Topic P02: Diagnostics of streptococci

To study: *Streptococcus* (from textbooks, www etc.)
From spring term: Microscopy, culture, biochemical identification, neutralization

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 (blood agar) Task 3	Size								
	Colour								
	Shape								
	Profile								
	Agar changes								
	Other								
PARTIAL CONCLUSION									
Task 4a: Optochin (viridans strep only)									
4b: STREPTOtest 16 (oral strep only)									
Task 5a: PYR test (haem. strep only)									
Task 5b: CAMP (haem. strep only)									
Task 5c: Agglutination (nAnB only)									
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 marker. Write your results in the table.

Task 2: Basic culture and biochemical tests – genus determination

a) Catalase test for the differentiation from staphylococci

Perform the catalase test with all the strains from Task 1 with the 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 (SB) agar for the differentiation of enterococci

The plate with SB agar has been inoculated with all the strains, each in one sector. However, only one of them is growing and that would be an enterococcus, not a streptococcus. Write the results of 2a and 2b in the table.
Note: The same thing can be done with bile-aesculin medium, too, but the colour of colonies is different.

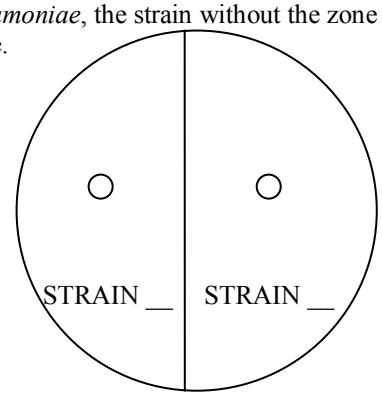
Task 3: Blood agar culture

The plates with blood agar again contain all strains. Observe all of them, but describe only the strains that were not excluded by tasks 1 and 2. Describe the colony morphology, and especially the haemolysis, partial haemolysis or viridation. Write your findings in the table.
Now write "Partial conclusion" to your table. Write "NO STREP" (no streptococcus), "HAEM STREP" (partial or total haemolysis) or "VIR STREP" (viridation) to each strain K to S.

Task 4: More detailed diagnostics of streptococci with viridation

a) Optochin test

Your task is to evaluate the result of the optochin test in the two strains shown to be streptococci with viridation. The optochin test does not differ from a common diffusion disc test but the effective drug (optochin) is not used for treatment any longer. The strain with the presence of the inhibition zone around the optochin disc is *S. pneumoniae*, the strain without the zone is an "oral streptococcus". Draw your result, and write "+" or "–" to the table. + = any susceptibility zone (not necessary to measure) – = no zone



b) Biochemical determination of "oral" streptococcus

In the strain found in Task 4a to be an "oral streptococcus", evaluate the results of a biochemical microtest (STREPTOtest 16), using methods learned in the summer term.

	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 5: Diagnostics of streptococci with partial or total haemolysis

This task will be done with the three strains proven to be streptococci with haemolysis (parts a, b); the last part (c) will be only performed with the one proven to be "non-A-non-B" streptococcus.

a) PYR test

PYR test is a strip-test, similar to the oxidase test. For reading the colour result, it is necessary to wait for about five minutes, then add a drop of "Reagent for PYR test" and wait another 30 sec. A positive result is indicated by the red colour of the reaction zone. This test is again positive in *S. pyogenes* (and in *Enterococcus*, as well). Negative result can be seen in *S. agalactiae* and in non-A-non-B streptococci.

*Note: Formerly **bacitracin test** was used instead of the PYR test. Its principle was identical with that of the optochin test, only with another type of antibiotic. Due to its low specificity, it's not in use any more.*

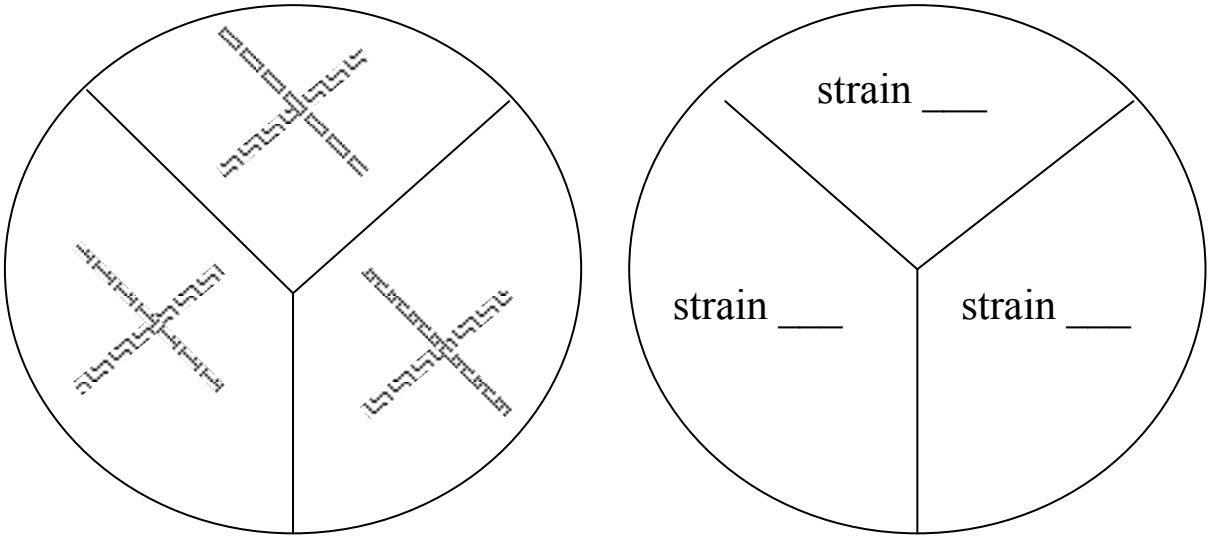
Fill in the following table, including drawing a result of the PYR test in all the three tested strains.

