Topic P01: Diagnostics of staphylococci

Materials for study (from textbooks, internet etc.): Diagnostics of genus *Staphylococcus*. From spring semester: Microscopy, culture, biochemical identification.

Task No 1 Microscopy of infectious material

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

Description (write names of objects, and connect by arrows with the objects on your picture)

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					
Culture (blod agar) Task 3	Size				
	Colour				
	Shape				
	Profile				
	Haemo-lysis				
	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: Test for hyaluronidase					
Task 7: STAPHYtest 16					
FINAL CONCLUSION					

Task No 2 Microscopy of microbial cultures

Gram stain pure cultures of given organisms, labelled by letters. Draw your findings here and write the results into the table above.

Strain K	Strain L	Strain M	Strain N

Task 3 Growth on blood agar (BA)

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

Task 4 Bacterial growth on BA with 10 % NaCl

Evaluate growth ability of given strains on BA with 10 % NaCl, that serves as a selective medium for staphylococci. Write „+“ for presence of growth and „–“ for absent growth.

Task No. 5: Catalase test

Evaluate presence of catalase enzyme. Using microbiological loop, take several colonies of given strains and mix them wit a drop of 3% H_2O_2 on slide. As you already know (Topic J04), positive reaction is characterized by

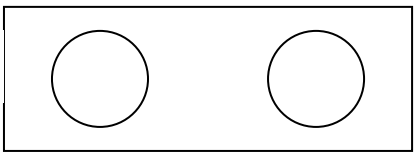
_____, while _____ is negative. Write „+“ for and „–“.

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

Task 6 Tests for *S. aureus* differentiation

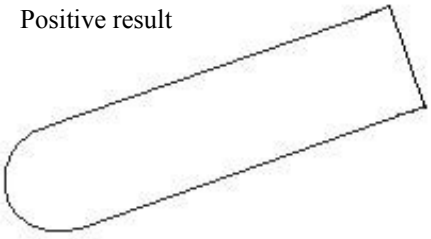
6a) Clumping factor test (test of bound plasmacoagulase)

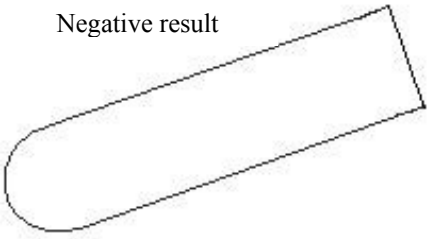
On the slide, a drop of diluted rabbit plasma was placed. Using microbiological loop suspend the examined staphylococcal strain in it. Draw your result here and write the conclusion to the table. (Boxes for strains proven not to be staphylocci should be left empty).

Strain _____  Strain _____

6b) Plasmacoagulase test (test of free plasmacoagulase)

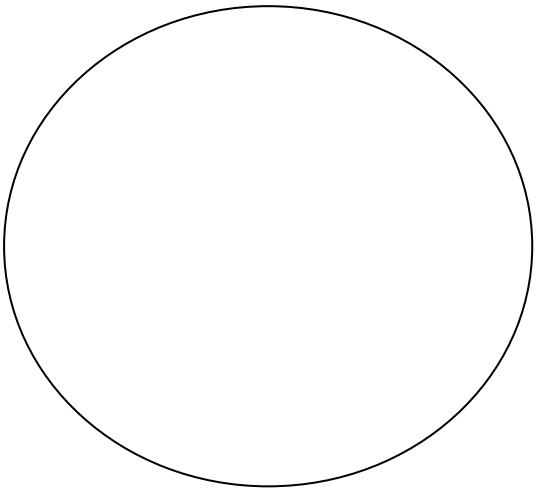
In 0.5 ml of 10× diluted rabbit plasma several colonies of examined straphylococcal strain was suspended. The suspension is incubated in a thermostat at 37 °C. The result is evaluated after 1, 2 and 24 hours. The reaction is considered positive, when rabbit plasma in the test tube is coagulated, i.e. the total volume of the test-tube is „gel-like“, at least partially. Write and draw the result of this reaction for given strains after 24 h of incubation.

Positive result 

Negative result 

6c) Hyaluronidase detection

On blood agar, about 2 cm large strip of *Streptococcus equi*, is inoculated. It is a capsule-forming bacterium. In 90° direction to this strip a line of a given *Staphylococcus* is to be inoculated. When the given staphylococcal strain produces hyaluronidase, it diffuses to the vicinity, and the capsule of *Streptococcus equi* is lysed overnight. You can see it as half-circular zone without „mucoidity“ in the strip of *Streptococcus equi.*
Draw the result of your reaction (including positive and negative reaction) and describe.



Task 7 More precise determination of staphylococci using biochemical tests

For staphylococcal identification there exists a set of biochemical tests. According to use instructions read the results of individual tests. Names of tests and their results for individual strains should be written, and according to a codebook find the species name of given staphylococcus.

	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 *Staphylococcus*						% 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 *Staphylococcus*						% of probability			T index	

Task 8 Susceptibility of staphylococci to antibiotics

Assess Susceptibility of given strains to chosen antibiotics using diffusion disc test. Susceptibility to given antibiotics is to be evaluated using the diffusion disc test. Evaluate Susceptibility to given antibiotics using comparison of found diameter of inhibition zone and reference zone. Write full names of antibiotics, diameter of zones and interpretation.

Antibiotic (full name, not abbreviation!)	*S. aureus*		*S. epidermidis*	
	Zone diameter (mm)	Interpretation	Zone diameter (mm)	Interpretation

Check-up questions

1. What are important characteristics of genus *Staphylococcus* (metabolic, morphological and growth characteristics)?

2. How can we differentiate *S. aureus* from so named „coagulase-negative staphylococci“ (CoNS)?

S. aureus	CoNS

3. In what more staphylococcal species, besides *S. aureus*, plasmacoagulase may be present?

4. What the clumping factor is?

5. What are the most important anti-staphylococcal antibiotics?

6. What does mean MRSA and what problems it brings to the therapy of staphylococcal infections?