

Name: \_\_\_\_\_

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**Diagnostics of some other gram-negative bacteria (*Neisseria*, *Moraxella*, *Bordetella*, *Legionella*, *Francisella*.....)**

**Table for major results of Task 1 to Task 4:**

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					
	Chocolate agar (“CA”) Growth Y/N					
	Description of colonies on BA+*					
Task 3	a) Oxidase test (+/-)					
	b) Indoxylacetate (INAC) test (+/-)					
<b>FINAL CONCLUSION (result of Task 4 – <i>Neisseria</i> Test, or result of Task 1 for the strain proven not to be G– cocci)</b>						

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

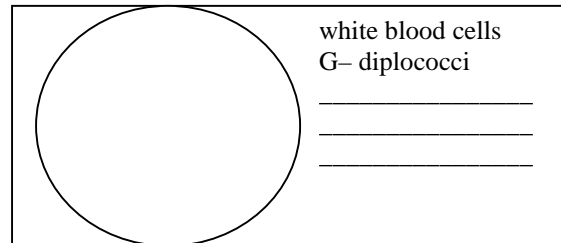
**Task 1: Microscopy of a clinical specimen and microscopy of strain**

**a) Observation of a urethral smear in gonorrhoea**

Observe and Gram stained smear.

Pay attention not only for bacteria, but also for the cells of macroorganism, especially 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 similarly looks also a smear of purulent CSF (cerebrospinal fluid) in meningococcal meningitis.



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

There are letter-labelled strains on the table. Gram-stain them and write your results to the 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 in the table, what bacteria grow on „nutritionally poor blood agar BA-“, „rich blood agar BA+“ and chocolate agar. Oral species of *Neisseri* as well as *Moraxella* and majority of G+ cocci are also able to grow on nutritionally poor BA. *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 of all strains on BA+. If the strain does not grow it should be described on chocolate agar. Write all your results to the table.

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### Task 3: Basic biochemical tests for gram-negative cocci

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

#### a) Oxidase test

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

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

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### Task 4: Species diagnostics of *Neisseria* and *Moraxella* using biochemical 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, so the proper test starts in the SECOND well! Dropping of Lugol solution was already done, you should not do it yourselves. Remark low biochemical activity of some *Neisseria*. Compare the result with grow requirements (the strain, found to be *N. gonorrhoeae*, should 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:
	×	1	2	4	1	2	4	1		Identification:
	×									
Strain:	H	G	F	E	D	C	B	A		
	NEC									Code:
	×	1	2	4	1	2	4	1		Identification:
	×									
Strain:	H	G	F	E	D	C	B	A		
	NEC									Code:
	×	1	2	4	1	2	4	1		Identification:
	×									
Strain:	H	G	F	E	D	C	B	A		
	NEC									Code:
	×	1	2	4	1	2	4	1		Identification:
	×									

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### Task No. 5 Susceptibility tests of G- cocci to 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, interpret the zones as susceptible (S) resistant (R) and dubious (D).

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

### Task No. 6 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: \_\_\_\_\_

### Check-up questions:

1. What specimens are recommended to take for gonorrhoea diagnostics? And how should they be transported to the laboratory?
  
2. When taking CSF specimen, can you see any differences between healthy person and a person with purulent meningitis? (Just at the patient, not in the laboratory.)
  
3. *Neisseria* and *Moraxella* are both gram-negative. Does this mean that they grow on Endo agar?

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4. What species of *Neisseria* are the less biochemically active? What sugars do they split?

5. What causative agent of meningitis is the most common one in pre-scholar age, what in teens, what in elder people?

6. What is the most typical source of tularaemia infection?