Name: Date:
Group Nr.:

LABORATORY PROTOCOL Nr.2

Topic: Direct detection and antibiotics

Task Nr.1: Cultivation

Principle:

Cultivation is based on the ability of microorganisms to multiply on inanimate media.

Conclusion:

McConkey agar	
Blood agar	

Name: Date:
Group Nr.:

Task Nr.2: Counting of colonies

Principle:

One colony will grows from one microbe cell in case the cells on the agar surface are well separated. This is achieved by appropriate dilution of the sample.

Procedure:

Add 0.1 ml of bacterial suspension to test tube 1 with 9.9 ml of phys. saline solution, after mixing transfer 0.1 ml suspension from test tube 1 to test tube 2, then from test tube 2 to test tube 3. From tubes 2 and 3, inoculate 0.1 ml of suspension on agar, spread it over the whole surface by loop. Incubate for 24 hours at 37°C and check the plate.

Microbial concentration (K) - calculation

Volume (V), dilution(D), and numbers of colonies (N)

K = N/V x D

Conclusion:

Tube	Dilution	Numbers of colonies	Microbial concentration
2			
3			

Task Nr.3: Determination of susceptibility of bacterial strain using disc diffusion method

Principle:

Determination of susceptibility of non-fastidious bacteria to antibiotics using antibiotic discs.

Procedure:

Incubate for 18 hours at 37°C and read the inhibition zone.

Name: Date:
Group Nr.:

Antibiotic discs	Cephoxitin 30 μg	Erythromycin 15 μg	Clindamycin 2 μg	Tetracyklin 30 μg	Chloramphenicol 30 μg	Gentamicin 10 μg
Zone size						
Breakpoint	C ≥22 / R <22	C ≥21 / R <18	C ≥22 / R <19	C ≥22 / R <19	C ≥18 / R <18	C ≥18 / R <18
Evaluation						

Conclusion:

Task Nr.4: Nitrocefin test

Principle:

We demonstrate the production of beta-lactamase, which cleaves the beta-lactam ring of antibiotics.

Procedure:

Moisten a strip of filter paper with a solution of nitrocefin and inoculate by the test strain. Check the color reaction.

Conclusion: