Topic P02: Diagnostics of streptococci

To study: Streptococcus (from textbooks, WWW etc.)
From spring term: Microscopy, culture, biochemical identification, neutralisation

Table for major results of Task 1 to Task 5 (to be filled step by step):

Strain		K	L	M	N	P	Q	R	S
Gram stain – Task 1									
Catalase test Task 2a									
Slanetz-Bartley medium – Task 2b									
Culture (blod agar) Task 3	Size								
	Colour								
	Shape								
	Profile								
	Agar changes								
	Other								
PARTIAL CONCLUSION									
Task 4a: Optochin (viridans strep only)									
Task 4b: Streptotest 16 (oral strep only)									
Task 5a: PYR test (haem. strep only)									
Task 5b: CAMP (haem. strep only)									
Task 5c (nAnB only) Agglutination									
FINAL CONCLUSION									

Task 1: Microscopy of suspicious strains

There are letter-labelled strains on the table. Gram-stain them and assess, which one is NOT a gram-positive coccus. To avoid confusion, label the slides using a dermograph. Write your results to the table.

Task 2: Basic culture and biochemical tests – genus determination

a) Catalase test for differentiation of staphylococci

Perform catalase test with all strains from Task 1 with exception of the strain proven not to be a G + coccus. Staphylococci should be catalase positive, streptococci and enterococci should be catalase negative.

b) Growth on Slanetz-Bartley agar for differentiation of enterococci

On the agar plate you have inoculated all strains, each in one sector. Nevetheless, only one of them is growing. This one should be an Enterococcus – so it is not a streptococcus. Write the results of 2a and 2b to the table.
Note: The same thing can be done with Bile-aesculin medium, too, but the colour of colonies is different.

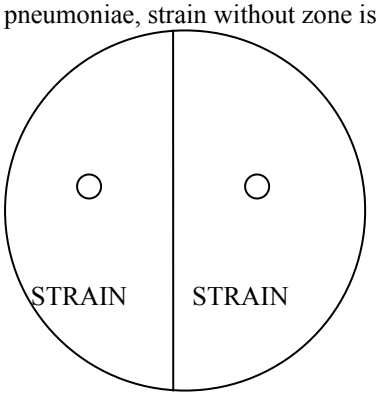
Task No. 3 Blood agar culture

On plates with blood agar, once more, you have all strains. Observe all of them, but describe only the strains that were not excluded by tasks 1 and 2. Describe colony morphology, and especially the haemolysis, partial haemolysis or viridation. Write your findings to the table.
Now write „partial conclusion“ to your table. To each strain K to S, write „NO STREP“ (no streptococcus) „HAEM STREP“ (partial or total haemolysis) or „VIR STREP“ (viridation)

Task No. 4 More detailed diagnostics of streptococci with viridation
a): Optochin test

Your task is to evaluate the result of optochin test in two strains shown to be viridans streptococci.

Optochin test is a test, that is not different from a normal diffusion disc test; the only difference is that the effective drug (optochin) is no more used for treatment. Strain with presence of inhibition zone around disc is *S.* pneumoniae, strain without zone is an „oral streptococcus“. Draw your result, and write „+“ or „–“ to the table.



b): Biochemical determination of the „oral“ streptococcus

In strain, found in Task 4a to be an „oral streptococcus“ evaluate the results of a biochemical microtest, using approaches known from summer semester.

	Tube	First row with 8 wells								Second row with 8 wells							
	VPT	1H	1G	1F	1E	1D	1C	1B	1A	2H	2G	2F	2E	2D	2C	2B	2A
	1	2	4	1	2	4	1	2	4	1	2	4	1	2	4	1	2
	Code:						Identification *Streptococcus* _____						% of probability			T index	

Task No. 5: Diagnostics of streptococci with partial or total haemolysis

This task will be done with three strains proven to be streptococci with beta-haemolysis (parts a, b); the last part will be only done with one of them proven to be „non-A-non-B“.

a): PYR test

PYR test is a strip-test, simillar to the oxidase test. For reading the colour result it is necessary to wait about five minutes, then add a drop of „Reagent for PYR test“ and wait another 30 s. Positive reaction is the red colour of the reaction zone. This strain is again positive in *S. pyogenes* (and in *Enterococcus*, too). Negative result can be seen in *S. agalactiae* and in non-A-non-B streptococci.

*Note: Formerly **Bacitracin test** was used inetead of PYR-test. Its principle was identical with that of optochin test, only with another antibiotic. Unfortunatelly, it is not specific enough, so we do not use it any more.*

