

Name:

Date:

Diagnostic of staphylococci

Task 1: Microscopy of infectious material

Observe preparation stained by Gram staining. Draw and describe your observations. Look for bacteria (their shape, colour and quantity), WBC and epithelia. Use immersion oil and immersion objective.

Task 2: Microscopy of microbial cultures

Stain according to Gram pure cultures of prepared microorganisms. Draw your observations. Use immersion oil and immersion objective.

Task 3: Growth on Blood Agar

Describe morphology of colonies of prepared bacterial strains on Blood Agar. Note colour and haemolytic properties of observed colonies.

No. of strain	Description of colony (size, colour, shape, surface, margins, haemolysis)

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Task 4: Growth of bacteria on blood agar supplemented with 10% NaCl

Evaluate the growing abilities of prepared strains on the Blood Agar supplemented with 10% NaCl, which is selective growing medium for staphylococci.

On Blood Agar supplemented with 10% NaCl grow strains:.....

Task 5: Catalase test

Show presence of enzyme catalase in observed strains. Take a loop of colony and submerge it into drop of 3% H₂O₂ on a microscopy glass. Write down the reaction in your strains.

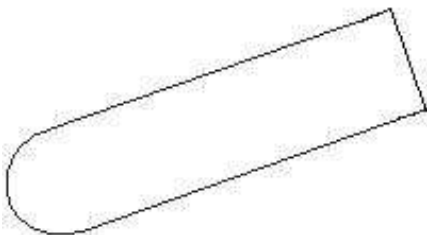
No. of strain	Reaction	Interpretation

**On the basis of tasks 2 - 5 choose strains, which belong to the genus *Staphylococcus*:
..... In the next tasks find out which staphylococci are here:.....**

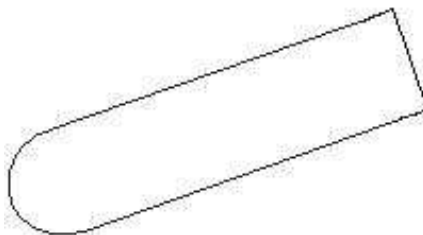
Task 6: Plasmocoagulase test

Into 0,5 ml 10x diluted rabbit plasma was suspended several colonies of examined strain of staphylococcus. The Suspension was incubated in 37 °C. The results are obtained after 1, 2 and 24 hours. Positive reaction is coagulation of rabbit plasma in the test tube, i.e. gelatination of the whole amount of rabbit plasma. Write down and draw the results after 24 hrs of incubation.

Positive:



Negative:



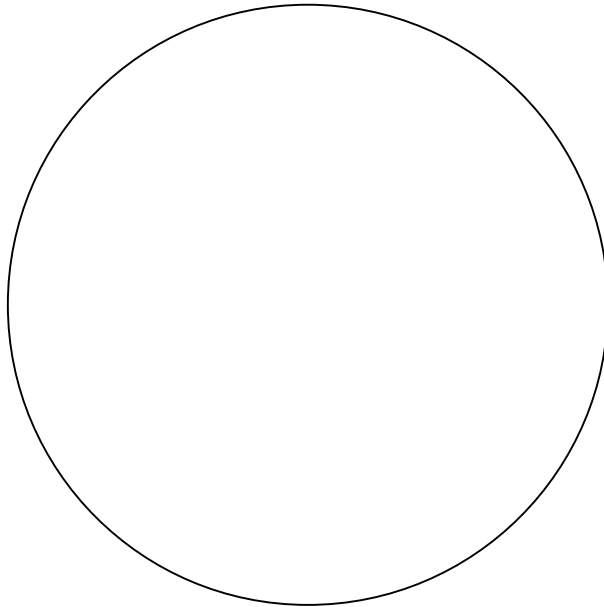
Task 7: Proof of hyaluronidase

On the blood agar we inoculate 1 cm wide line of *Streptococcus equi*, bacterium, which produces large capsule with presence of hyaluronic acid. To this line we inoculate line with examined strain of staphylococcus. If examined strain produces hyaluronidase, diffuses to the surrounding area and causes lysis of capsules of *Str. equi*. This can be seen as semicircle in

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the slimy line of *Str. equi*. Draw result of reaction (including positive and negative control) and describe.



Strains of staphylococci were determined to be:

Task 8: Susceptibility of staphylococci to antibiotics

Examine susceptibility of your strains of staphylococci to selected antibiotics on the basis of disk diffusion test. The susceptibility evaluate by comparing diameter of inhibition zone with the break-point zone of the antibiotics.

Antibiotic	<i>S. aureus</i>		<i>S. epidermidis</i>	
	Zone diameter (mm)	Intepretation	Zone diameter (mm)	Intepretation

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Check-up questions:

1. What are important characteristics of staphylococci (metabolic, morphologic and growth properties)?

2. How to distinguish between *S. aureus* and so-called coagulase-negative staphylococci?

<i>S. aureus</i>	coagulase-negative staphylococci

3. Which infections caused by members of genus *Staphylococcus* you can meet in dentistry?

4. Which are the most important anti-staphylococcal antibiotics?

5. What is the MRSA and which complications in therapy it brings?