

Name:
Group Nr.:

Date:

LABORATORY PROTOCOL Nr.4

Topic: Enterobacteria, Pseudomonas

Task Nr.1: Biochemical identification of Enterobacteria – Enterotest24

Principle:

The differentiation of enterobacteria is based on the species' typical biochemical activity. The tests use the detection of metabolic products, usually based on a change in the pH of the growth medium.

Procedure:

Create a suspension with a turbidity of 1 McFarland, add 100µl of inoculum to each well of the test, drip the designated wells with paraffin oil (anaerobic conditions). Incubate for 24 hours at 37°C and check the reactions.

Conclusion:

Task Nr.2: Serotypization of enterobacteria

Principle:

The indirect method (slide agglutination) uses the complex structure of enterobacteria (O - somatic, H - flagellar antigens) to distinguish the serotype (combination of O and H antigen). It is used to identify pathogenic serotypes.

Procedure:

Make a suspension in a drop of phys. saline solution, add a drop of serum next to the suspension and mix it. Check the formation of precipitate.

Conclusion:

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Task Nr.3: O/F test

Principle:

Oxidization/fermentation of glucose is used for differentiation of metabolism type and for differentiation of aerobic (oxidization) and anaerobic (fermentation) bacteria.

Procedure:

Inoculate two tubes with the test strain by inserting loop to the bottom of the tubes, drip one tube with paraffin oil. After incubation, we observe a color change of the medium in the test tubes. Incubate for 24 hours at 37°C and check the reaction.

Conclusion:

Task Nr.4: Test of oxidase production

Principle:

Detection of cytochrome oxidase by color reaction of N, N-dimethyl-1,4-phenylenediamine with α -naphthol to give indolphenol blue.

Procedure:

Directly imprint the strip testing zone onto one or several colonies of testing culture. Evaluate the color change.

Conclusion:

Task Nr.5: Isolation of *Pseudomonas aeruginosa* pigment

Principle: Pyocyanin is a blue-green pigment soluble in both water and chloroform, which has the properties of a siderophor. The pigment can be extracted into chloroform, by changing the pH it is possible to change the color of the solution.

Procedure:

Add 1 ml of chloroform into the tube with 2ml of inactivated *Pseudomonas aeruginosa* broth culture. Tightly cover the tube with cape and carefully shake.

Conclusion: