Topic P04: Diagnostics of *Enterobacteriaceae* and bacterial agents of gastrointestinal infections

To study: *Enterobacteriaceae, Vibrionaceae, Campylobacter, Helicobacter* (from textbooks, www etc.)
From spring term: Microscopy, culture, biochemical identification, antigenic 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									
Colonies (blood 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									
Other media Task 4a	XLD agar								
	MAL agar								
	CIN agar								
ENTEROtest 16 (Task 4b)									
Antigenic 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 in the table. A strain that is NOT a 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 important Gram-negative rods growing on Endo agar (differentiation of *Enterobacteriaceae*, *Vibrionaceae* and G– non-fermenters)

a) Reading of an examination on oblique triple sugar iron 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 the strains growing on Endo were inoculated on Hajna medium. Examine 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 demonstrate an 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 the exception of *Plesiomonas*) oxidase-negative.

Make partial conclusion after tasks 1 to 3. Which bacteria are *Enterobacteriaceae*? Tasks 4 and 5 will be only performed with strains proven to be *Enterobacteriaceae*.

Task 4: Genus and species determination of *Enterobacteriaceae*

a) Culture of *Enterobacteriaceae* on other media

You have already seen what the colonies look like on BA and Endo agar. Describe shortly the appearance of the colonies on CIN, XLD and MAL media.

b) Biochemical properties of *Enterobacteriaceae*

Evaluate the results of the ENTEROtest 16 incubated a day before. Check, whether the results correspond with other already performed 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 just *Salmonella* sp. (or *Salmonella enterica*) as a result. For this strain, count the percent of probability as a total of all % of probability of all three individual salmonellae found in the book corresponding to your code; T index should be taken from the first *Salmonella* taxon in the codebook. All the names of bacteria should be copied to the table preceding Task 1.

	Tube	First row								Second row							
	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 5: Antigenic analysis to intra-species diagnostics of *Eterobacteriaceae*

We will perform the antigenic analysis in strains of bacteria, where it is performed routinely. Antigenic analysis in *Enterobacteriaceae* is performed mainly for one of two reasons:

(a) To differentiate antigenic types with elevated virulence – especially in *E. coli* to differentiate e.g. EPEC, ETEC and EIEC.

(b) For epidemiological reasons, sometimes in combination with (a) reasons – *Salmonella, Shigella, Yersinia* etc.

a) Excluding EPEC

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

b) Assessing the serovar in *Salmonella*

In the strain identified as *Salmonella enterica*, perform the antigenic analysis using the slide agglutination and determine the serovar. Let us suppose that in the patient there has already been found a strain of the serovar Enteritidis and now we only want to make sure, that this is the same strain again. Perform a test with body antigen O: 9 and flagellar antigens H: g, m. Write the result in the table.

Task 6: Antibiotic susceptibility tests of *Enterobacteriaceae*

On your table, you will find diffusion disc tests for the strains found to be *Enterobacteriaceae*. There is no test for *Salmonella* – such isolates are usually stool origin and so antibiotic treatment is not indicated. Write full names of antibiotics according to the card and measure susceptibility zones for the all tested strains. Borderline zones are written on the cards; using them, interpret the strains as susceptible (S), resistant (R) and dubious (D).

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

Task 7: Diagnostics of *Campylobacter*

Observe the cultivation appearance of the strain that grew 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 the four main conditions needed for the cultivation of *Campylobacter*:

(a) Special medium with charcoal and addition of antibiotics and antimycotics to prevent growth of other microbes.
(b) Microaerophilic conditions.
(c) Temperature elevated to 42 °C, which corresponds to the body temperature of birds – natural hosts.
(d) The extension of the cultivation to 48 hours.

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

Description of colonies	Result of oxidase test	More notes

Task 8: Urease test in the diagnostics of *Helicobacter*

In the diagnostics of helicobacters we use the urease test, performed directly on a biopsy specimen of gastric mucosa (not with a cultured strain – an exception!). The specimen is put in a medium containing urea and an indicator. The positive result is red, the negative one yellow.
Out of the two specimens (X and Y) find the positive one.
Result: Positive urease test was found in specimen ____, negative in specimen ____.

Task 9: Diagnostics of the *Vibrionaceae* family

Vibrionaceae is a bacterial family similar to *Enterobacteriaceae*, but oxidase-positive. We use special media to culture *Vibrionaceae*. Mutual differentiation is possible using the same biochemical tests as with *Enterobacteriaceae*. Even Enterotest 16 can be used, but a special codebook would be required. Antigenic analysis can be used as well. Draw here what a *Vibrio* looks like microscopically, and add some more properties according to the slideshow.

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