

Topic P01: Diagnostics of staphylococci

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

From spring term: Microscopy, culture, biochemical identification.

Task 1: Microscopy of infectious material

In your microscope, observe the Gram stained smear. Describe and draw the observed objects. Note the presence of bacteria (their shape, staining and quantity) and also WBCs, epitheliae and their mutual ratio.

	Description (write the objects names and draw arrows to them in your picture): <hr style="border: 0; border-top: 1px solid black; margin: 5px 0;"/> <hr style="border: 0; border-top: 1px solid black; margin: 5px 0;"/> <hr style="border: 0; border-top: 1px solid black; margin: 5px 0;"/>
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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.

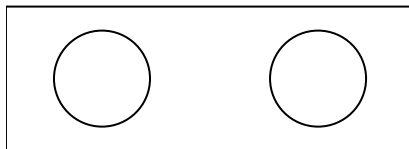
Now, fill in the line “Partial conclusion“. Write STAPH for strains found to be staphylococci and OTHER for strains that do not belong to the *Staphylococcus* genus.

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)

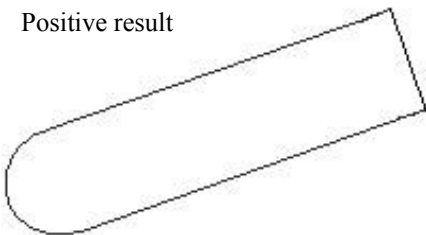


Strain _____
(letter)
positive – negative
(delete as appropriate)

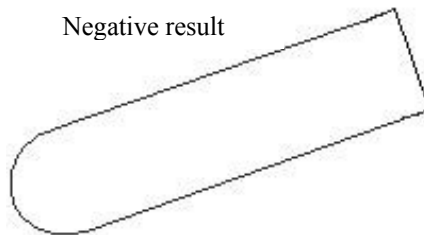
6b) Plasmacoagulase test (test of free plasmacoagulase)

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 drawn inclined in order to enable you to draw the difference between a liquid (horizontal level) and a gel (no horizontal level).

Positive result



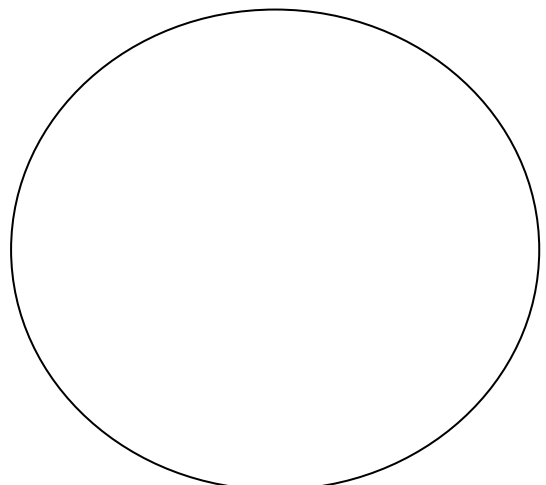
Negative result



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 typicalness 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 comparing the measured diameter of the inhibition zone and the reference zone. Write down full names of the antibiotics, the zone diameter and the interpretation. In case of total absence of a zone of susceptibility do not measure, cross the field “zone diameter” and write directly an “R”.

Antibiotic	<i>S. aureus</i>		<i>S. epidermidis</i>	
	Zone diameter (mm)	Interpretation*	Zone diameter (mm)	Interpretation*
Cefoxitin (FOX)* (ref. zone see below)				
Erythromycin (E) (ref. zone 21 mm)				
Clindamycin (DA) (ref. zone 22 mm)				
Co-trimoxazole (SXT) (ref. zone 17 mm)				
Tetracycline** (TE) (ref. zone 22 mm)				
Chloramphenicol (C) (ref. zone 18 mm)				

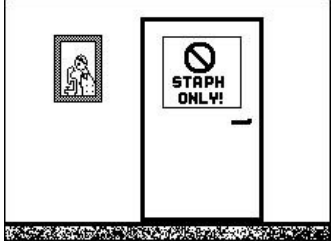
S = susceptible, R = resistant, D = dubious (plus/minus, the measured zone just equal to the reference zone)

*interpreted as oxacillin and other beta-lactams; ref. zone is 22 mm for *S. aureus* and 25 mm for *S. epidermidis*

**result of this test is also valid for doxycycline

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		