Name: Date:
Group Nr.:

LABORATORY PROTOCOL Nr.5

Topic: Hemophilus, Neisseria, Mycobacteria, Corynebacteria

Task Nr.1: Identification of Hemophilus – factors V and X

Principle:

Particular *Haemophilus* species grow on agar only around the disc containing the required growth factor.

Procedure:

Inoculate massively both strains on the Müeller-Hinton agar, each on its half. Put discs containing growth factor X, V a X+V on each half of agar with inoculated strains. Incubate for 24 hours at 37°C, (in 5-10% CO_2) and check the growth around the disks.

Conclusion:

Task Nr.2: : Identification of Hemophilus – satellitism

Principle:

Hemophilus grows on blood agar only in the presence of *Staphylococcus aureus*, in its presence factor X is released and factor V produced.

Procedure:

Inoculate massively both strains of *Haemophilus* on the blood agar with the sterile loop, each strain on one half of agar. Inoculate a line of the *Staphylococcus aureus* strain in the middle, throuhg boths sides of the agar. Incubate for 24 hours at 37°C, (in 5-10% CO_2) and check the growth.

Conclusion:

Name: Date:
Group Nr.:

Task Nr.3: Identification of Neisseria – oxidase test

Princip:

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

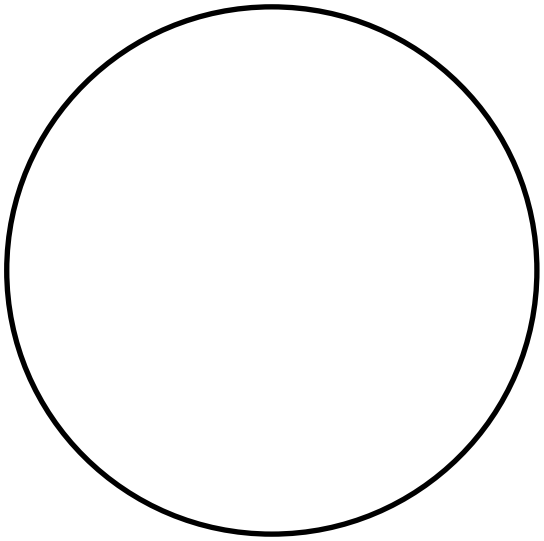
Procedure:

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

Conclusion:

Task Nr.4: Identification of Neisseria – microscopy

Conclusion:



Task Nr.5: Identification of Corynebacteria

Principle:

Phospholipase D producing strain inhibits staphylococcal β-hemolysin and reduces or completely cancels the influence of β-hemolysin on blood cells. A positive test is demonstrated by suppression of β-hemolysis by the test strain at the intersection of the lines with the *Staphylococcus aureus* strain.

Procedure:

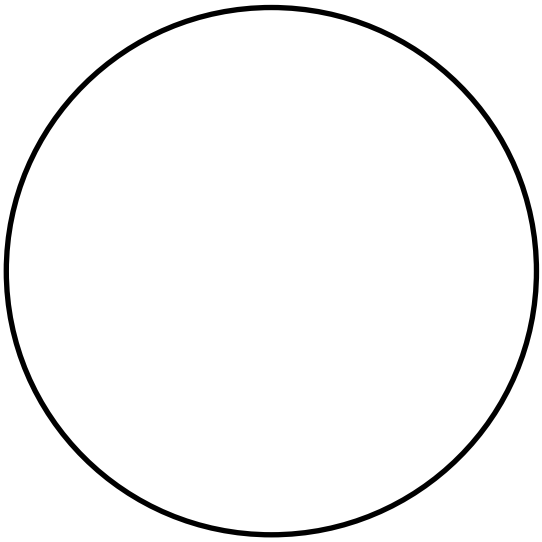
Inoculate a line of tested strain on blood agar. Inoculate a perpendicular line of *Staphylococcus aureus.* Incubate for 24 hours at 37°C and check.

Conclusion:

Name: Date:
Group Nr.:

Task Nr.6: Identification of Corynebacteria – microscopy

Conclusion:



Task Nr.7: Identification of *Listeria monocytogenes*

Principle:

L. monocytogenes is mobile when cultivated up to 25°C. A typical somersault movement can be observed in the native specimen.

Procedure:

On the slide, we put a drop of broth with Listeria culture, cover with coverslip, and observed at 40x magnification.

Conclusion:

Name: Date:
Group Nr.:

Task Nr.8: Identification of Mycobacteria

Principle:

Acid-resistant staining according to Ziehl-Neelsen - heating of concentrated carbolfuchsin, decolorization with acid alcohol and staining of the preparation with malachite green.

Conclusion: