GENERAL MICROBIOLOGY – practical - week 10

Testing of Biochemical Properties of Bacteria(Part I)

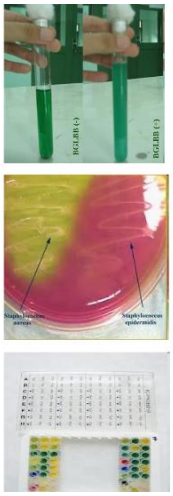
Tests for biochemical properties and metabolic activity testing

Aim – final identification
Method – subcultivation on the series of testing diagnostic meida

-liquid media – with chemical structure – substrate and indicator,

-solid media with biochemical – metabolic substrate and indicator

-diagnostic disc with substrate, micromethods – liquid media with substrate and indicator in microwells
Algorithm – of chosen procedures



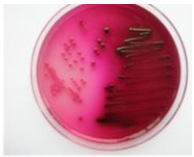
1. Demonstration of bacterial biochemical properties testing on Endo agar, Deoxycholate-citrate agar (DCA) and Mannitol salt agar(MSA).

Proteus vulgaris, Proteus mirabilis,Salmonellatyphi,Shigella dysenteriae, Escherichia coli,Klebsiella pneumoniae, Staphylococcus aureus, Staphylococcus epidermidis.

Endo agar:

Endo agar is a differential and slightly selective culture medium for the detection of coliform and other enteric microorganisms.The selectivity of Endo agar is due to the sodium sulfite/basic fuchsin combination which results in the suppression of gram-positive microorganisms. Endo agar is culture medium for the **differentiation of lactose fermenters from the nonfermenters**. Coliforms ferment the lactose producing pink to rose-red colonies and similar coloration of the medium. The colonies of organisms which do not ferment lactose are colorless to faint pink against the pink background of the medium.

ENDO agar



- differential
- selective
- detection of coliform and other enteric microorganisms

5. ENDO agar

E. coli *Shigella dysenteriae* *Salmonella typhi* *Proteus vulgaris*

Typical colonial morphology on Endo Agar is as follows:

E. coli.....pink to rose-red, green metallic sheen - LACTOSE FERMENTER

Klebsiella.....large, mucoid, pink- LACTOSE FERMENTER

Proteus.....colorless to pale pink - LACTOSE NON- FERMENTER

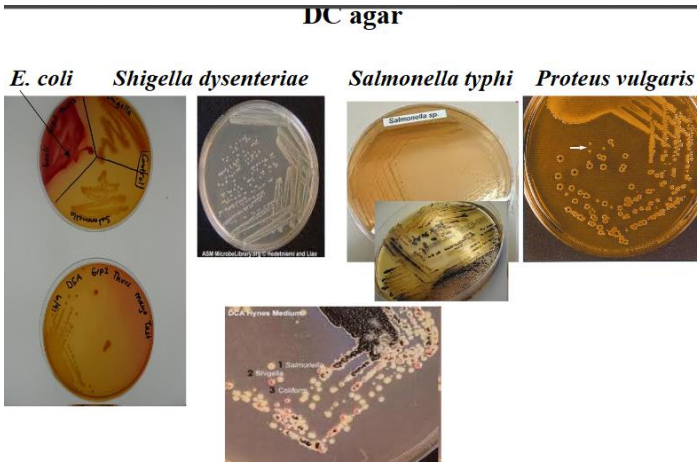
Salmonella.....colorless to pale pink - LACTOSE NON- FERMENTER

Shigella.....colorless to pale pink - LACTOSE NON- FERMENTER

Gram-positive bacteria .....no growth to slight growth

DCA:

Deoxycholate Citrate Agar is a selective medium recommended for the isolation of enteric pathogens particularly *Salmonella* and *Shigella* species. This medium is selective for enteric pathogens owing to increased concentrations of both citrate and deoxycholate salts. Sodium deoxycholate at pH 7.3 to 7.5 is inhibitory for Gram-positive bacteria. Citrate salts, in the concentration included in the formulation, are inhibitory to gram-positive bacteria and most other normal intestinal organisms. Lactose helps in differentiating enteric bacilli, as lactose fermenters produce red colonies while lactose non-fermenters produce colourless colonies. Coliform bacteria, if present form pink colonies on this medium. The degradation of lactose causes acidification of the medium surrounding the relevant colonies and the pH indicator neutral red changes its colour to red. *Salmonella* and *Shigella* species do not ferment lactose but *Salmonella* may produce H2S, forming colorless colonies with or without black centers.



