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 Tasks 1 to 5 and 8 to 9 (to be filled step by step):

Strain	K	L	M	N	P	Q	R	S	T	U	V	W
Gram stain – Task 1												
Culture – task 2 (basic characteristics only)												
Catalase test Task 3a												
Slanetz-Bartley medium – Task 3b												
Bile-aesculin 3c												
Task 4a: Optochin (viridans strep only)												
Task 5a: PYR test (haem. strep only)												
Task 5b: CAMP (haem. strep only)												
Task 5c: Agglutination (nAnB only)												
Task 8: arabinose test												
Task 9: Growth on BA at 4 °C												
FINAL CONCLUSION												

Task 1: Microscopy of suspicious strains

There are letter-labelled strains on the table. Gram-stain them. Differentiate G+ cocci, G+ bacilli and G– bacteria (those should not be studied anymore). In G+ bacilli mention also whether they are slender or robust, arranged in palisades or not and bearing spores or not. Label your slides with letters.

Task 2: Blood agar culture

The plates with blood agar again contain all strains. Observe all of them. Describe just the most typical characteristics (size, colour and mostly partial haemolysis/total haemolysis/viridation/no haemolysis).

Task 3: 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. Among G+ rods, listeriae, corynebacteria and bacilli are all catalase-positive, but some others (e. g. arcanobateria) would 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 in the table.

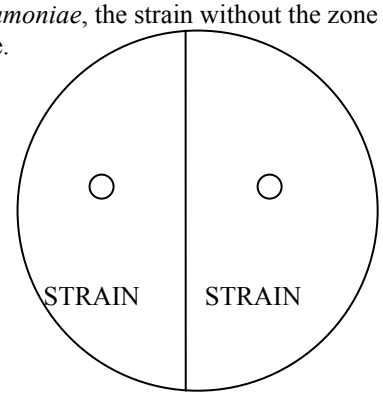
c) Growth on bile-aeskulin agar for differentiation of enterococci and listeriae

You have all your strains cultured on your Petri dish. Nevertheless, only three of them do grow. In case of G+ cocci, they should be enterococci. In case of G+ rods, they should be listeriae. Fill in the table.

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

Not performed in this special practical session. The reading is very similar to that of STAPHYtest 16. Follow the teacher's explanation.

Task 5: Diagnostics of streptococci with partial or total haemolysis

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

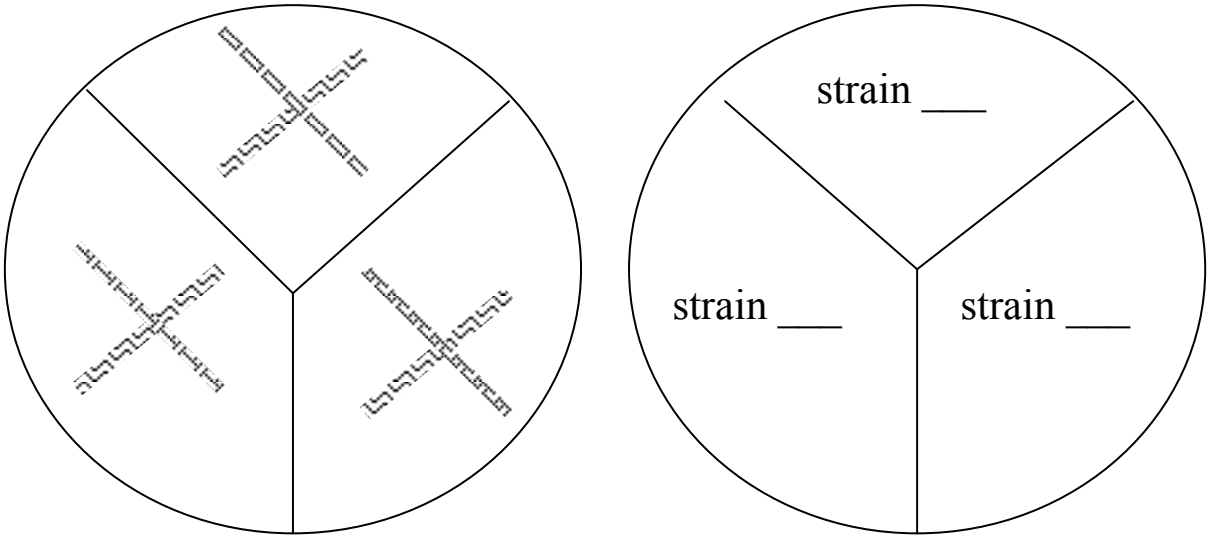
a) PYR test

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.
Look at 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).
For time reasons, in this double dental practical session, assess antibiotic susceptibility for strain of *Streptococcus pyogenes* (strain L) only.
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 beta-hemolytic streptococci group A (less commonly also other groups) caused infections. In risk for late sequellae, 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 interprete the risk of late sequellae 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	○	○	○	○	○	○	○	○	○	○	○	○		
P6	○	○	○	○	○	○	○	○	○	○	○	○		
P7	○	○	○	○	○	○	○	○	○	○	○	○		

Task 8: Differentiation of enterococci

a) Arabinose test for species determination of two most common enterococci

Examine the two strains proven to be enterococci in the previous tasks. Observe the test tubes with the result of the arabinose test. Yellow colour means positiveness (typical for *Enterococcus faecium*) and green colour means negativity (typical for *Enterococcus faecalis*).

b) Biochemical test for species determination of enterococci from important clinical materials (allowing to find more than the two most important species)

In important cases, it is recommended to use a more precise species determination method than the arabinose test. We use a biochemical test in a microtitration panel, in this country usually „EN-COCCUStest“. In this practical session it is not performed. It is simillar, but simplier than STAPHYtest 16 from P01: it has only one row (not two rows) and no additional test-tube test.

Task 9: More methods for diagnostics of *Listeria*

a) Growth of listeriae at 4 °C

Observe a plate with blood agar where the strains of Gram-positive rods were inoculated, and the plates were then cultivated at refrigerator temperature. Write the results in the main table.

b) Demonstration of *Listeria monocytogenes* growth on a chromogenic medium

Examine the appearance of listerial growth on a chromogenic medium. The medium is specific for this species. In medical microbiology, we do not use the chromogenic media for *Listeria* very often; however, it plays an important role in food industry.

Result: On the medium called_____*L. monocytogenes* has_____-coloured colonies.

Note: For practical reasons, antibiotic testing for listeriae, corynebacteria and enterococci is not performed here. The test uses to be performed as usually, only for *Listeria* and *Corynebacterium* it would be necessary to use MH agar with blood. For enterococci, use of normal MH agar would be OK.

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