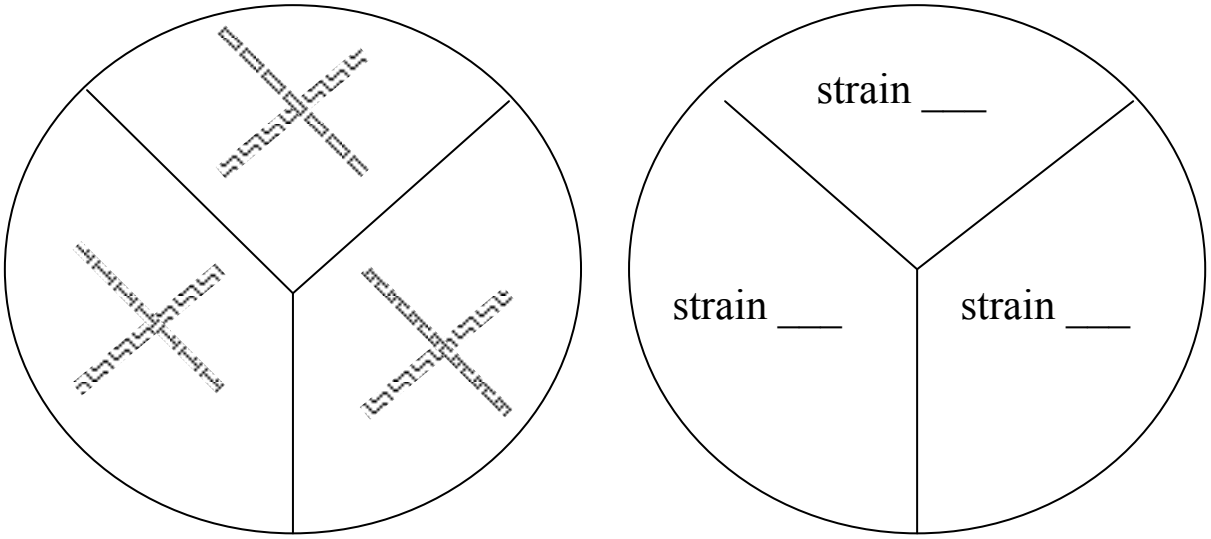
Fill the following table, including drawing a result of PYR test in all three tested strains.

Strain (write the letter)	Strain (write the letter)	Strain (write the letter)
Interpretation: negative – positive	Interpretation: negative – positive	Interpretation: negative – positive

b): CAMP test.

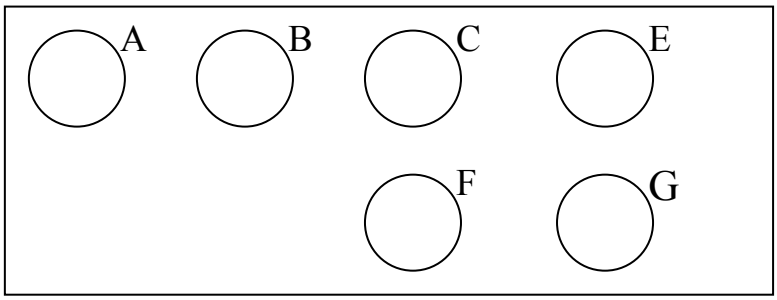
Note: This test has nothing to do with cyclic adenosinmonofosfate, therefore it is CAMP test and not cAMP test. Its name is derived from names of its inventors.

CAMP test is based on haemolytical synergism between *S. aureus* beta-haemolyzin producing strain, and *S. agalactiae* strain. Positive result has the form of two triangular zones („butterfly shape“) of complete hemolysis at crossing of both two strains. A small zone of another shape is considered negative. Draw your result (picture is on the following page):



c): Demonstration of agglutination test for detailed diagnostics of mainly non-A-non-B streptococci

Strains with both CAMP test and bacitracin and/or PYR test negative belong to the „non-A-non-B“ group. Draw the result of streptococcal agglutination from your dataprojection. Now, write the results of tasks 5 a), b) and c) to the table, and after that, **make a final conclusion of tasks 1–5.**



Task No. 6 Antibiotic susceptibility tests in streptococci

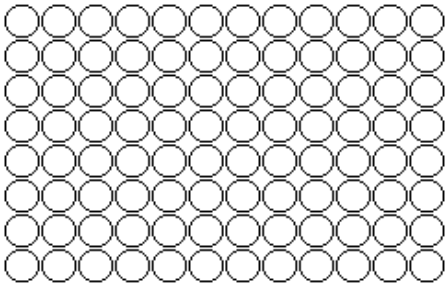
Evaluate the susceptibility tests (diffusion disc tests) for antibiotics in the strains of streptococci that you consider to be pathogens or possible pathogens (count with strains from specimens from the upper respiratory tract). To the table, write the abbreviations of antibiotics and for all tested strains measure the susceptibility zone in mm. On the card, you can see the borderline values of diameters – according to them, interprete the zones as susceptlible (S), resistant (R) or dubious (D).

Strain →								
Antibiotic (full name)	Zone ∅ (mm)	Interpr.	Zone ∅ (mm)	Interpr.	Zone ∅ (mm)	Interpr.	Zone ∅ (mm)	Interpr.

Task No. 7 Diagnostics of late sequellae of streptococcal infections – ASO determintion

On a side table in a wet chamber you will find a microtitration plate. It includes a positive control, sera 1 to 5. Determine the titers and interprete the risk for pacients. (For precise instructions, see the spring term – J09)

Result: Interpretation of the result:



Check-up questions:

1. Which species of *Streptococcus* is aranged in pairs? What is the shape of cocci in this species?

2. Which *Streptococcus* is PYR test positive? And which whole genus of G+ cocci is positive, too?

3. Is is useful to perform routinelly detailed diagnostics of oral streptococci? When it has its importance?

4. Why optochin test and diffusion disc test employs MH agar with blood and not common MH agar?

5. Can we use CAMP test in *Staphylococcus aureus* diagnostic? Why?

6. Why in beta-haemolytical streptococci biochemical test is rarely used for detailed diagnostics?

7. In which streptococci late sequellae take part, and what are the late sequellae?

8. Which streptococci cause infections wiht skin symptomas? What is the name of the infections?