MSA: Mannitol salt agar (MSA) is both a selective and differential media used for the isolation of *Staphylococci* from mixed cultures.MSA Components7,5% NaCl–selects for species of *Staphylococcus*. This concentration of salt is too high for most other bacteria to withstand and,therefore, inhibits their growth. Mannitol–alcohol of the carbohydrate mannose.

Mannitol fermentation produces acid end products which turn the medium yellow.Yellow indicates mannitol positive and no color change indicates mannitol negative.

Phenol red pH indicator–yellow in acid pH (the same indicator that is used in phenol red carbohydrate fermentation broths).On MSA, only pathogenic *Staphylococcus aureus* produces small colonies surrounded by yellow zones. The reason for this color changeis that *S. aureus* have the ability to ferment the mannitol, producing an acid, which changes the indicator color from red to yellow. The growth of other types of bacteria is usually inhibited. This growth differentiates *S.aureus* from *S.epidermidis,* whichforms colonies with red zones.

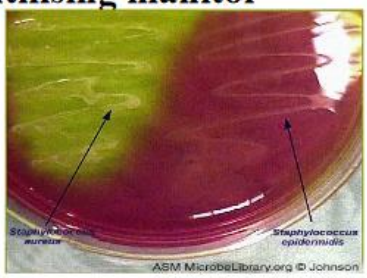
G+cocci:

Staphylococcus aureus a Staphylococcus epidermidis on the selective-diagnostic medium Salt mannit: selectively NaCl allowed growing of staphylococci that tolerate it while others do not

mannitol is the diagnostic substrate utilised by St. aureus which metabolised it, formed acid that makes the medium becomming acid and change the pH and indicator color (from pink → yellow)

St. aureus changes the original red color to yellow,

St. epidermidis is growing on the medium, tolerates salt without changing the pH and indicator color - not utilising manitol



2. Triple Sugar Iron Agar (Hajn´s Agar): composition, use, demonstration

The Triple Sugar-Iron(TSI)agar test is designed to differentiate among the different groups or genera of the Enterobacteriaceae. To facilitate the observation of carbohydrate utilization patterns, TSI Agar contains three fermentative sugars, lactoseand sucrosein 1% concentrations andglucose in 0.1% concentration. Due to the building of acid during fermentation, the pH falls. The acid base indicator (phenol red) is incorporated for detecting carbohydrate fermentation that is indicated by the change in color of the carbohydrate medium from orange red to yellow in the presence of acids.

In case of oxidative decarboxylation of peptone, alkaline products are built and the pH rises. This is indicated by the change in colour of the medium from orange red to deep red.

Sodium thiosulfateand ferrous ammonium sulfatepresent in the medium detects the production of hydrogen sulfide. Sodium thiosulfate is reduced to hydrogen sulfide, and hydrogen sulfide reacts with an iron salt yielding the typical black iron sulfide. Ferric ammonium citrate is the hydrogen sulfide (H2S) indicator.

TSI agar

- The **Triple Sugar Iron** or **TSI**
- to test microorganism's ability to ferment sugars and to produce hydrogen sulfate.
- It is often used in the selective identification of enteric bacteria including Salmonella and Shigella.
- test tube that contains
- agar
- pH-sensitive dye (phenol red),
- 1% lactose, 1% sucrose, 0.1% glucose,
- sodium thiosulfate and ferrous sulfate

Principle

Carbohydrate fermentation is indicated by the production of gas and a change in the colour of the pH indicator **from red to yellow**. To facilitate the detection of organisms that only ferment glucose, the glucose concentration is one-tenth the concentration of lactose or sucrose.

Surface of tube medium – aerobe environment, lactose negative bacteria do not ferment, it is alkaline, red

Lower part - in anaerobe environment, enterobacteria ferments glycids – acidic – yellow or black H2S .

1 negative control 2 Ps.aeruginosa : net fermenting - red

3 Shigella sonnei: H2S - negat.,gas – negat., TSI – acid/alcalic red/yellow

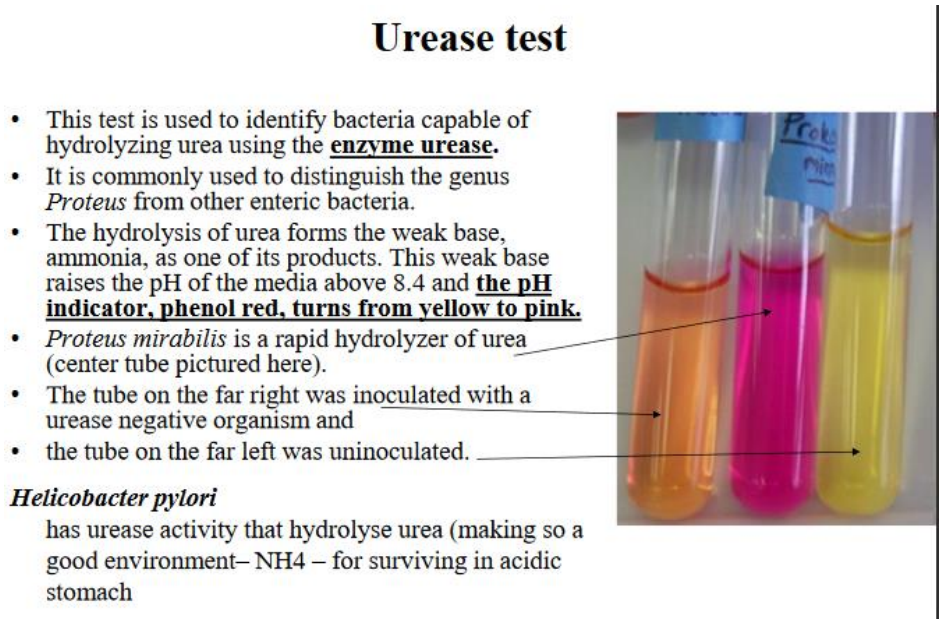
4 Salmonella typhi: H2S – pozit., gas–negat., TSI – acidic/alcalic red/yellow

5 Escherichia coli: H2S – negat.,gas -posit., TSI – acid/acidic red/red

6 Proteus mirabilis: H2S – posit, gas - negat., TSI – acid /acid red/red

3. Urease test – biochemical properties of *Helicobacter pylori.*

Helicobacter pylori has urease activity that hydrolyse urea (making so a good environment– NH4 –for surviving in acidic environment -stomach).