Strain (write the letter)	Strain (write the letter)	Strain (write the letter)
Interpretation: negative – positive *(delete as appropriate)*	Interpretation: negative – positive *(delete as appropriate)*	Interpretation: negative – positive *(delete as appropriate)*

b) CAMP test

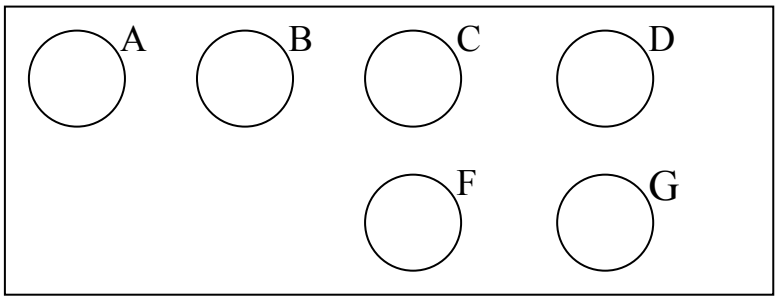
Note: This test has nothing to do with cyclic adenosinmonophosphate, therefore it is CAMP test and not cAMP test. Its name is derived from the names of its inventors.

The CAMP test is based on haemolytical synergism between *S. aureus* beta-haemolysin producing strain, and *S. agalactiae* strain. The positive result has the form of two triangular zones ("butterfly shape") of complete haemolysis at the crossing of both strains. A small zone of a different shape is considered negative. Draw your result (the picture is on the following page):



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

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



Task 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 (for the sake of simplification, consider the strains as originating from the upper respiratory tract). For the strain determined as a "non-A-non-B" streptococcus we do not perform the test, as its pathogenicity is low; for the strain determined as *S. agalactiae* (usually UTI origin) we have to use a special set of antibiotic, containing also special drugs for UTI treatment (e. g. nitrofurantoin).
Interpret the strains as susceptible (S), intermediary (I) or resistant (R) to given antibiotics.

Strain →							
Antibiotic	Susceptible if	Inter-mediate if	Resistant if	Zone ∅ (mm)	Interpre-tation	Zone ∅ (mm)	Interpre-tation
Penicillin P	≥ 18 mm		< 18 mm				
Erythromycin E	≥ 21 mm	18–20 mm	< 18 mm				
Clindamycin DA	≥ 17 mm		< 17 mm				
Chloramphenicol C	≥ 19 mm		< 19 mm				
Tetracycline* TE	≥ 23 mm	20–22 mm	< 20 mm				
Vancomycin VA	≥ 13 mm		< 13 mm				

Strain →					
Antibiotic	Susceptible if	Inter-mediate if	Resistant if	Zone ∅ (mm)	Interpre-tation
Penicillin P*	≥ 18 mm		< 18 mm		
Tetracycline* TE	≥ 23 mm	20–22 mm	< 20 mm		
Vancomycin VA	≥ 13 mm		< 13 mm		
Nitrofurantoin F	≥ 15 mm		< 15 mm		

*interpreted as ampicillin

Task 7: Diagnostics of late sequels of streptococcal infections – ASO determination

Principle – repetition of J07: *Antibodies prevents hemolysin (streptolysin O – i.e. antigen) to hemolyse rabbit RBC. ASO levels increase after hemolytic streptococci group A (less commonly also other groups) caused infections. In risk for late sequels, ASO increase over 200 I. U. (international units) is seen*.

On a side table, you will find a microtitration plate in a wet chamber. It includes a positive control and several sera. Determine the ASO values *(ASO value = the last positive well; absence of haemolysis means positivity, haemolysis means negativity)* and interpret the risk of late sequels of streptococcal infections.

	100	120	150	180	225	270	337	405	506	607	759	911	ASO value (IU)	Interpretation
K+	○	○	○	○	○	○	○	○	○	○	○	○		
P1	○	○	○	○	○	○	○	○	○	○	○	○		
P2	○	○	○	○	○	○	○	○	○	○	○	○		
P3	○	○	○	○	○	○	○	○	○	○	○	○		
P4	○	○	○	○	○	○	○	○	○	○	○	○		
P5	○	○	○	○	○	○	○	○	○	○	○	○		

Vocabulary to this topic:

In this protocol (and some textbooks)	In some other textbooks
viridation	alpha-haemolysis
partial haemolysis	beta-haemolysis
total haemolysis	
no haemolysis/absence of haemolysis	gamma-haemolysis