BIOCHEMICAL ACTIVITIES OF BACTERIA

10th lab

Objectives

To know some biochemical tests which are routinely used in the identification of the different types of bacteria

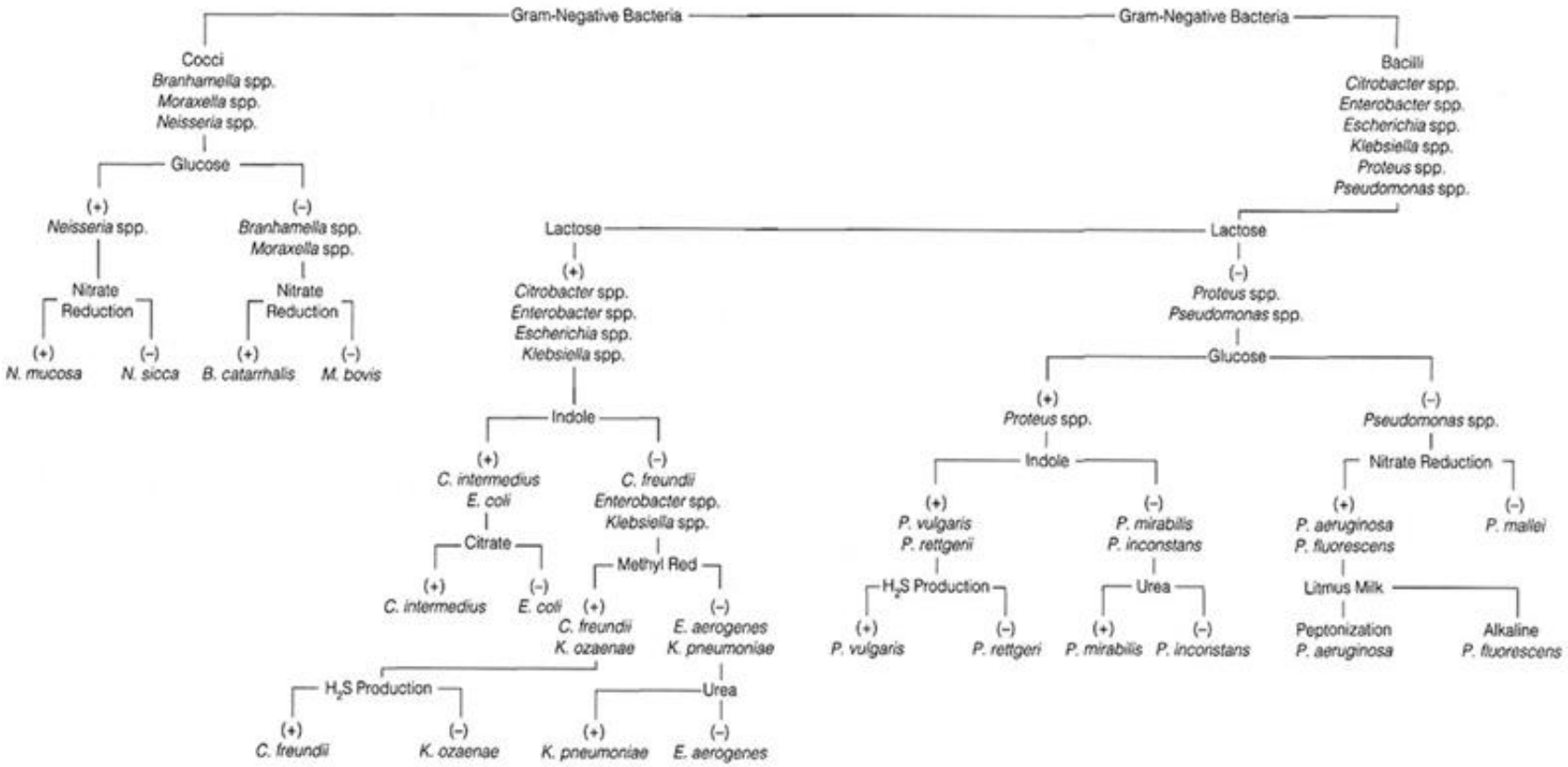
Tests to know

- H_2S production in SIM
- Indole
- Motility

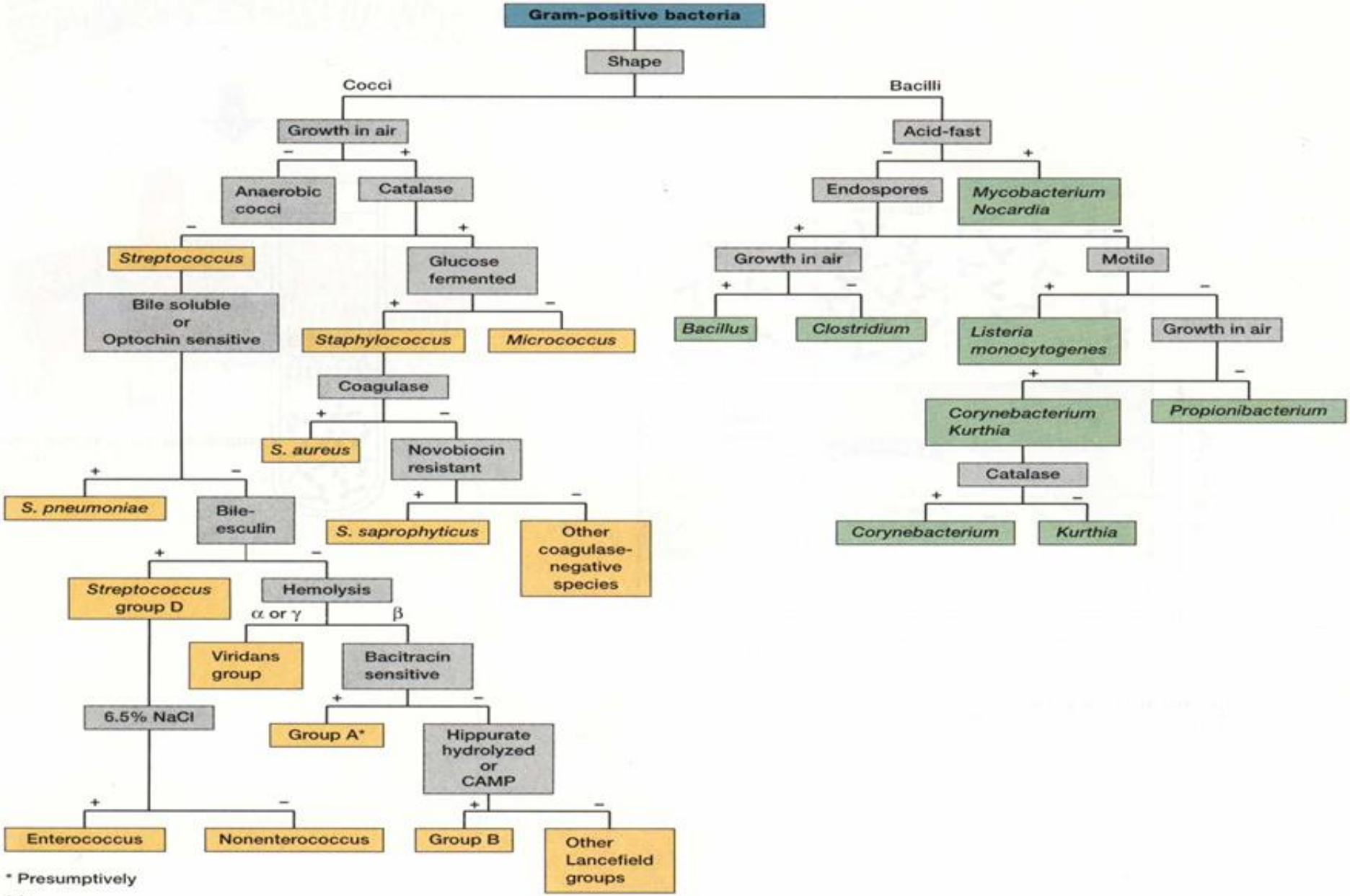