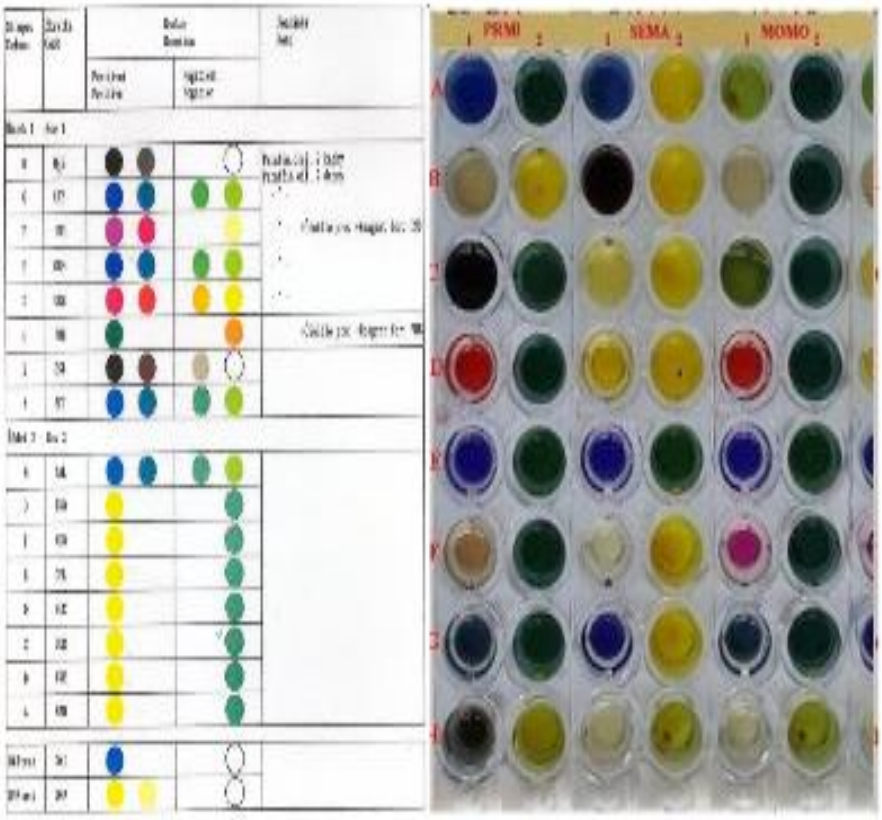


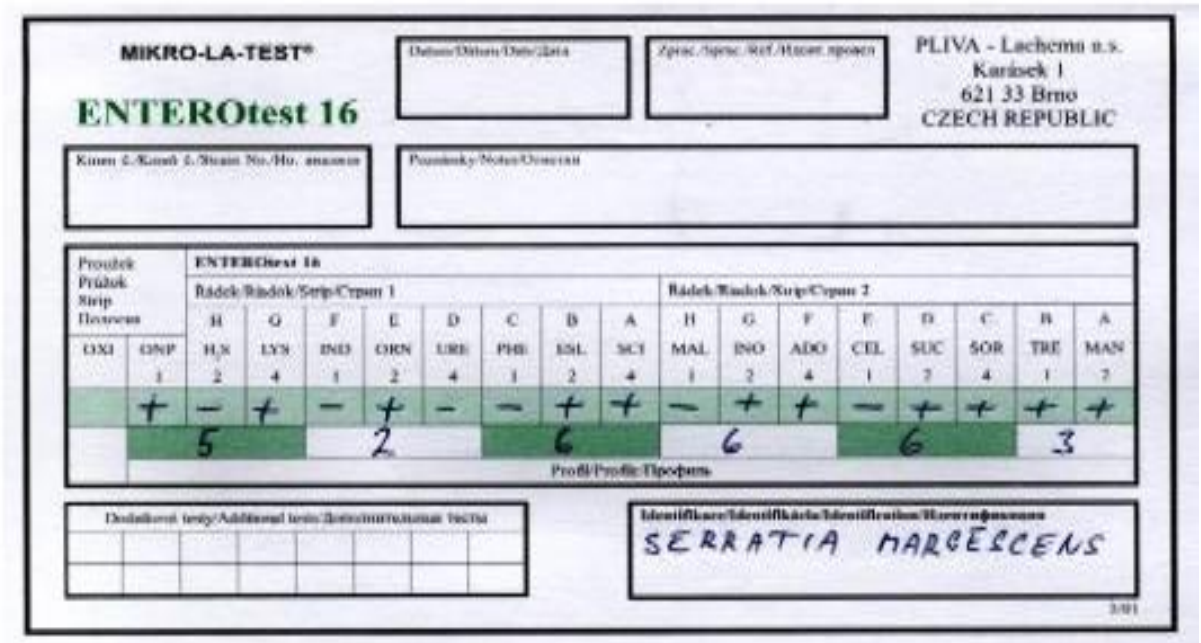
This test is used to identify bacteria capable of hydrolyzing urea using the enzyme urease.The hydrolysis of urea forms the weak base, ammonia, as one of its products. This weak base raises the pH of the media above 8.4 and the pH indicator, phenol red, turns from yellow to pink.

4. ENTEROTEST - Principle

The final identification of Enterobacteriaceae studies the phenotypical demonstration of those biochemical properties, which are specific for the individual pathogens. Enterotest is a system that permits the differentiation of Enterobacteriaceae by several simultaneous biochemical reactions. Microorganisms are identified by colour change after 18 -24 hours of incubation at 35 ± 2°C(according to Colour Scale for ENTEROtest 16).

Group of biochemical tests aligned that they allow numeric identification based on statistical probability of the result of one test. In the positive result the well is attributed the cipheraccording to the position in the triplet (1, 2 or 4). Addition of ciphers in triplet gives the number and each result of the triplet gives a subsequent one position of the code that is the combination of numbers of tested triplets. This code is corresponding to one bacteria (526663 –*Serratia marcescens*).For the identification use the differentiation table, or the Code book or the Identification programme





SOURCES:

Kompaniková, Neuschlová, Sadloňová: Special Bacteriology – basic laboratory tests. Available from: https://www.jfmed.uniba.sk/fileadmin/jlf/Pracoviska/ustav-mikrobiologie-a-imunologie/VLa/Special_Bacteriology_-_basic_laboratory_tests.pdf

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