Topic P03: some more gram-positive bacteria (enterococci, listeriae, corynebacteria, bacilli)

To study: *Enterococcus, Listeria, Corynebacterium, Bacillus* (from textbooks, WWW etc.)
From spring term: Microscopy, culture, biochemical identification

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									
Culture (blod agar) Task 2	Size								
	Colour								
	Shape								
	Profile								
	Agar changes								
	Other								
Catalase test Task 3a									
Slanetz-Bartley medium – Task 3b									
Bile-aesculin medium – Task 3c									
Arabinose test Task 4a									
EnCoccus test Task 4b									
Growth in refrigerator Task 5a									
FINAL CONCLUSION*									

**In G+ bacilli, write genus name only. Species level diagnostic would require more tests, that could not be performed in our practital.*

Task 1: Microscopy of suspicious strains

There are letter-labelled strains on the table. Gram-stain them and write your results to the table. Do not forget to write important details („rods in palisades“, „robust, spore forming rods“ etc.). To avoid confusion, label the slides using a dermograph. The bacteria not being gram-negative are to be excluded from all remaining tasks.

Task 2: Morphology of colonies of G+ cocci and bacilli

Describe the colonies as usually. Do not describe collonies of bacteria proven not to be G+ cocci or bacilli.
In strains, microscopically found to be gram-positive rods, try to guess, to which genus the bacterium might belong, according to followindg description:
Bacillus – large, flat, dry, felt-like colonies, „spreading“ through the agar surface, sometimes with a massive haemolysis, sometimes with no haemolysisat all. Microscopically very robust rods, sometimes with finding of central or subterminal spores, that may, but must not be larger then the diameter of the rod.
Listeria – colourless to greyish colonies, very simillar to those of *Enterococcus*, with or without haemolysis, microscopically tinier than *Bacillus*, not arranged in pallisades, rather in short chains.
Corynebacterium (and related genera) – greyish or whitish colonies simillar to those of *Staphylococcus*, but less or more smaller, usually ahaemolytical, in microscopy rather smaller than previous, but club-shaped and arranged in palisades.

Task 3: Several common biochemical and culture tests

a) Catalase test

Perform catalase test for all strains proven to be G+. Mention, that *Listeria, Corynebacterium* and *Bacillus* are

positive, but some of conyneforms other than *Corynebacterium* (e. g. *Arcanobacterium*) are catalase negative.

b) Growth on Slanetz-Bartley medium

On your plate, the same strains as in Task 1 are cultivated in sectors. Positive strains should be not only growing, but also maroon colour of colonies. *Enterococcus* is the only G+ bacterium growing on this medium. Write your result to the table.

c) Growth on Bile aesculin medium

Unlike Slanetz-Bartley medium, Bile-aesculin medium enables not only growth of *Enterococcus* (diagnostic for this genus among G+ cocci), but also *Listeria* (diagnostic among G+ bacilli). Write your result to the table.

Task 4: Mutual differentiation of enterococci

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

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

b) Biochemical test for species determination of enterococci from important clinical materials (able to find more then two most importand species)

In important cases, we use rather a better species determination method than the arabinose test. We use a biochemical test in a microtitration panel, in Czechia usually „EN-COCCUStest". Notice, that the arabinose test takes part in this test, too. Mention, that EN-COCCUS test is simple in comparison with Staphytest 16 and Streptotest 16. Read the results of the EN-COCCUStest according to the instruction sheet in both strains from the previous task. Fill in the table bellow and write your result to the main table.

Strain:	H	G	F	E	D	C	B	A	Code:	
									Identification:	
	1	2	4	1	2	4	1	2	% of probability:	
									Typicity index:	
Strain:	H	G	F	E	D	C	B	A	Code:	
									Identification:	
	1	2	4	1	2	4	1	2	% of probability:	
									Typicity index:	

Task No. 5 More methods for diagnostics of *Listeria*

a): Growth of Listeria at 4 °C

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

b): Demonstration of *Listeria monocytogenes* growth on a chromogene medium

Examine the picture of listerial growth on a chromogenic medium. The medium is specific fot this species. In medical microscopy we do not use the chromogenic media for *Listeria* too often; it has, however, a big importance in food industry.

Result: On the medium called_____*L. monocytogenes* has_____coloured colonies.

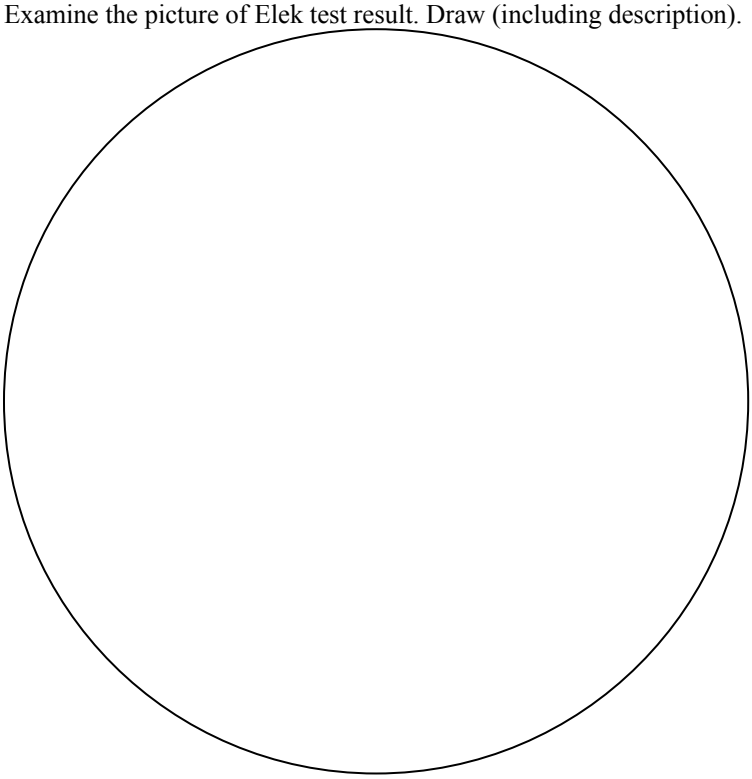
Task No. 6: Susceptibility tests of enterococci and gram-positive rods to antibiotics

On your table, you will find diffusion disc tests for strains found to be *Enterococcus faecalis, Enterococcus faecium, Listeria* sp. and *Corynebacterium* sp. There is no test for Bacillus sp.– the findings of this genus is usually interpreted as environmental contamination and thus not tested.
Write abbreviations of antibiotics according to the card and measure susceptibility zones for all tested strains. Borderline zones are written on the cards; using them, interprete the strains as susceptible (S) resistant (R) and dubious (D).

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

Task No. 7 Demonstration of Elek test

Examine the picture of Elek test result. Draw (including description).



Check-up questions:

1. Which gram-positive rods are spore forming? Are the spores allways visible?

2. Why there are no cephalosporins in the set of antibiotics? (The set is primarilly used for enterococci.)

3. What are the VRE and what is their importance?

4. Which bacteria (other than *Listeria*) grow at the refrigerator temperature?

5. What are the metachromatic granula and what is (rather „was“) the importance of their staining in diphtheria diagnostics?

67. What is the use of *Bacillus stearothermophilus* and *Bacillus subtilis* in medical microbiology (See J05)