SIM

- Lactose ,Glucose fermentation
- Starch hydrolysis
- Lipids

Gram Negative Identification Flow Chart



NG: No growth; G: Growth; A/G: Acid and gas; A: Acid only



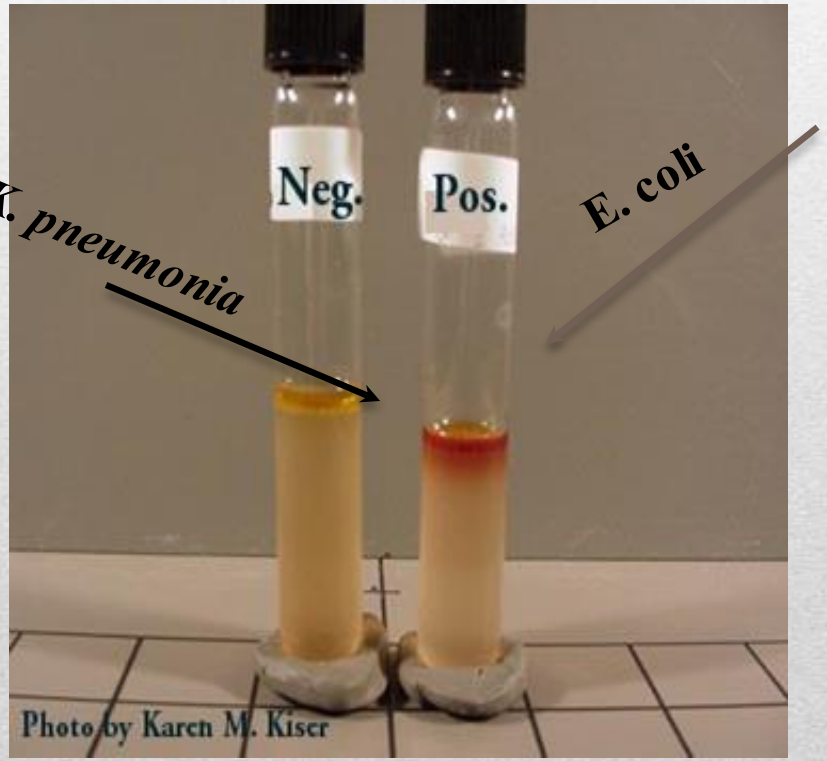
Dichotomous Keys for Clinically Important Genera
Figure 34.7 *a*

SIM

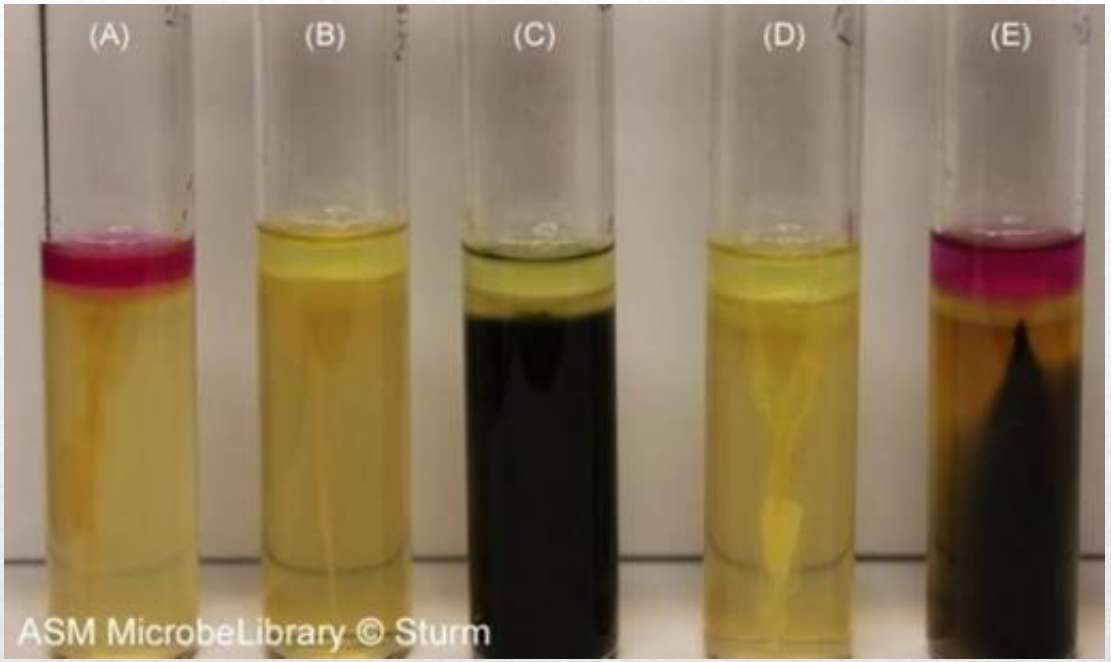
SIM Agar , a rich source of the amino acid tryptophan. Indole positive bacteria such as *Escherichia coli* produce tryptophanase, an enzyme that cleaves tryptophan, producing indole and other products. When Kovac's reagent (p-dimethylaminobenzaldehyde) is added to a tube with indole in it, a dark pink color develops.

Procedure

1. **Using a wire needle , inoculate test organism two-thirds into the medium with stab motion.**
2. **Examine tubes after incubation for motility and H2S production.**
3. **Add 2-3 drops of Kovac's Reagent to each tube. Record as indole positive if a pink or red color appear, or as indole negative if there is no color change.**



SIM results interpretation



A: Escherichia coli - Negative for H2S, Positive for Indole, motile
B: Klebseila pneumonia- Negative for H2S, Negative for Indole, none motile
C: Salmonella arizonae - Positive for H2S, Negative for Indole, Positive for motility
D: Enterobacter aerogenes - Negative for H2S, Negative for Indole, Positive for motility
E: Proteus vulgaris - Positive for H2S, Positive for Indole, Positive for motility

TSI

- **Sugar fermentations**
- **Gas production**
- **Hydrogen sulfide production**

Purpose

Triple sugar iron (TSI) agar is a differential medium used in determining carbohydrate fermentation, H_2S production and Gas from carbohydrate metabolism.

- Bacteria can metabolize carbohydrates aerobically (with oxygen) or fementatively (without oxygen). TSI differentiates bacteria based on their fermentation of lactose, glucose and sucrose and on the production of hydrogen sulfide.

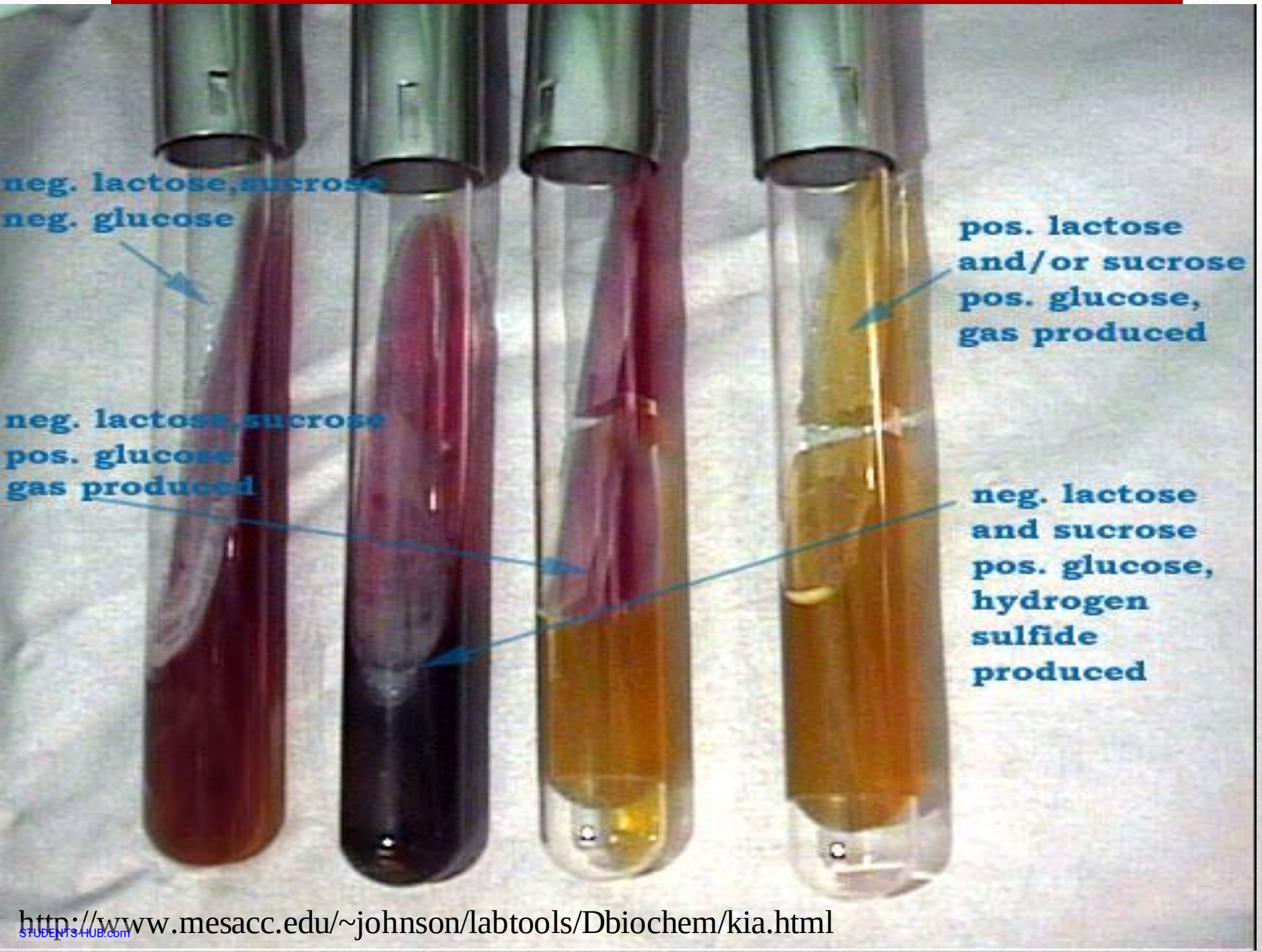
Pancreatic digest of casein	10.0 g
Peptic digest of animal tissue	10.0 g
Glucose	1.0 g
Lactose	10.0 g
Sucrose	10.0 g
Ferrous sulfate or ferrous ammonium sulfate	0.2 g
NaCl	5.0 g
Sodium thiosulfate	0.3 g
Phenol red	0.024 g
Agar	13.0 g
Distilled water	1,000 mL

Procedure

1- Use a straight inoculating loop to pickup an isolated colony.
2- Inoculate the TSI slant by first stabbing the butt down to the bottom, withdraw the needle, and then streak the surface of the slant.
3- Read results after incubation at 37°C for 18 to 24 h.

http://www.microbelibrary.org/component/resource/laboratory-test/2842-triple-sugar-iron-agar-protocols

http://www.mesacc.edu/~johnson/labtools/Dbiochem/kia.html



http://www.mesacc.edu/~johnson/labtools/Dbiochem/kia.html

Starch hydrolysis

Starch agar is a differential medium that tests the ability of an organism to produce certain exoenzymes, including **a-amylase**, that hydrolyze starch.

Starch agar is a simple nutritive medium with starch added. Since no color change occurs in the medium when organisms hydrolyze starch, we add **Iodine** to the plate after incubation. **Iodine turns blue, purple**, or **black** in the **presence of starch**. A clearing around the bacterial growth indicates that the organism has hydrolyzed starch.

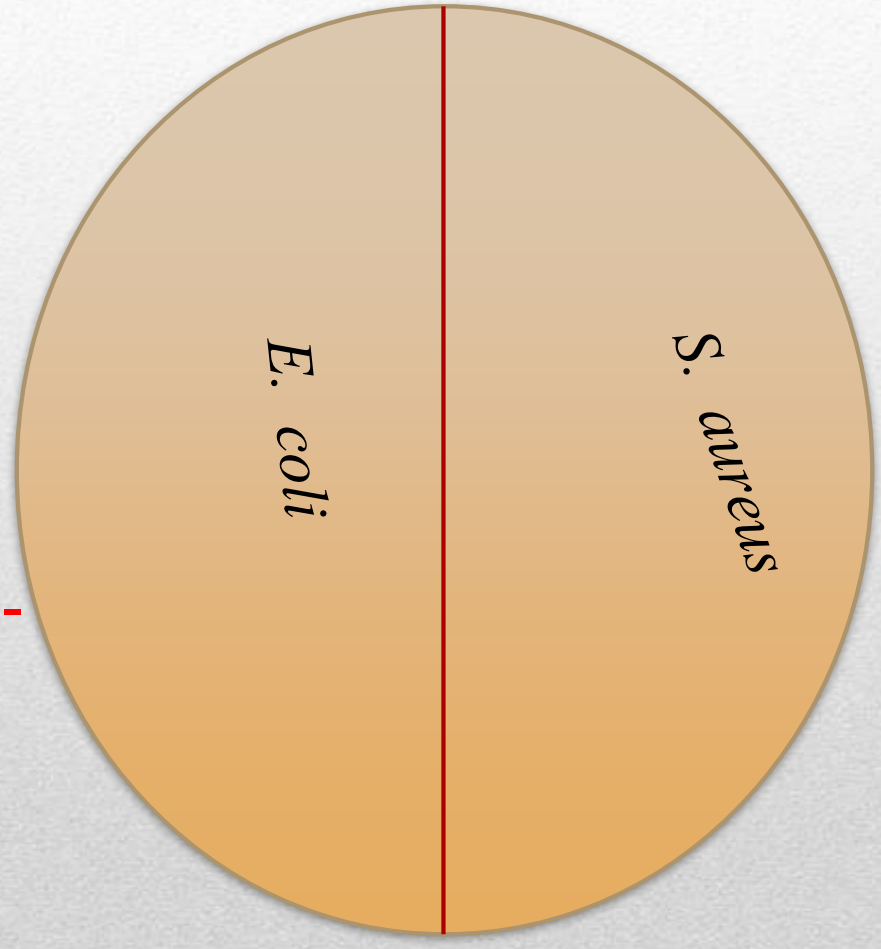
http://biosci.usc.edu/courses/2002-fall/bisc300.html

