Topic P04: Diagnostics of enterobacteria and bacterial agents of gastrointestinal infections

To study: *Enterobacteriaceae, Vibrionaceae, Campylobacter, Helicobacter* (from textbooks, WWW etc.)
From spring term: Microscopy, culture, biochemical identification, antigen analysis

Table for major results of Task 1 to Task 5 (to be filled step by step):

Strain		K	L	M	N	P	Q	R	S
Gram stain – Task 1									
Culture (blod agar and Endo agar) Task 2	Size on BA								
	Colour on BA								
	Other on BA								
	Size on Endo								
	Colour on Endo								
	Other on Endo								
Hajna medium Task 3a									
Oxidase test Task 3b									
PARTIAL CONCLUSION									
More media Task 4a	XLD agar								
	MAL agar								
	CIN agar								
EnteroTest 16 (Task 4b)									
Antigen analysis (Tasks 5a and 5b)									
FINAL CONCLUSION									

Task 1: Microscopy of suspicious strains

There are letter-labelled strains on the table. Gram-stain them and write your results to the table. Strain that is NOT G– rod should not be used in tasks 3 to 5 (but in Task 2 it should be described, for comparison)

Task 2: Cultivation on blood agar and Endo agar

Using standard procedure, describe colonies of all strains on blood agar and Endo agar. If the strain on the medium does not grow, write a zero to the corresponding cell of the table. Bacteria, that do not grow on any of the media and morphologically look like curved gram-negative rods, might be *Campylobacter* – see later. A G– rod, that does not grow on any of the media, but is not curved, will be studied in P05. For comparison describe also the strain, that appeared morphologically as a gram-positive coccus.

Task 3: Group diagnostics of the most imporant gram-negative rods growing on Endo agar (differentiation of enterobacteriae, *Vibrionaceae* and G– non-fermenters)

a) Reading of an examination on oblique agar according to Hajna

Agar according to Hajna is a combined diagnostic medium. Nevertheless, in this task we will mostly search for biochemically non-active, neither glucose nor lactose splitting and sulphan non forming rods – the gram-negative non-fermenting bacteria („non-fermenters“). All strains, growing on Endo, were inoculated on Hajna medium. Have a look to the result. Where the medium remained fully red, it is a biochemically non-active strain – very likely, a gram-negative non-fermenter. This strain will not be used in Task 4 and Task 5.

b) Oxidase test

The teacher will do as a demonstration oxidase test for all Gram-negative, on Endo agar growing bacteria. Oxidase-positive are members of family *Vibrionaceae* and some gram-negative non-fermenters; the *Enteobacteriaceae* are (with exception of *Plesiomonas*) oxidase negative.
Make partial conclusion after tasks 1 to 3. What bacteria are enterobacteria? Tasks 4 and 5 will be only performed with strains proven to be enterobacteria.

Task 4: Genus and species determination of enterobacteria

a) Culture of enterobacteria on more media

You have already seen, how the colonies look like on BA and Endo agar. Add shortly your description of appearance of the colonies on CIN, XLD and MAL.

b) Biochemical behaviour of enterobacteria

Evaluate given results of ENTEROtest 16, beeng incubated a day berfore. Check, wethrer the results with other, already done tests; e.g. strains with sulphan formation lead to black colour of Hajna medium, *Yersinia* has tiny pink colonies, *Salmonella* pale transparent colonies with black centre on XLD and MAL medium…
For the strain found to be *Salmonella*, write *Salmonella* sp. only. Count % of probability as a total of all % of probability of individual Salmonellas at the code; T index should be taken from the first *Salmonella.*

	ONPG	1H	1G	1F	1E	1D	1C	1B	1A	2H	2G	2F	2E	2D	2C	2B	2A
Strain:																	
	1	2	4	1	2	4	1	2	4	1	2	4	1	2	4	1	2
	Code:						Identification						% of prob.			T index	

	ONPG	1H	1G	1F	1E	1D	1C	1B	1A	2H	2G	2F	2E	2D	2C	2B	2A
Strain:																	
	1	2	4	1	2	4	1	2	4	1	2	4	1	2	4	1	2
	Code:						Identification						% of prob.			T index	

	ONPG	1H	1G	1F	1E	1D	1C	1B	1A	2H	2G	2F	2E	2D	2C	2B	2A
Strain:																	
	1	2	4	1	2	4	1	2	4	1	2	4	1	2	4	1	2
	Code:						Identification						% of prob.			T index	

	ONPG	1H	1G	1F	1E	1D	1C	1B	1A	2H	2G	2F	2E	2D	2C	2B	2A
Strain:																	
	1	2	4	1	2	4	1	2	4	1	2	4	1	2	4	1	2
	Code:						Identification						% of prob.			T index	

Task No. 5 Antigen analysis to intra-species diagnostics of enterobacteriae

We will perform the antigen analysis in strains of bacteria, where it is performed routinelly. Antigen analysis is performed in enterobacteria mainly for one of two reasons:
(a) to diferenciate antigen types with elevated virulence – especially in *E. coli* to differenciate EPEC, STEC etc.
(b) of epidemiological reasons, sometimes in combination with (a) reasons – *Salmonella, Shigella, Yersinia* etc.

a) Excluding of EPEC

In strain identified as *Escherichia coli*, perform antigen analysis using slide agglutination with two polyvalent sera (one nonavalent, one trivalent). If both results will be negative, the strain does not belong into EPEC group.

b) Assessing the serovar in *Salmonella*

In strain identified as *Salmonella enterica*, perform antigen analysis using slide agglutination and discover the serovar. Let us suppose that in the pacient there was allready found a strain of serovar Enteritidis and now we only want to be sure, that it is the same strain once more. Perform a test with body antigen O: 9 and flagellar antigen H: g, m. Write the result to the table.

Task No. 6: Susceptibility tests of enterobacteria to antibiotics

On your table, you will find diffusion disc tests for strains found to be *Enterobacteriaceae*. Write abbreviations of antibiotics according to the card and measure susceptibility zones for all tested strains. Borderline zones are written on the cards; using them, interprete the strains as susceptible (S) rezistant (R) and dubious (D).

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

Task No. 7 Diagnostics of *Campylobacter*

Observe the cultivation appearance that did not grow neither on BA nor on Endo agar and which, according to the morphology, is supposed to be a *Campylobacter* (because of being curved), on a special medium. Remember four main conditions for cultivation of *Campylobacter*: (a) special medium with charcoal and addition of antibiotics and antimycotics to prevent growth of other microbes, (b) microaerofilic conditions, (c) temperature elevated to 42 °C, what correspons to body temperature of birds – natural hosts, and (d) prolongation of the cultivation to 48 hours.

Describe the colonies write down the result of oxidase test (teacher will perform it as a demonstration). For *Campylobacter* a retarded positivity is typical, e. g. the strip becomes blue, but not immediatelly, but after a while.

Description of colonies	Result of oxidase test	More notes

Task No. 8: Urease test in diagnostics of *Helicobacter*

In diagnostics of helicobacters we use the urease test, performed directly with a bioptic specimen of gastric mucosa. A pozitive result is red, negative yellow. Among two specimens (X and Y) find the positive one.

Result: Positive urease test was found in specimen ____, negative in specimen ____

Task No. 9 Diagnostics of the family *Vibrionaceae*

Vibrionaceae is a bacterial family simillar to *Enterobacteriaceae*, but oxidase-positive. We use special media to culture *Vibrionaceae*. Mutual differentiation is possible through biochemical tests like for enterobacteria. even Enterotest 16 could be used, but a special codebook would be required. Antigen analysis could be used, too.

Draw here, how a *Vibrio* looks like microscopically, and add some more properties according to the slideshow.

Microscopy:	Most important solid medium for *Vibrio*:	
	Most important liquid medium for *Vibrio*:	
	The two most important serovars of *V. cholerae*	
	The two most important biovars of *V. cholerae* O1	

Check-up questions:

1. Do you know, what is the result of the catalase test in enterobacteria?

2. For practical reasons, one medium used in diagnostics of enterobacteria is missing: – selenite broth. What type of medium is it and what is its use? (See practical J03)

3. Do you know at least some antigenic types of EPEC?

4. What would be difference in Task 5b, when the patient would have no evidence of previous *Salmonella*?

5. What pathogen is diagnosed by Widal reaction? Is it a direct, or indirect method? Which type of reaction is it?

6. Is it recommended to use antibiotic treatment for intestinal infection? Why?

7. Do you know, what the Urea breath test is and what is its principle?

8. In which clinical material it is more likelly to find *Salmonella* Typhi in typhoid fever rather than in the stool?