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):

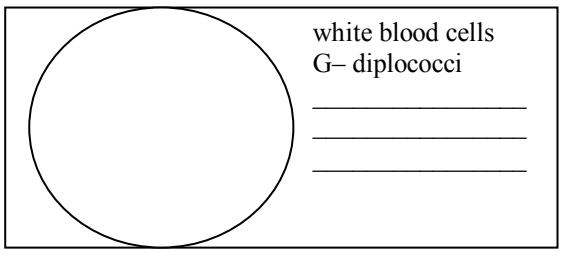
Strain		K	L	M	N	P
Gram stain of a strain – Task 1b						
Culture – task 2	"Common" BA ("KA") Growth Y/N					
	"Rich" BA+ ("KA+") Growth Y/N					
	Chocolate agar ("ČA") Growth Y/N					
	Description of colonies on BA+*					
Task 3	a) Oxidase test (+/–)					
	b) Indoxylacetate (INAC) test (+/–)					
FINAL 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.

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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.

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Task 4: Species diagnostics of *Neisseria* / *Moraxella* (*Branhamella*) (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. For all the tested strains, measure the susceptibility zones. In your protocol, you have limit zones – according to them, interpret the zones as susceptible (S), resistant (R) and intermediate (I).

a) Susceptibility of meningococcus (strain ___) to antibiotics

Actually, susceptibility to penicillin is tested by E-test, and other susceptibilities by diffusion disc test in meningococcus.

Antibiotic (breakpoint values in μg/ml)	MIC (μg/ml)	Interpr.
Penicillin (P) S ≤ 0.06 R > 0,25		

Antibiotic (zones in mm)	∅ zone (mm)	Interpretation
Cefotaxime (CTX) S ≥ 34 R < 34		
Meropenem (MEM) S ≥ 30 R < 30		
Azithromycin (AZM) S ≥ 20 R < 20		
Ciprofloxacin (CIP) S ≥ 35 R < 33		

b) Susceptibility of gonococcus (strain ___) to antibiotics

Actually, in gonococcus, susceptibility to penicillin and cefotaxime is tested by E-test, and other susceptibilities by diffusion disc test.

Antibiotic (breakpoint values in μg/ml)	MIC (μg/ml)	Interpr.
Penicillin (P) S ≤ 0.06 R > 1		
Cefotaxime S ≤ 0.12 R > 0.12		

Antibiotic (zones in mm)	∅ zone (mm)	Interpretation
Cefuroxime (CXM) S ≥ 31 R < 26		
Azithromycin (AZM) S ≥ 25 R < 25		
Tetracycline (TE) S ≥ 38 R < 30		
Ciprofloxacin (CIP) S ≥ 41 R < 28		

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

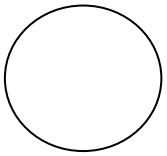
Look at the videoclip. In our example, the pathogen was found to be _____

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

a) Diagnostics of *Bordetella*

*Diagnostics of pertussis is now based on serology (agglutination and ELISA), that always requires **two** specimens of sera. Another diagnostic method is PCR. Nevertheless, cultivation is still important and classic method.*

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: _____

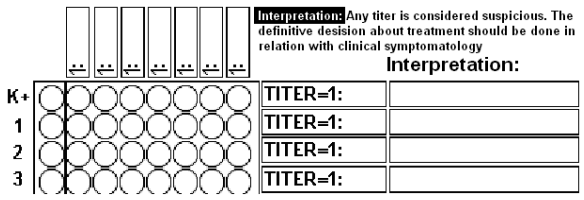
b) Demonstration of a culture medium for *Legionella*

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 indirect 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). The first well is a "technical well", after the line diluted sera continue in dilutions 1 : 10, 1 : 20, 1 : 40 etc. Try to interpret the results, if you know that any titre is interpreted as "suspicious", of course, except positive control.



d) Diagnostics of antibodies against brucellosis

Diagnostics of brucellosis (Bang disease – caused by *B. abortus*) was performed using indirect diagnostics – ELISA in both IgG and IgM antibodies. The absorbance was measured by a spectrophotometer and the results were converted into "positive", "borderline" or "negative" values using an expert system. Results can be found on your table. Try to make a final conclusion to individual patients.

Patient	IgM result	IgG result	Final conclusion
Alice			
Bob			
Claudia			
David			

Note: Brucellosis is quite rare disease and many laboratories, including our laboratory, does not perform the diagnostics. Therefore the worksheets used for this task are not real Brucella diagnostics worksheets, but adapted worksheets of another serology reaction. On the other hand, the true worksheets for Brucella diagnostics would look the same of very similar.