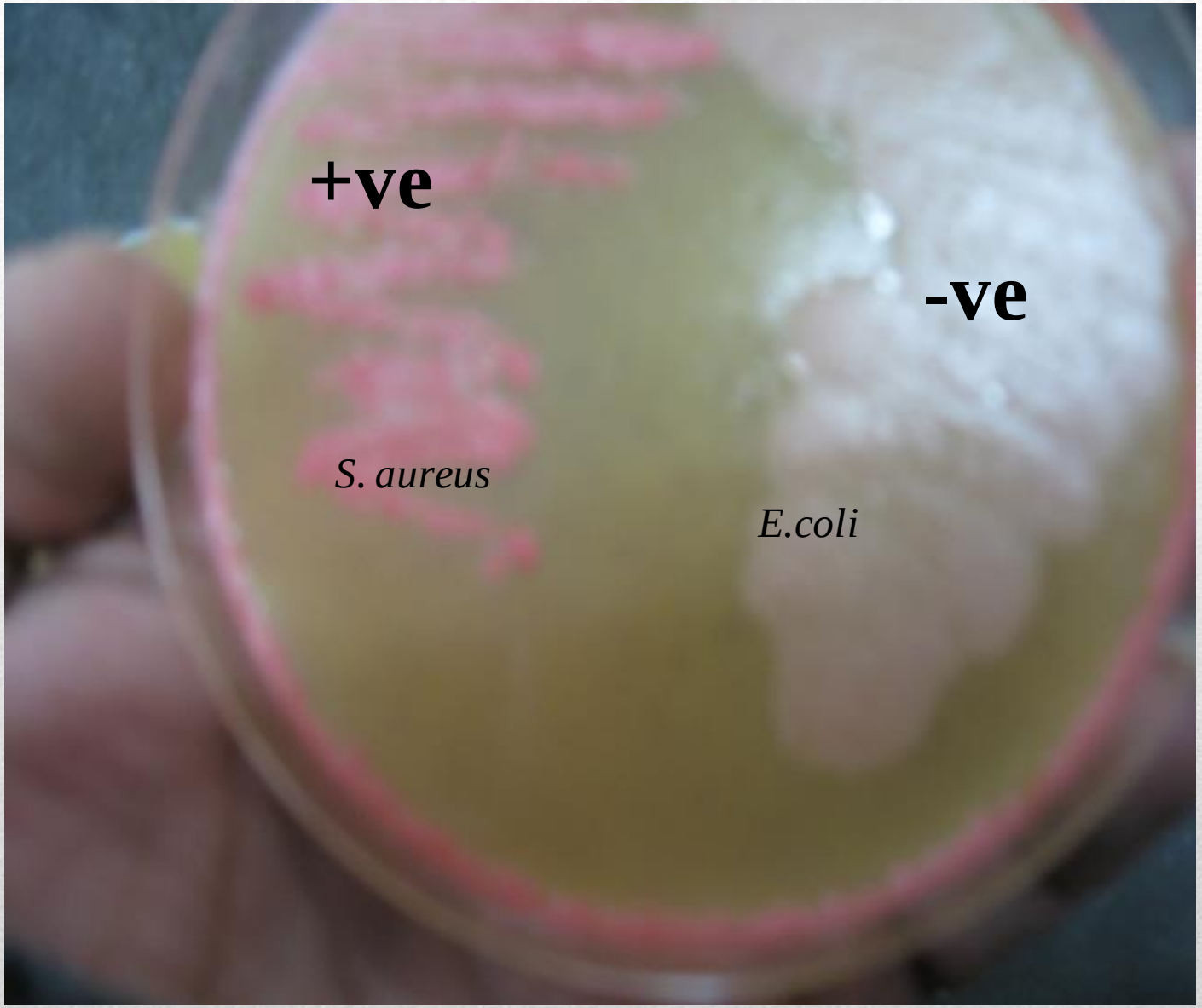
http://faculty.ccbcmd.edu/courses/bio141/labmanua/lab8/lab8.html

Lipid hydrolysis

Some bacteria produce enzyme that hydrolyzes triglycerides into free fatty acids and glycerol which can then be taken up by the cell and metabolized more in glycolysis.

lipase could be detected by growing the bacteria on **neutral red fat agar** -- Neutral red (pH indicator that turns bright red at a low pH)





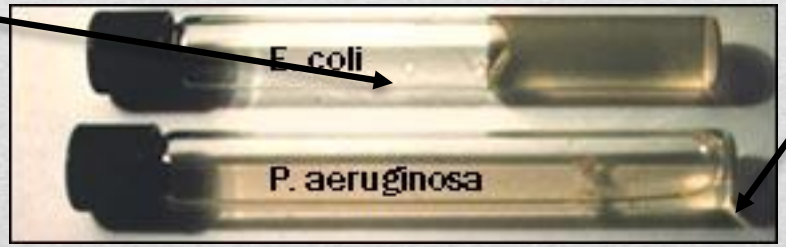
Gelatin Liquefaction

Can bacteria use gelatin as source of carbon ?

Yes or No

How is **gelatinase** activity determined?

Negative result:-
Solid in ice bath or refrigerator after 5 minutes.



