Lab class no. 4 – Evaluation of disc diffusion test and MIC, determination of MBC, determination of concentration of bacteria by cultivation

(in pairs)

Aims:

- *Evaluation of disc diffusion test*
- *Evaluation of Escherichia coli sensitivity to chloramphenicol (MIC)*
- *Determination of minimal bactericidal concentration of chloramphenicol (MBC)*
- *Determination of concentration of Escherichia coli by cultivation*

Material

Paper ruler, transluminator, Nutrient broth – solid in Petri dish and liquid, bacteriological loop, microbiological tubes, tube with calibration, vortex, automatic pipette

Working procedure

1. Evaluation of disc diffusion test

- *E. coli* growth was inhibited by chloramphenicol and around discs there are inhibition zones - measure their diameter using paper ruler and evaluate sensitivity or resistance according to EUCAST - write down to protocol no. 3.

2. Evaluation of Escherichia coli sensitivity to chloramphenicol (MIC)

- MIC is lowest concentration of antibiotics still able to inhibit growth of bacteria
- evaluate MIC of chloramphenicol from turbidity in wells
- compare with EUCAST and wrote to protocol no. 3, if the bacteria are sensitive or resistant

3. Determination of minimal bactericidal concentration of chloramphenicol (MBC)

- Petri dish with Nutrient broth agar divide into quadrants (on the bottom side) - number of quadrants correspond to number of wells in microtitration plates without turbidity
- using bacteriological loop inoculate all 4 wells of corresponding concentration into the quadrant
- in the same manner inoculate the other concentrations without turbidity (e.g. 8 ug/ml, 16 ug/ml, 32 ug/ml) - always with new loop!

4. Determination of microbiological quality of a medicinal product (determination of number of microbes)

- in pairs determine concentration of bacteria in liquid sample of *Micrococcus luteus*
- prepare set of 5 microtubes, add to each 900 µl of Nutrient broth
- mix the sample of bacteria by vortex and take out 100 µl of sample to the first tube; mix well, take out 100 µl again and transfer to second tube and so on
- mix all 5 tubes again, take out 100 µl from each and transfer to agar plate using tip (divided in 5 segments on bottom by marker)

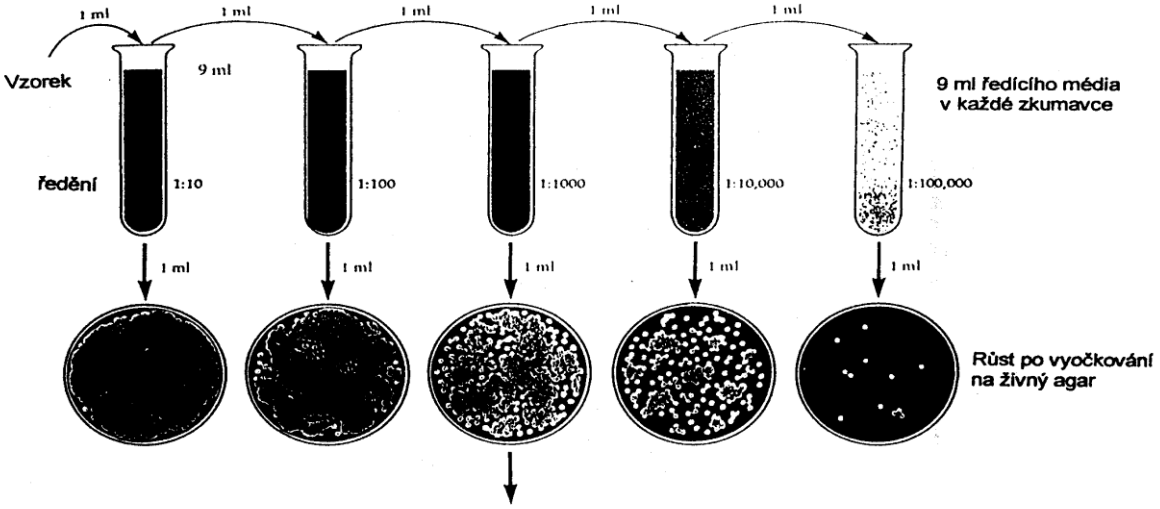
Cultivate of all plates at 37°C for 24 hours. Then the plates will be kept in fridge.

Protocol no. 4 will contain:

- definition of MIC and MBC; in last lab class you are going to fill in MBC value to your protocol - evaluate chloramphenicol as bacteriostatic or bactericidal
- in last lab class determine concentration of *E. coli* in unknown sample; draw the scheme of dilution into your protocol.

Miscellaneous agents	MIC breakpoint (mg/L)		Disk content (µg)	Zone diameter breakpoint (mm)		Notes
	S ≤	R >		S ≥	R <	Numbered notes relate to general comments and/or MIC breakpoints. Lettered notes relate to the disk diffusion method.
Chloramphenicol	8	8	30	17	17	1. Quality control of colistin must be performed with both a susceptible QC strain (*E. coli* ATCC 25922 or *P. aeruginosa* ATCC 27853) and the colistin resistant *E. coli* NCTC 13846 (*mcr-1* positive).
Colistin[1]	2	2		Note[A]	Note[A]	2. Agar dilution is the reference method for fosfomycin. MICs must be determined in the presence of glucose-6-phosphate (25 mg/L in the medium). Follow the manufacturers' instructions for commercial systems.
Daptomycin	-	-		-	-	3. Trimethoprim:sulfamethoxazole in the ratio 1:19. Breakpoints are expressed as the trimethoprim concentration.
Fosfomycin iv	32[2]	32[2]	200[B]	24[C,D]	24[C,D]	
Fosfomycin oral (uncomplicated UTI only)	32[2]	32[2]	200[B]	24[C,D]	24[C,D]	A. Use an MIC method.
Fusidic acid	-	-		-	-	B. Fosfomycin 200 µg disks must contain 50 µg glucose-6-phosphate.
Metronidazole	-	-		-	-	C. Zone diameter breakpoints apply to *E. coli* only. For other Enterobacteriaceae, use an MIC method.
~~Mupirocin~~						D. Ignore isolated colonies within the inhibition zone (see pictures below).
Nitrofurantoin (uncomplicated UTI only), *E. coli*	64	64	100	11	11	
Nitroxoline (uncomplicated UTI only), *E. coli*	16	16	30	15	15	
Rifampicin	-	-		-	-	
Spectinomycin	-	-		-	-	
Trimethoprim (uncomplicated UTI only)	2	4	5	18	15	
Trimethoprim-sulfamethoxazole[3]	2	4	1.25-23.75	14	11	

Sériové ředění vzorku a přímé stanovení počtu živých bakterií (kolonie tvořících jednotek – KTJ; colony forming units – CFU)



Sériové ředění vzorku Výpočet: počet kolonií na misce x ředění vzorku = KTJ/ml