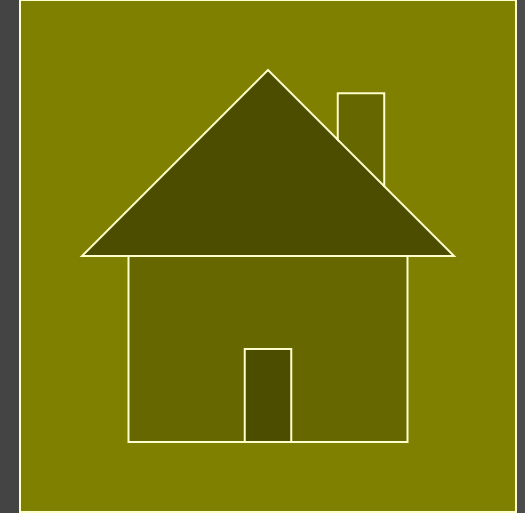
- In staphylococci, the drug of choice is **oxacilin**, in UTI **cefalosporins of first generation**. Often used **macrolids** are good in allergic persons only, **lincosamids** are good in locomotor system infections and **aminoglycosides** in combination only. **Glykopeptidic antibiotics (vankomycin 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