Topic PZ03: 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									
Task 2 Colonies on the blood agar	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 diagnostics would require further tests that could not be performed in our practicals.*

Task 1: Microscopy of suspicious strains

There are letter-labelled strains on the table. Gram-stain them and write your results in 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 wax pencil. The bacteria not being Gram-negative are to be excluded from all the remaining tasks.

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

Describe the colonies as usually. Do not describe colonies of bacteria proven not to be G+ cocci or bacilli.
In the strains microscopically found to be Gram-positive rods try to guess to which genus the bacterium might belong, according to the following description:
Bacillus – large, flat, dry, felt-like colonies, "spreading" through the agar surface, sometimes with a massive haemolysis, sometimes with no haemolysis at all. Microscopically very robust rods, sometimes with central or subterminal endospores, that may, but not necessarily, be larger than the diameter of the rod.
Listeria – colourless to greyish colonies, very similar to those of *Enterococcus*, with or without haemolysis, microscopically tinier than *Bacillus*, not arranged in palisades, rather in short chains.
Corynebacterium (and related genera) – greyish or whitish colonies, similar to those of *Staphylococcus* but sometimes considerably smaller, usually nonhaemolytic, microscopically rather smaller rods than in the previous genera, but club-shaped and arranged in palisades.

Task 3: Several common biochemical and culture tests

a) Catalase test

Perform catalase test for all the strains proven to be G+. Note, that *Listeria, Corynebacterium* and *Bacillus* are catalase positive, but some other coryneforms (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 of pink to maroon colour of colonies. *Enterococcus* is the only G+ bacterium growing on this medium. Write your result in the table.

c) Growth on bile-aesculin medium

Unlike the Slanetz-Bartley medium, bile-aesculin medium enables not only the growth of *Enterococcus* (allowing the diagnostics of this genus among G+ cocci), but also *Listeria* (diagnostic among G+ bacilli). In a positive case, you see black colonies. Write your result in the table.

Task 4: 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“. Note, that the arabinose test takes part in this test, too, and, that the EN-COCCUS test is simpler 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 in the main table.

Strain:	H	G	F	E	D	C	B	A	Code:
	1	2	4	1	2	4	1	2	Identification:
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	1	2	4	1	2	4	1	2	Identification:

Task 5: 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 (*does grow – does not grow*) 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.

Task 6a: 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* and *Listeria* sp. There is no test for *Enterococcus faecium* – majority of clinical isolates come from stool, so there is no need to perform antibiotic susceptibility testing. Nevertheless, UTI isolates are also not uncommon (see Task 6b). There is also no test for *Corynebacterium* sp. – let us suppose that our strain is a skin isolate, i. e. it is a part of normal microflora. And finally, there is no test for *Bacillus* sp. – the findings of this genus are usually interpreted as environmental contamination and thus not tested.

Write the names of the antibiotics according to the card and measure the susceptibility zones for all the tested strains. Borderline zones are written on the cards; using them, interpret the strains as susceptible (S), resistant (R) or dubious (D).

Strain letter →					*Notes:*
Antibiotic	Zone ∅ (mm)	Interpr.	Zone ∅ (mm)	Interpr.	**result is also valid for doxycycline*
Ampicillin (AMP) (ref. zone 10 mm)					***quinupristin + dalfopristin, combination of two streptogramin antibiotics*
Nitrofurantoin (F) (ref. zone 15 mm)					****only suitable for enterococci if combined with beta-lactamic antibiotics*
Vancomycin (VA) (ref. zone 12 mm)					
Tetracycline* (TE) (ref. zone 19 mm)					
Q. + D.** (QD) (ref. zone 22 mm)					
Gentamicin (CN)*** (ref. zone 8 mm)					

Task 6b: Demonstration of antibiotic susceptibility test for *Enterococcus faecium*

On the side table you can see a test for *E. faecium*. Write the name of antibiotic that is used as drug of choice for *E. faecalis* infections, but cannot be used for *E. faecium* because of primary resistance: _____

Task 6c: Demonstration of a VRE strain

On the side table or in the slideshow you can also see a VRE strain. Using your memory and/or protocols from spring semester, write what that abbreviation stands for: _____

Task 7: Demonstration of Elek test

The principle of the Elek test is precipitation between the toxin of a toxic strain and the antitoxin from a paper strip with the antiserum. Both the toxin and the antitoxin diffuse through the agar plate. Students of dental medicine do not preform this test.