

Topic P01: Diagnostics of staphylococci

Materials for study (from textbooks, www etc.): Diagnostics of the *Staphylococcus* genus.

From spring term: Microscopy, culture, biochemical identification.

Notice: All microscopic preparations are supposed to be observed not only in the CX31, but also in the CX33 microscope with camera and your picture should be captured to the PC and shown to your teacher as a proof that the task has been really done. At the end of the practical session you have to clean your microscope, to switch it off and to cover it, and also to delete all previously captured photos. Any manipulation with microscopes and computers, except what is a part of your tasks or what is done after bid by your teacher, is forbidden! Especially the use of USB devices is strictly forbidden, including for the computer used by the teacher!

Task 1: Microscopy of infectious material

In your microscope, observe a Gram stained smear of the blood culture. Describe and draw the observed objects.

Blood culture is a specimen of blood, mixed with transport-cultivation medium and sent to the laboratory. The complete vessel is cultured in an automated cultivator and in case of positivity, (not only) microscopy is performed. More about blood cultures and haemocultivation see in P13 practical session.

Note the presence of bacteria (their shape, staining and quantity), red blood cells, eventually also other objects. Do not forget to draw your picture in colours and to describe it. All “rules” from J01 practical session are still valid!



Table for major results of Task 2 to Task 7 (to be filled step by step):

Strain		K	L	M	N
Gram stain – Task 2					
Task 3 Colonies on the blood agar (BA)	Size				
	Colour				
	Shape				
	Profile				
	Haemolysis				
Other					
Task 4: Growth on BA + 10% NaCl					
Task 5: Catalase test (write + or –)					
PARTIAL CONCLUSION					
Task 6a: Clumping factor test (+/–)					
Task 6b: Plasma-coagulase test (+/–)					
Task 6c: Hyaluronidase test (+/–)					
Task 7: STAPHYtest 16					
FINAL CONCLUSION					

If you perform a test for some strains only (e. g. K + L, but not M + N), score out not used fields.

Task 2: Microscopy of microbial cultures

Gram stain the pure cultures of the presented organisms, labelled with letters. Draw your findings below and write the results in the table above.

Strain K	Strain L	Strain M	Strain N
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Task 3: Growth on blood agar (BA)

Fill in the table for Task 3. In “Other” write all other specific characteristics.

Task 4: Bacterial growth on BA with 10% NaCl

Evaluate the growth ability of the presented strains on BA with 10% NaCl serving as a selective medium for staphylococci. Write “+” for the presence of growth and “-” for its absence.

Task 5: Catalase test

Evaluate the presence of the catalase enzyme. Using microbiological loop, take several colonies of the presented strains and mix them with a drop of 3% H₂O₂ on the slide. As you already know (Topic J04), a positive reaction is characterized by _____, while _____ is negative.

Fill in the table on Page 1/1 for Task 5; write “+” or “-” for results of strains K, L, M, N.

Now, fill in the line “Partial conclusion“. Write STAPH for strains found to be staphylococci and OTHER for strains that do not belong to the *Staphylococcus* genus.

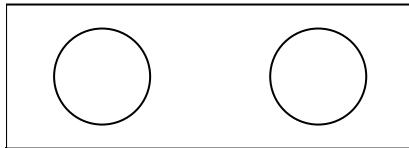
Task 6: Tests for the *S. aureus* differentiation

6a) Clumping factor test (test of bound plasmacoagulase)

Place a drop of diluted rabbit plasma on a slide. Using microbiological loop, suspend the examined staphylococcal strain in it. Draw your results below, fill in the comment and write the conclusion in the table.

Strain _____
(letter)

positive – negative
(delete as appropriate)

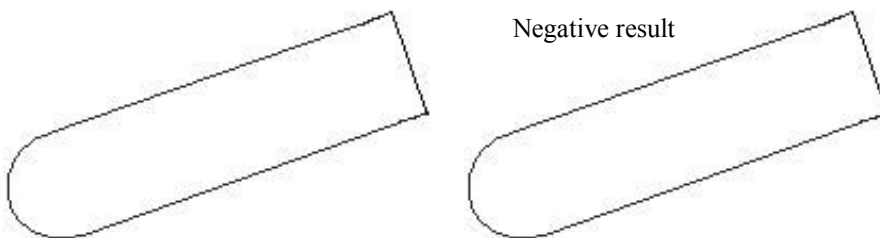


Strain _____
(letter)

positive – negative
(delete as appropriate)

6b) Plasmacoagulase test (test of free plasmacoagulase)

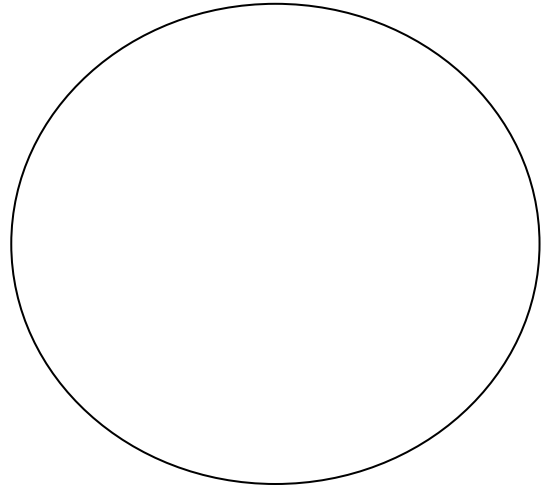
Several colonies of the examined staphylococcal strain were suspended in 0.5 ml of 10× diluted rabbit plasma. The suspension was incubated in an incubator at 37 °C. The result was evaluated after 1, 2 and 24 hours. The reaction is considered positive when the rabbit plasma in the test tube is coagulated, i.e. the total volume of the test-tube is at least partially “gel-like”. Write and draw the results of this reaction for the presented strains after 24 h of incubation. The test tube is on purpose drawn inclined in order to enable you to draw the difference between a liquid (approximately horizontal level) and a gel (no horizontal level).



6c) Hyaluronidase detection

On blood agar, about 2 cm broad band of capsule forming *Streptococcus equi* was inoculated. Perpendicularly to this band, a strip of an examined *Staphylococcus* strain was inoculated. When the staphylococcus produces hyaluronidase, it diffuses into the surrounding agar overnight and the capsule of *Streptococcus equi* made from hyaluronic acid is lysed. This can be observed as a half-circular zone without mucosity in the mucous *Streptococcus equi* band. Draw the positive and negative results of the test and describe them.

Attention! The principle of this test has NOTHING to do with haemolysis! If you see it, you may draw it, nevertheless it is not important for this task. Follow the teacher’s instructions and do the task only after his/her explanation!



Task 7: More precise determination of staphylococci using biochemical microtest (STAPHYtest 16)

For the identification of staphylococci, there is a set of biochemical tests. Read the results of the individual tests according to the guidelines or coloured pattern. Write down the results of the tests and according to a codebook find the species name of the examined staphylococcus. (Strain K is partially filled already.) Do not forget to fill in also % of probability and typicality index for individual strains!

	Tube	Proper test – first row with 8 wells								Proper test – second row with 8 wells							
	VPT	1H	1G	1F	1E	1D	1C	1B	1A	2H	2G	2F	2E	2D	2C	2B	2A
K	+	+	+	–	–	–	+	+	–								
	1	2	4	1	2	4	1	2	4	1	2	4	1	2	4	1	2
	7			0			3										
	Code:						Identification <i>Staphylococcus</i>						% of probability			T index	
	VPT	1H	1G	1F	1E	1D	1C	1B	1A	2H	2G	2F	2E	2D	2C	2B	2A
L	+																
	1	2	4	1	2	4	1	2	4	1	2	4	1	2	4	1	2
	7																
	Code:						Identification <i>Staphylococcus</i>						% of probability			T index	

Task 8a: Susceptibility of staphylococci to antibiotics

Assess the susceptibility of the presented strains to the selected antibiotics using the diffusion disc test. Evaluate the susceptibility to the given antibiotics by measuring the diameter of the inhibitory zone and comparison with the table. Interpret the strains as susceptible (S), intermediary (I) or resistant (R) to given antibiotics.

Strain →							
Antibiotic	Susceptible if	Inter-mediate if	Resistant if	Zone Ø (mm)	Inter-pretation	Zone Ø (mm)	Inter-pretation
Cefoxitin FOX*	≥ 22/25 mm**	18–20 mm	< 22/25 mm**				
Erythromycin E	≥ 21 mm	18–20 mm	< 18 mm				
Clindamycin DA	≥ 22 mm	19–21 mm	< 19 mm				
Co-trimoxazole SXT	≥ 17 mm	15–16 mm	< 15 mm				
Tetracycline*** TE	≥ 22 mm	19–21 mm	< 19 mm				
Chloramphenicol C	≥ 18 mm	18–20 mm	< 18 mm				

*interpreted as oxacillin, eventually also more beta-lactams

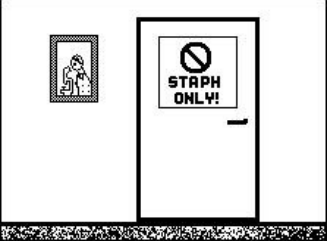
**22 mm valid for *S. aureus*, 25 mm valid for coagulase-negative staphylococci

***the result is valid also for doxycycline

Important note: In some tests you may find seventh disk in the middle – MUP (mupirocin). It is a locally administered antimicrobial stuff (antiseptic) that serves e. g. for at least temporary elimination of MRSA strains (see below) from nasal cavity. Susceptibility test to mupirocin is performed especially at findings of *S. aureus* from nasal swab. As mupirocin is not present in all your tests, we do not read it in our practical session (just for your information, the strain is susceptible at zone ≥ 18 mm and resistant at < 18 mm). – One consequence of presence of mupirocin in the middle is worsened “readability” of other zones. If it is not possible to measure the diameter, measure the radius (guess the middle of the disc) and multiply by two.

Task 8b: Demonstration of screening medium for MRSA

Evaluate given strains (on the side table) if they are/are not MRSA. (You may have only one or two strains, then let free the not used rows.)

Strain	This strain is a	
	MRSA – MRSKN – MS staphylococcus*	
	MRSA – MRSKN – MS staphylococcus*	
	MRSA – MRSKN – MS staphylococcus*	
*delete as appropriate: MRSA = methicillin resistant <i>S. aureus</i> , MRSKN = methicillin resistant coag.-negative staph., MS = methicillin susceptible		