

Name: \_\_\_\_\_

Date: \_\_\_\_\_

## Diagnostic of streptococci

**Table for summary results for tasks 1 - 6**

Strain		K	L	M	N	P	Q	R	S	T
Gram staining – Task 1										
Catalase – Task 2a										
Slanetz-Bartley – Task 2b										
Cultivation (Blood agar) Task 3	Size									
	Colour									
	Shape									
	Profile									
	Changes of agar									
	Other									
<b>Preliminary result</b>										
Task 4a: Optochine test (only str. with viridation)										
Task 4b: Streptotest 16 (only str. with viridation)										
Task 4c: Suscept. to bacitracin (str. with viridation)										
Task 5a: PYR test (only haemolytic st.)										
Task 5b: CAMP (only haemolytic st.)										
<b>DEFINITIVE RESULT</b>										

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**Task 1: Microscopy of microbial cultures**

Stain according to Gram pure cultures of prepared microorganisms. Use immersion oil and immersion objective. Draw your observations and write results into table. In strains, which are not Gram-positive cocci cross out the rest of the table.

**Task 2: Basic culture and biochemical tests – identification of the genus****a) Catalase test – differentiation of staphylococci**

Examine strains of Gram-positive cocci by catalase test. Staphylococci are catalase positive, streptococci and enterococci negative. Write results in your table.

**b) Growth on bile-aesculin agar – differentiation of enterococci**

There are all strains inoculated on the plate. The strain, which is growing on this medium, is enterococcus. Write results in your table.

**Task 3 – Cultivation on Blood Agar**

Describe morphology of colonies of prepared bacterial strains on Blood Agar. Note colour and haemolytic properties of observed colonies. Write the strain properties in your table.

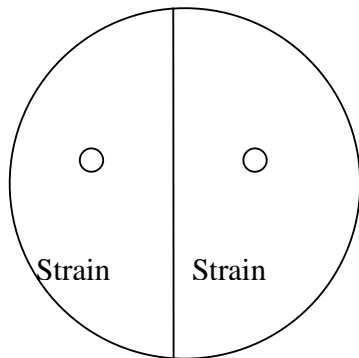
**Fill in the preliminary result – decide which strains are streptococci. To all strains write „NO STR“ (no streptococcus), „HAEMOL STR“ (partial of full haemolysis) or „VIRID STR“ (streptococcus with viridation).**

**Task 4 – Species identification of streptococci with viridation****a) Optochine test**

Evaluate the optochine test in your two streptococci with viridation. Strain with susceptibility to optochine is *S. pneumoniae*, the strain with no zone around optochine disc is „oral streptococcus“. Draw the result and write in your table (+ / -).

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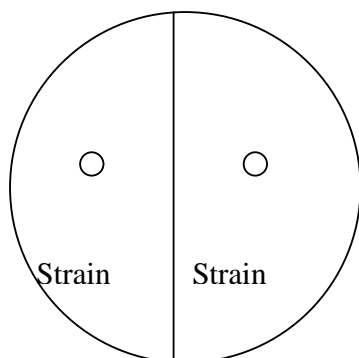
**b) Biochemical identification of „oral“ streptococcus**

In the strain of oral streptococcus read the biochemical profile and evaluate the result with knowledge from spring term.

VPT	1	1	1	1	1	1	1	1	2	2	2	2	2	2	2	2
	H	G	F	E	D	C	B	A	H	G	F	E	D	C	B	A
1	2	4	1	2	4	1	2	4	1	2	4	1	2	4	1	2
Code:			Identification				% of probability				T index					
			<i>Streptococcus</i>													

**c) Susceptibility to bacitracin**

*Streptococcus mutans*, streptococcus with high cariogenic potential is resistant to bacitracin. Evaluate which of your strains is *Streptococcus mutans*. Write the results in your table.



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**Task 5 Identification of streptococci with partial or full haemolysis**

Work only with three strain identifies as beta-haemolytic streptococci.

**a) PYR test**

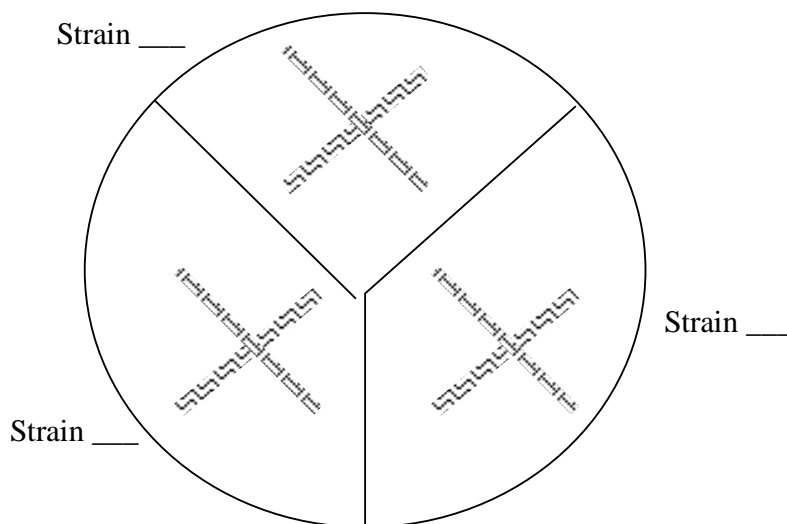
PYR test is strip test similar to oxidase test, but the reaction is slower, so the result can be read after 5 minutes and addition of PYR-indicator. Positive result is reddish or pinkish colour of the colony. This test is positive in *S. pyogenes* (and enterococci). Negative is in *Streptococcus agalactiae* and non-A-non-B streptococci.

Draw the result and fill the table.

Strain ____	Strain ____	Strain ____
<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
negative – positive	negative – positive	negative – positive

**b) CAMP test**

CAMP test is based on haemolytic synergy between the strain of *S. aureus* with production of beta-haemolysine and strain of *S. agalactiae*. Positive result is butterfly-like shape of zones of full haemolysis in the crux of both strains. Small zone of other shape is considered as negative result. The CAMP test is negative in *S. pyogenes* and non-A-non-B streptococci. Draw the result of the test.



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**Task 6 – Susceptibility tests in streptococci**

Read and evaluate the susceptibility tests (disc diffusion tests) to antibiotics in *Streptococcus pyogenes* and *Streptococcus agalactiae*.

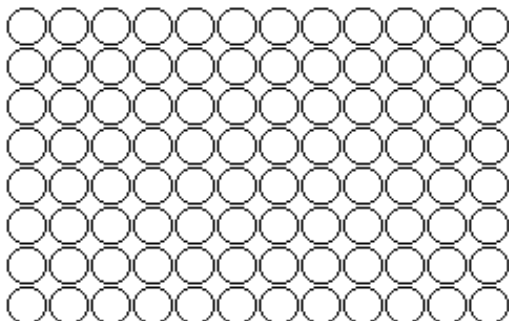
Kmen				
Antibiotikum (celý název)	Ø zóny (mm)	Interpr.	Ø zóny (mm)	Interpr.

**Task 7 – Diagnostic of late sequelae of streptococcal infection – determination of ASO**

On a side table in a wet chamber you will find a microtitration plate. It includes a positive control, sera X, Y and Z. Determine the titers and interpret the risk for patients. (For precise instructions, see the spring term – J08)

Result:

Interpretation of the result:



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**Check-up questions:**

1. Why it was difficult to see the arrangement of cocci in our task (pairs, clusters, chains)?
2. Which test besides cultivation on Bile-aesculin or Slanetz-Bartley medium might be employed in *Enterococcus* differentiation? One disadvantage: not only the enterococci, but one streptococcus is positive, too. Which one?
3. Is it useful to perform routine 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?