

Topic P06: Diagnostics of some more 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	“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 – <i>Neisseria</i> Test, 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+)

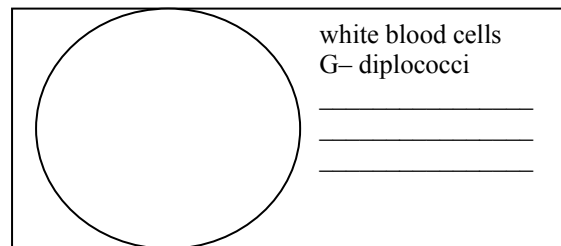
Task 1: Microscopy of a clinical specimen and microscopy of strain

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 similar is also a 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

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 and not 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 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 blood agar only).

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

Task No. 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, interpret 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 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 Ø (mm)	Interpr.	Zone Ø (mm)	Interpr.	Zone Ø (mm)	Interpr.	Zone Ø (mm)	Interpr.

Check-up questions:

1. What are the most recommended specimens 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?

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?

7. There exist also gram-negative cocci and bacilli, that were not studied in practical lessons, but may be important. Find in textbooks or www, what diseases are caused by following microorganisms:
 - a) *Bartonella quintana*
 - b) *Bartonella hensellae*
 - c) members of HACEK group (and write also the names of all bacteria belonging to this group).