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

To study: Enterobacterales, *Vibrio, Aeromonas, Campylobacter, Helicobacter* (from textbooks, www etc.)
From spring term: Microscopy, culture, biochemical identification, antigenic analysis

Summary sheet 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								
Triple sugar agar (Hajna) Task 3a									
Oxidase test Task 3b									
PARTIAL CONCLUSION									
XLD agar Task 4a									
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 summary sheet. **Attention, strain N, more difficult for staining, is already stained, i. e. you do not have to stain it.** 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 summary sheet. 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. **Do not use Petri dishes labelled "Úkol č. 2" for other tasks than Task 2.**

Task 3: Group diagnostics of the most important Gram-negative rods growing on Endo agar (differentiation of enterobacteria, group *Vibrio/Aeromonas* and G– non-fermenters)

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

Triple Sugar Iron (TSI) agar modified according to A. A. Hajna is a combined biochemical test-tube test. 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 that 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 the *Vibrio/Aeromonas* group and some Gram-negative non-fermenters; the Enterobacterales are (with the exception of *Plesiomonas*) oxidase-negative.

Make partial conclusion after tasks 1 to 3. Which bacteria are enterobacteria? Tasks 4 and 5 will be only performed with strains proven to belong to the order Enterobacterales.

Task 4: Genus and species determination of enterobacteria

a) Culture of enterobacteria on other media

You have already seen what the colonies look like on BA and Endo agar. Describe in the summary sheet shortly the appearance of the colonies on XLD medium. Also look at MAL medium on the side table – it is very likely to the appearance of XLD medium (there exist more similar media, for example DC agar). Look also at CIN medium – *Yersinia* has tiny pink colonies, other bacteria are different or do not grow at all. Results on MAL and CIN are not written in the summary sheet.

b) Biochemical properties of enterobacteria

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 TSI medium, *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 summary sheet 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 *Enterobacteriaceae*

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 (as a final result, write "E. coli, non-EPEC").

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 summary sheet.

Task 6: Antibiotic susceptibility tests of enterobacteria

On your table, you will find diffusion disc tests for the strains found to be enterobacteria There is no test for *Salmonella* – such isolates are usually stool origin and so antibiotic treatment is not indicated. The antibiotic set is one of suitable sets for urinary tract infections (UTI). Interpret the strains as susceptible (S), resistant (R) and intermediate (I) to given antibiotics. **The interpretation is done according to actual EUCAST# recommendations, so for some antibiotics we have only "susceptible" or "resistant", for some other we also have intermediate (I).**

Strain (write its letter) →									
Antibiotic	Suceptible if	Inter-mediate if	Resistant if	Zone ∅ (mm)	Interpre-tation	Zone ∅ (mm)	Interpre-tation	Zone ∅ (mm)	Interpre-tation
Ampicillin AMP	≥ 14 mm		< 14 mm						
Cephazolin KZ	≥ 15 mm		< 14 mm						
Co-trimoxa-zole SXT	≥ 16 mm	13–15 mm	< 13 mm						
Nitrofuran-toin F	≥ 11 mm		< 11 mm						
Tetracycline TE*	≥ 15 mm	12–14 mm	< 12 mm						
Cefuroxime CXM	≥ 18 mm		< 18 mm						
Norfloxacin NOR	≥ 22 mm	19–21 mm	< 19 mm						

*also valid for doxycycline

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 *Vibrio–Aeromonas* group

Vibrio and *Aeromonas* are bacteria similar to enterobacteria, but oxidase-positive. We use special media to culture them. Mutual differentiation is possible using MALDI-TOF, or using the same biochemical tests as with Enterobacterales. 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:	

#EUCAST = The European Committee on Antimicrobial Susceptibility Testing

Important note: In the recent practice of laboratories of clinical microbiology, the most common test for identification of Enterobacterales, *Campylobacter, Helicobacter, Vibrio* and *Aeromonas* is MALDI-TOF. That means, all bacteria from the today practical session are mostly tested using this method. Just for educational and practical reasons it was not included in the practical session. On the other hand, laboratory workers should be skilled also in alternative methods of identification, so for illustration of the diagnostic algorithm is was useful to get the image of these methods.