# Topic P01: Diagnostics of staphylococci

## Task 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 | | | | | |
| Task 3 Culture (blod agar) | 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 | | | | | |
| **FINAL CONCLUSION** | | | | | |

## Task 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 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

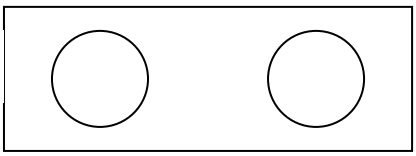
_____, while _____ is negative. Write „+“ for and „–“.

**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

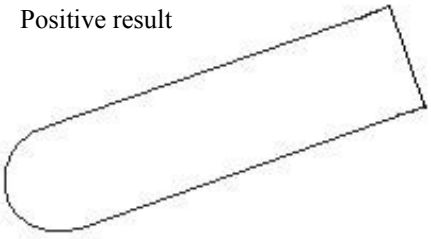
### 6a) Clumping factor test (test of bound plasmacoagulase)

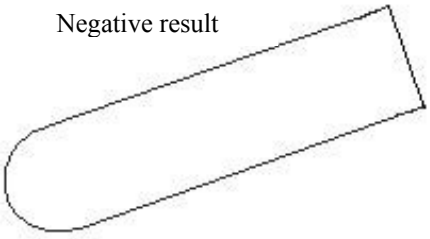
On the slide, a drop of diluted rabbit plasma was placed. Using microbiological loop suspend the examined staphylococcal strain in it. Draw your result here and write the conclusion to the table. (Boxes for strains proven not to be staphylocci should be left empty).

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.

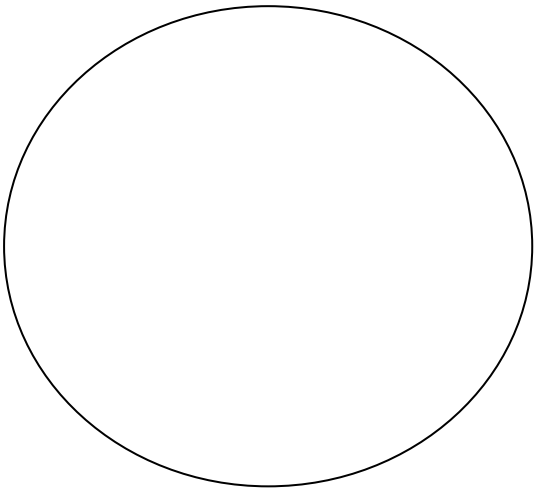
Positive result 

Negative result 

### 6c) Hyaluronidase detection

On blood agar, about 2 cm large band of *Streptococcus equi*, is inoculated. It is a capsule-forming bacterium. In 90° direction to this band, 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. **Attention! The principle of this tast has NOTHING to do with haemolysis! If you see it, you may draw it, nevertheless it is not important for this task. Follow teacher‘s instructions and do the task only after his/her explanation!**



## Task 7: Determination of staphylococci using biochemical microtest (STAPHYtest 16)

Only for Medical Microbiology students

## 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 |
| | | | | |
| | | | | |
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