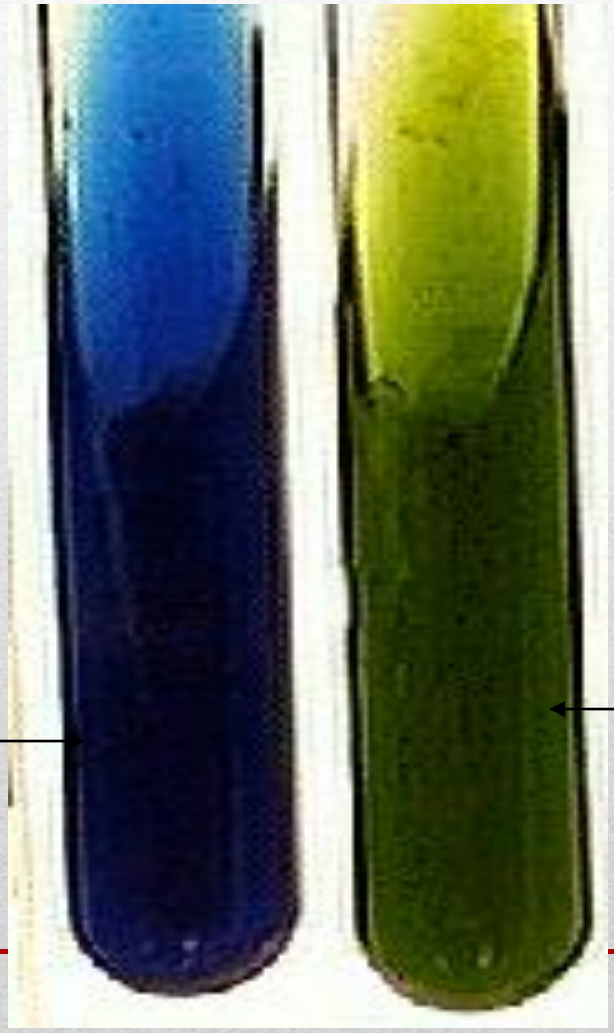
Positive result:
Liquid in ice bath or refrigerator after 5 minutes.

Stab straight to bottom with an inoculating needle. Incubate at 37C for 24 hr . Put in ice bath or refrigerator for 5 minutes, read results.

Citrate Utilization

- Citrate is an organic molecule that can be utilized by bacteria that produce the enzyme **citrase**. Citrase is produced by some bacteria such as *K.pneumonae*, *E. aerogenes* but not by others like *E. coli*
- **Media and Reagent:** Simmon's Citrate Agar. It has citrate as the only carbon source and pH indicator bromothymol blue
- **Method:** Inoculate the slant and incubate at 37°C for 24-48 hours.
- **Expected results:**
  - **Positive test:** Growth and color changes to blue
  - **Negative test:** No growth and color remains green

Results for Citrate Test



Positive

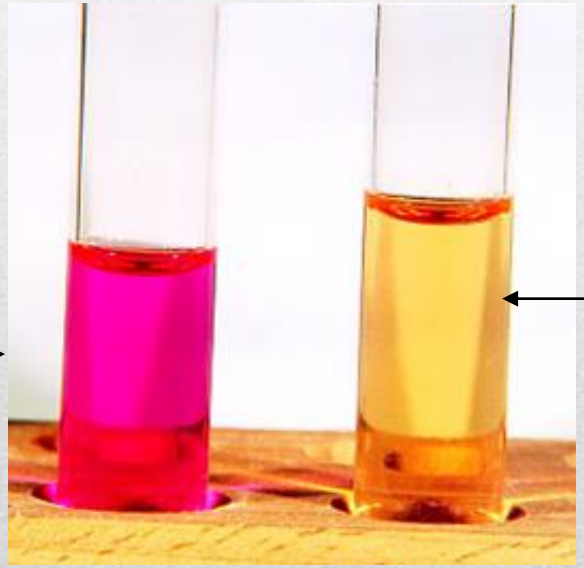
Negative

Urea Utilization

- Some bacteria produce urease, an enzyme capable of breaking down urea and produce alkaline end products. This distinguishes ***Proteus*** from other bacteria
- **Media and Reagent:** Urea Broth with phenol red
- **Method:** Inoculate the media with a loop and incubate at 37°C for 24 hours.
- **Expected Results:**
  - **Positive test:** production of alkaline end products = pinkish red color
  - **Negative test:** No color change

Results for Urea Test

Positive



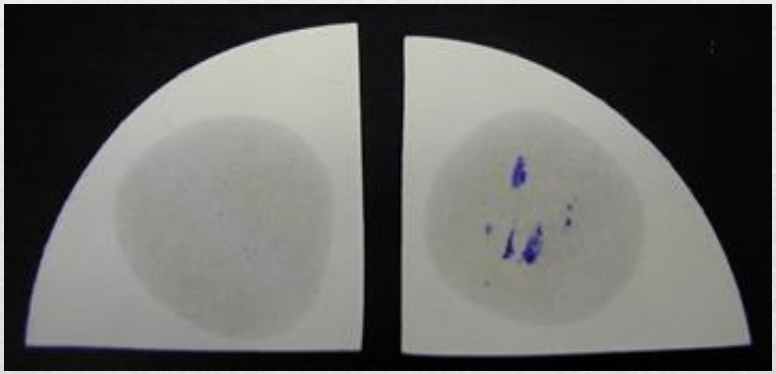
Negative

Oxidase Activity

- The oxidase test is a biochemical reaction that assays for the presence of **cytochrome oxidase**.
- In the presence of an organism that contains the cytochrome oxidase enzyme, the reduced colorless reagent becomes an oxidized colored product

Oxidase Activity

- The reagent:(N,N,N',N' tetramethyl –p-phenylenediamine) is used to do the test
- The test must be interpreted within 10 to 20 sec, many organisms in this family can give delayed false positive reaction .
- The use of metal loops (due to iron oxide on its surface) to transfer the colonies for the test gives false positive. **Only wooden applicator sticks can be used**.



www.mesacc.edu

Nitrate Reduction

- This test is used to determine the ability of an organism to reduce nitrate (NO3) to nitrite (NO2) using the enzyme **nitrate reductase**.

Nitrate Reduction

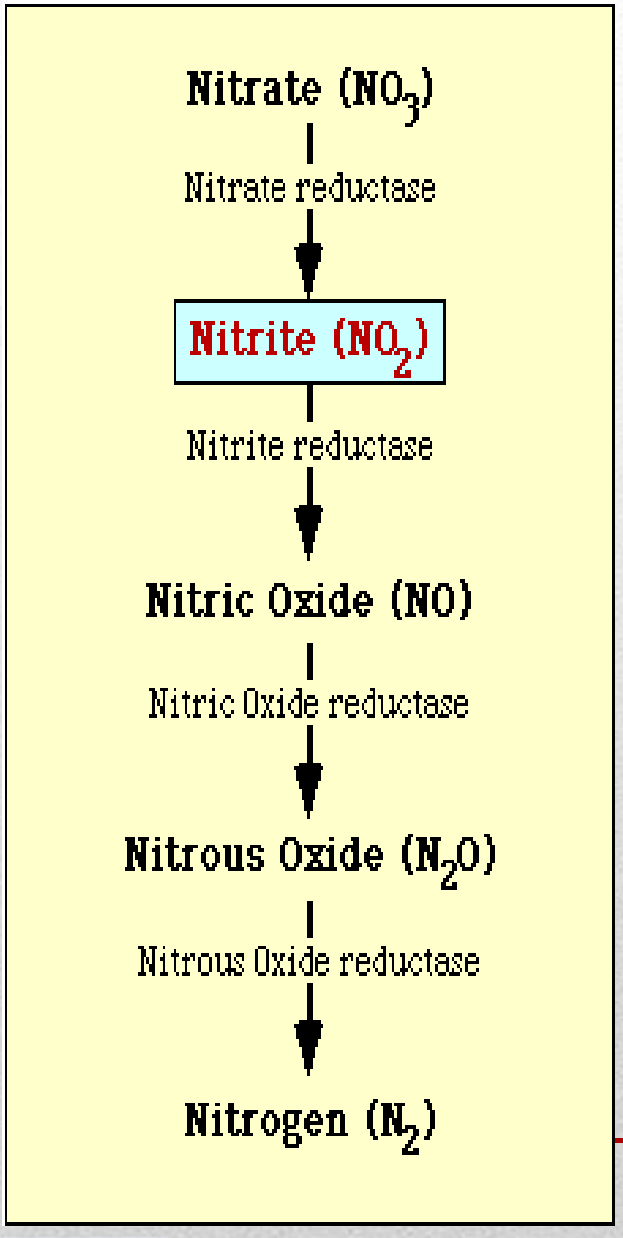
- It can be done on any basal medium that support the growth of the organism and contains 0.1% KNO3.

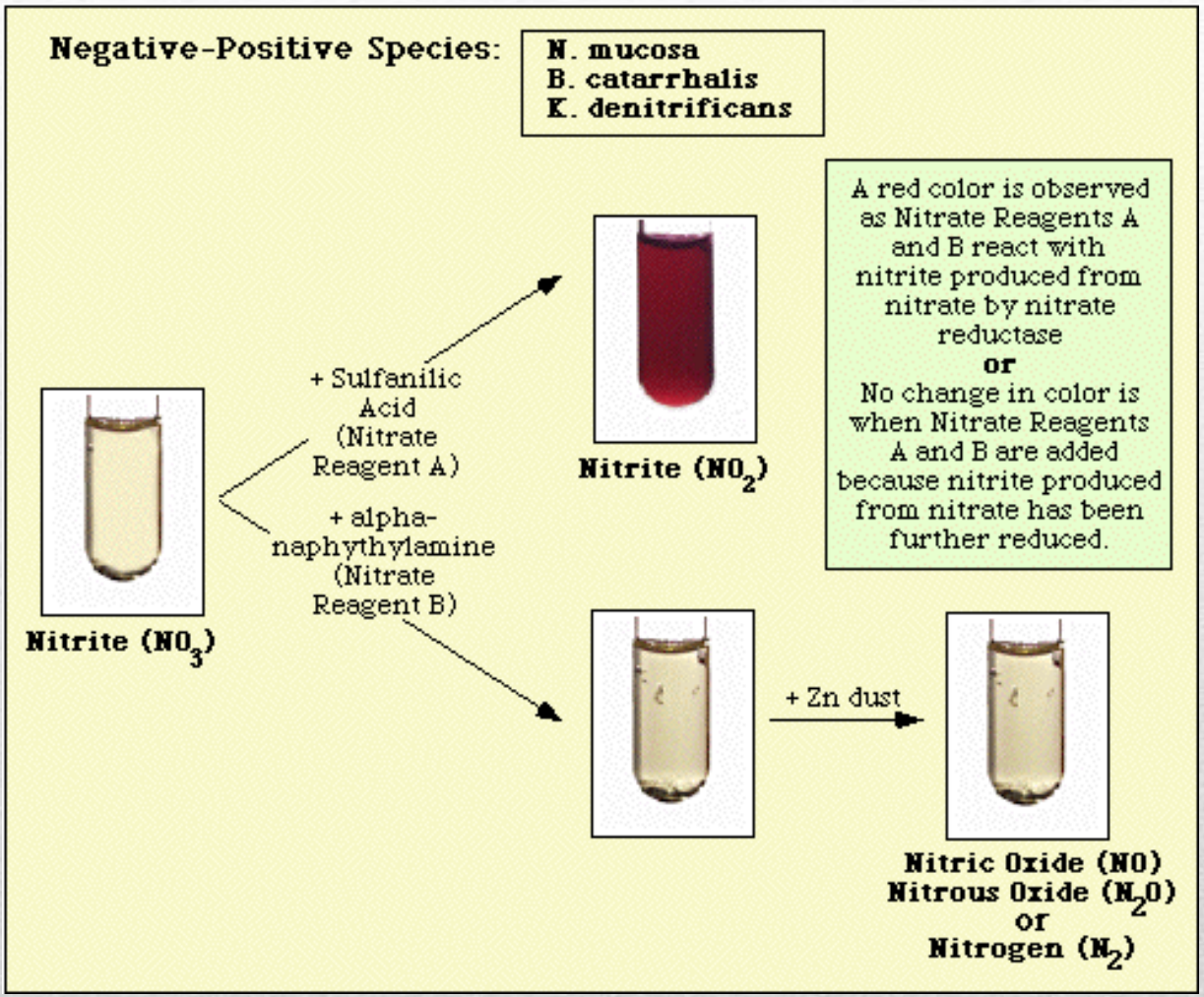
- $NO_3 \xrightarrow{\text{Nitrate reductase}} NO_2 + H_2O$

- Detection of nitrate reduction can be done by:
  - **Addition of alpha-naphtyl amine and sulfanilic acid will form diazonium compound (red color).**
  - **If color did not develop, it means one of two possibilities:**
    - **NO3 is not reduced to NO2 or**
    - **NO3 is reduced to NO2 and further reduced to Nitrogen gas**

➢ **To check for gas production: add a pinch of zinc dust, the development of red color indicates that nitrate reduction did not take place.**

No color development indicates that NO3 was reduced to NO2 and further reduced to nitrogen gas





http://biosci.usc.edu/courses/2002-fall/documents/bisc300-lab_NitrateReduction.jpg

Catalase

- Catalase is an enzyme found in most bacteria. It catalyzes the breakdown of hydrogen peroxide to release free oxygen.
- $2\ H_2O_2$ ---------> $2\ H_2O + O_2$
- **Procedure**: Add one drop of H_2O_2 to a glass slide with a loopful of growth from each culture to be tested. The development of bubbles is indicative of a positive catalase test.
- **The test is performed on a blood-free medium. Why??**

5-5 Catalase test. The test is performed by adding 3% hydrogen peroxide (H_2O_2) to a colony on a glass slide or by adding colony paste on a wooden stick to a drop of H_2O_2 on a slide, as shown here. The appearance of bubbles indicates that the enzyme, catalase, has hydrolyzed H_2O_2 into oxygen plus water. Staphylococci and micrococci are differentiated from other aerobic gram-positive cocci by a positive catalase test (*right*). No bubbles appear in a negative test result (*left*).

Color atlas of diagnostic microbiology Mosby, 1997

Coagulase

- Coagulase is an enzyme that catalyzes the formation of a fibrin clot in plasma.
- The presence of coagulase can be detected by heavily inoculating the test organism into rabbit plasma and incubating the mixture for 4 to 24 hours.
- Any degree of clotting during this time, from a loose clot suspended in the plasma to a solid, immovable clot is a **positive** result.
- *Staphylococcus aureus* produces coagulase enzyme while *Staphylococcus epidermidis* does not.

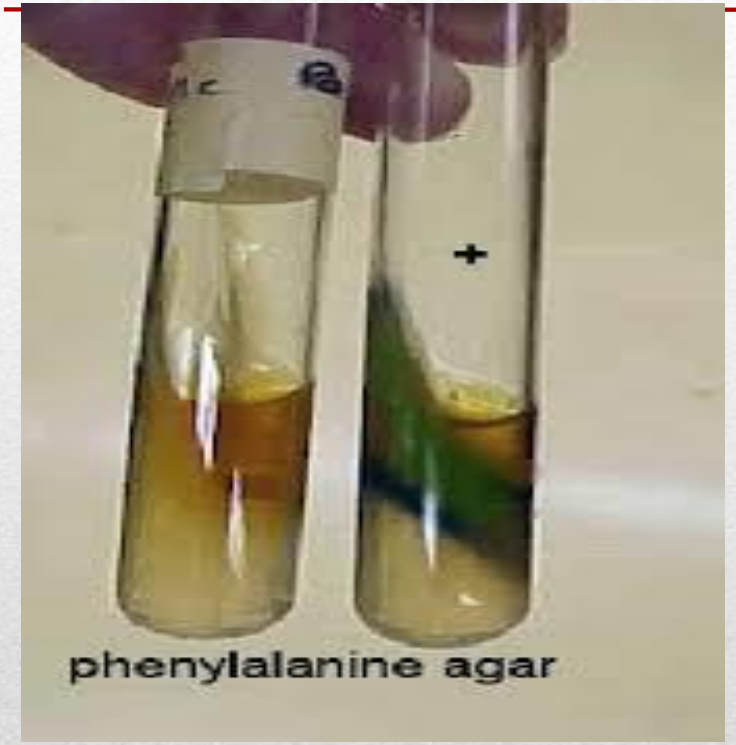
Coagulase Results

- **Reading Results:**
  - If the organism is has coagulase it will clump the plasma.
  - If the organism does not have coagulase it will not clump the plasma.

PAD

- Used to detect whether the bacterai produces the enzyme **phenylalanine deaminase**, which is needed to use the amino acid **phenylalanine** as a carbon and energy source for growth.

***Proteus mirabilis* --positive for PAD**



What is the purpose of the test