Topic P06: Diagnostics of some more gram-negative bacteria (*Neisseria, Moraxella, Bordetella, Legionella, Francisella…..*)

Table for major results of Task 1 to Task 4 (to be filled step by step):

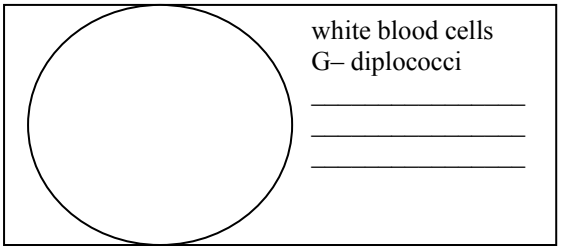
Strain		K	L	M	N	P
Gram stain of a strain – Task 1b						
Culture – task 2	"Poor" BA ("KA") Growth Y/N					
	"Rich" BA+ ("KA+") Growth Y/N					
	Chocolat agar ("ČA") Growth Y/N					
	Description of colonies on BA+*					
Task 3	a) Oxidase test (+/–)					
	b) Indoxylacetate (INAC) test (+/–)					
FINAL CONCLUSION (result of Task 4 – NEISSERIAtest, or result of Task 1 for the strain proven not to be G– cocci)						

*Use ChA (Chocolate agar) for bacteria not growing on BA+ (blood agar+)

Task 1: Microscopy of a clinical specimen and microscopy of strain

a) Observation of a urethral smear in gonorrhoea

Observe and Gram stained smear.
Pay attention not only for bacteria, but also for the macroorganism cells, especially leucocytes, and the position of bacteria in relation with the leucocytes. Mention, that cocci are not present in all white blood cells. Draw your result and draw lines connecting the description with the objects in your picture.
Note: Very simillar is also and smear from CSF in meningococcal meningitis.



b) Microscopy of suspicious strains – search for gram-negative cocci

There are slides with Gram-stained preparations on your table. Observe them and write your results to the table. Strain that is NOT G– coccus should not be used in tasks 3 and 4 (but in Task 2 it should be described, for comparison).

Task 2: Cultivation on agar media

Mark to your table, what bacteria grow on „poor blood agar", „rich blood agar" and chocolat agar. Oral species of *Neisseria*, but also *Moraxella* and majority of G+ cocci are able to grow on all media. *Neisseria meningitidis* („meningococcus") can only grow on „rich" blood agar. *Neisseria gonorrhoeae* (gonococcus) is not able to grow on blood agar at all, chocolate agar is needed. After that, describe the colonies on rich blood agar; the one not growing should be described on chocolat agar. Write all your results to the table.

Task 3: Basic biochemical tests in gram-negative cocci

Both tests will be done as a demonstration at a side table. Write your results to the table.

a) Oxidase test for differenciation of *Neisseria* or *Moraxella* from other G- cocci

Your teacher will touch several colonies of strains identified as G- cocci by the oxidase diagnostic strip. Blue colour should appear in several seconds, when positive. Draw a positive and a negative result.

b) Indoxylacetate test for differenciation of *Moraxella catarrhalis* from *Neisseria*

The procedure is simillar as that of oxidase test, but the strip should be moistened in advance, the colour is rather blue-green and not blue and it is not visible immediatelly, but it is necessary to wait several minutes. Draw a positive and a negative result.

Task 4: Species diagnostics of *Neisseria* and *Moraxella* (*Branhamella*) using biochemical identification tests

In strains, found to be gram-negative cocci, read the biochemical microtest (NEISSERIAtest by Lachema) inoculated one day before. Read it according to the scheme. The first well contains negative control (NEC), so the proper test starts in the SECOND well! Dropping of Lugol solution was allready done, you should not do it yourselves. Remark low biochemical activity of some Neisseria. Compare the result with cultivation conditions (the strain, found to be *N. gonorrhoeae*, shoud grow on chocolate agar only; the strain, found to be *N. meningitidis*, on chocolate and modified blood agar only).

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

Task 5: Susceptibility tests of G- cocci to antibiotics

Perform in vitro susceptibility testing of gram-negative cocci to suitable antibiotics.

Evaluate the diffusion disc susceptibility tests to antibiotics in strains found to be gram-negative cocci and that are pathogenous. Into the table, write the abbreviation of the antibiotics according to a card and for all tested strains measure the susceptibility zones. On your card, you have limit zones – according to them, interprete the zones as susceptible (S) resistants (R) and dubious (D).

Note: It is recommended to perform nitrocephin test as a proof of beta-lactamase, instead of diffusion disc test for testing susceptibility to penicillin (Neisseria) and ampicilin (Moraxella). To simplify the task for students, this recommendation were not taken into account.

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

Task 6: Direct detection of antigens of causative agents of meningitis in the cerebrospinal fluid (demonstration of a diagnostic kit and observation of a videoclip)

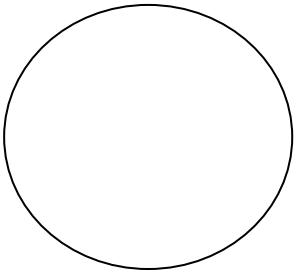
Meningococcal meningitis is a severe disease. It is not possible to wait for culture, so we need a quick diagnostic method. Besides microscopy, latex agglutination is one very important method for this purpose.

Only General Medicine

Task 7: Diagnostics of *Bordetella*, *Brucella*, *Legionella* and *Francisella*

a) Culture diagnostics of *Bordetella*

There is a special medium for *Bordetella pertussis*, an a special way of inoculation is used here. Unlike many other bacteria, *Bordetella* is resistant to penicillin; so we start by making a drop of penicillin solution in the middle of the agar plate. The swab is mixed with the drop, and inoculated in a spiral form. Then the loop is used to make radial rays. Write down the name of the medium, and re-draw the way of its inoculation from your slideshow.



Name of the medium: _____

b) Demonstration of a culture medium for *Legionella*

Only for General Medicine

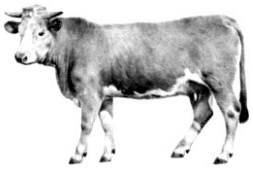
c) Antibody detection in tularemia

Only for General Medicine

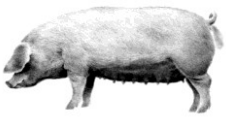
d) A Thought for Brucella

Diagnostics of *Brucella* is difficult and it is not easy in practice, as diseases caused by *Brucella* are not common in today Central Europe. Nevertheless, brucellosis still exists in many parts of the world. It is necessary to know at least, what is the connection between the species and the host animal.
Connect a picture of a typical host withe the name of a corresponing species of *Brucella*.

Brucella mellitensis



Brucella abortus



Brucella suis