Topic P06: Diagnostics of some other Gram-negative bacteria (*Neisseria, Moraxella, Bordetella, Legionella, Francisella…*)

To study: *Haemophilus, Neisseria, Moraxella, Bordetella, Legionella, Francisella* (from textbooks, www etc.)
From spring term: Microscopy, culture, biochemical identification, agglutination

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

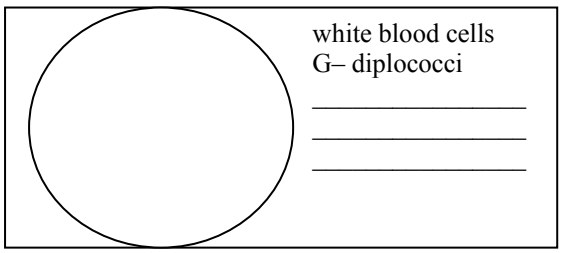
Strain		K	L	M	N	P
Gram stain of a strain – Task 1b						
Culture – task 2	"Common" BA ("KA") Growth Y/N					
	"Rich" BA+ ("KA+") Growth Y/N					
	Chocolate agar ("ČA") Growth Y/N					
	Description of colonies on BA+*					
Task 3	a) Oxidase test (+/–)					
	b) Indoxylacetate (INAC) test (+/–)					
FINAL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

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

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

a) Observation of a urethral smear in gonorrhoea

Observe a Gram-stained smear.
Pay attention not only to the bacteria, but also to the macroorganism cells, especially leucocytes, and the position of the bacteria in relation to the leucocytes. Notice that cocci are not present in all white blood cells. Draw your results and draw arrows connecting the description with the object in your picture.
Note: The smear from CSF in meningococcal meningitis is very similar.



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

There are slides with Gram-stained preparations on your table. Observe them and write the results into 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 in your table which bacteria grow on "common blood agar", "rich blood agar" and chocolate agar. Oral species of *Neisseria* but also *Moraxella* and majority of G+ cocci are able to grow on all media. *Neisseria meningitidis* (meningococcus) can grow only on rich blood agar. *Neisseria gonorrhoeae* (gonococcus) is not able to grow on blood agar at all, the chocolate agar is required. After that, describe the colonies on the rich blood agar; the one not growing should be described on the chocolate agar. Write all your results into the table.

Task 3: Standard biochemical tests in Gram-negative cocci

Both tests will be performed as a demonstration at a side table. Write the results into the table.

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

Your teacher will touch several colonies of strains identified as G– cocci with the oxidase diagnostic strip. When positive, blue colour should appear in several seconds. Draw the positive and the negative result.

\+ _____ − _____

b) Indoxylacetate test for the differentiation of *Moraxella catarrhalis* from *Neisseria spp.*

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

\+ _____ − _____

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

In the strains found to be Gram-negative cocci read the biochemical microtest (NEISSERIAtest Lachema) inoculated on the previous day. Read according to the scheme. The first well contains the negative control (NEC), so the proper test starts at the SECOND well! Dropping the Lugol solution has been already done, you should not do it yourselves. Note the low biochemical activity of some neisseriae. Compare the result with the cultivation conditions. The strain, found to be *N. gonorrhoeae*, should grow on chocolate agar only; the strain, found to be *N. meningitidis*, on chocolate and modified ("rich") 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 pathogenic Gram-negative cocci. Write the abbreviation of the antibiotics according to the card into the table and for all the tested strains, measure the susceptibility zones. On your card, you have limit zones – according to them, interprete the zones as susceptible (S), resistant (R) and dubious (D).

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

Strain →						
Antibiotics (full name)	Zone ∅ (mm)	Interpre-tation	Zone ∅ (mm)	Interpre-tation	Zone ∅ (mm)	Interpre-tation

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

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

a) Demonstration of a latex agglutination kit

Observe the kit and write down the names of bacteria that can be found using this method.

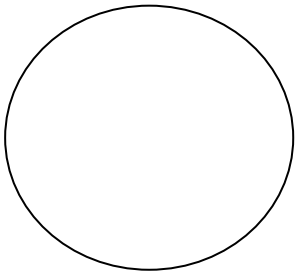
b) Videoclip

Look at the videoclip. In our example, the pathogen was found to be _____

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

a) Culture diagnostics of *Bordetella*

There is a special medium for *Bordetella pertussis*, and 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: _____

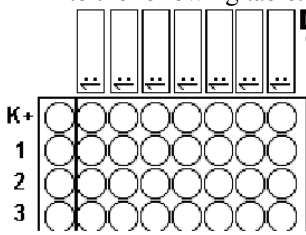
b) Demonstration of a culture medium for *Legionella*

Observe the culture medium for *Legionella*. Write down some data about it:

Abbreviation	What the individual letters of the abbreviation mean	Colour

c) Antibody detection in tularemia

On the side table you will find a wet chamber with the result of direct diagnostics of *Francisella* using agglutination. The wells with a positive reaction show the presence of agglutinate (a larger aggregate of irregular shape), the wells with a negative reaction show bacterial sedimentation (smaller, intensively white round disc). Fill into the following table.



Interpretation: Any titer is considered suspicious. The definitive desision about treatment should be done in relation with clinical symptomatology

	Interpretation:
TITER=1:	
TITER=1:	
TITER=1:	
TITER=1:	

d) A note about Brucella

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

Connect the picture of a typical host with the name of a corresponding *Brucella* species.

Brucella mellitensis *Brucella abortus* *Brucella suis*