Lab #9 Biochemical activities of bacteria

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

Tests to perform

- H_2S production in SIM
- Indole
- Motility
- Lactose ,Glucose fermentation
- Starch hydrolysis
- Lipids

Biochemical Tests:

- They are done to measure the presence or absence of specific enzyme.
- It help identifying bacterial strains.
- Type of biochemical reaction act as a (thumbprint) bas on :

1- each species has a different DNA

2- different protein enzymes

3- different and unique sets of biochemical reaction

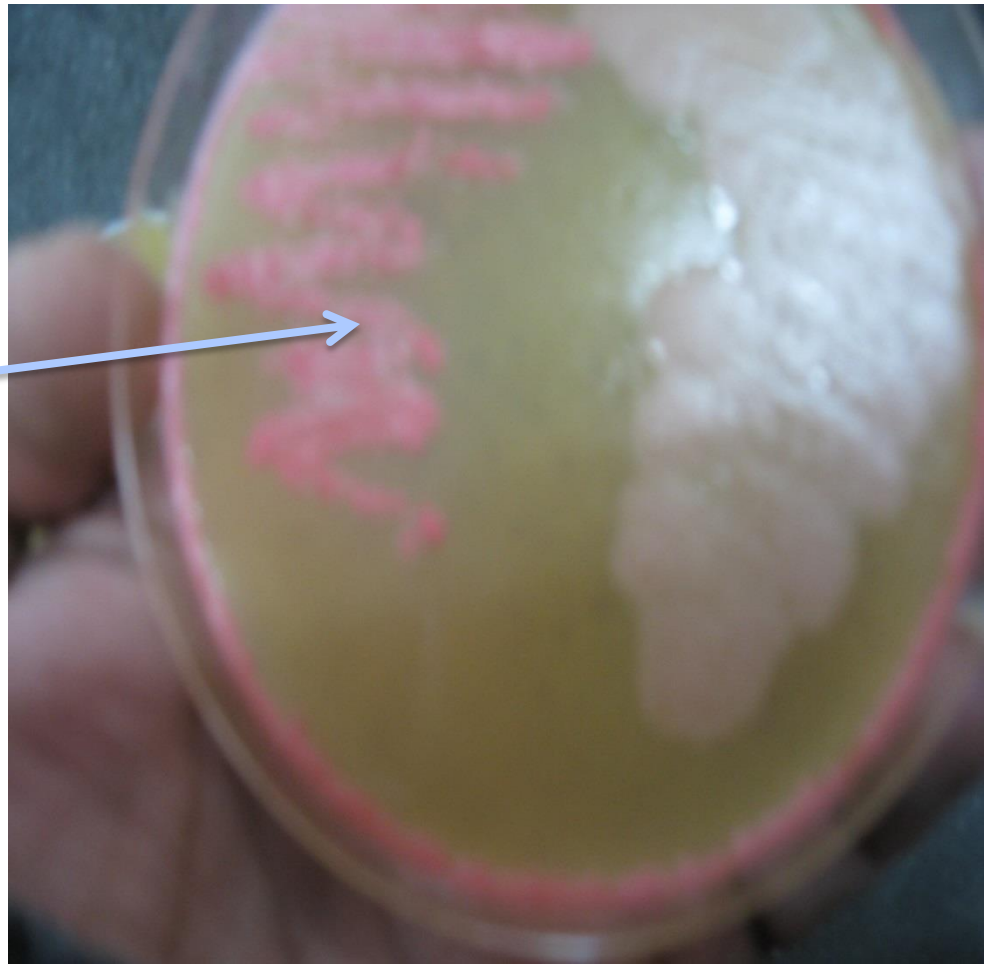
Lipid hydrolysis:

- 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)

Lipid hydrolysis:

Positive for lipid hydrolysis



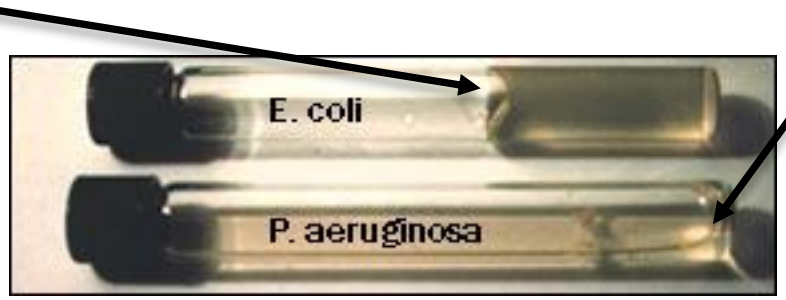
Gelatin liquefaction:

- Some bacteria can use gelatin as a source of carbon.
- **Protease** break the peptide bond between amino acids.
- Gelatin is semi-solid at room temperature. and melt at higher temperature such as 37C.
- In order to take the results, the tubes must be placed in the refrigerator for 5-10 minutes.

Gelatin Liquefaction

Can bacteria use gelatin as source of carbon ?

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.

TSI

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.

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

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

TSI: Triple Sugar Iron Test.

- Triple sugar:
  - 1% lactose, 1% sucrose and 0.1% glucose.
  - The indicator is phenol red
  - A/A or yellow/ yellow → the bacteria can ferment the three sugars.
  - K/K or (red/red)→ non fermenters bacteria.
- Sulfur reduction:
  - The indicator is iron.
- Gas production:
  - When Peptone is utilized aerobically it produce ammonia gas.

SIM: Sulfur, Indole and Motility test

- Sulfur reaction:

❖ Ferrous ammonium sulfate and sodium thiosulfate (together serve as indicator for the production **(H2S).**

❖ H2S react with iron and form **ferric sulfide. (black precipitate).**

- Indole test:

❖ Some bacteria can hydrolyze Tryptophan and produce **Indole** by **Tryptophanase.**

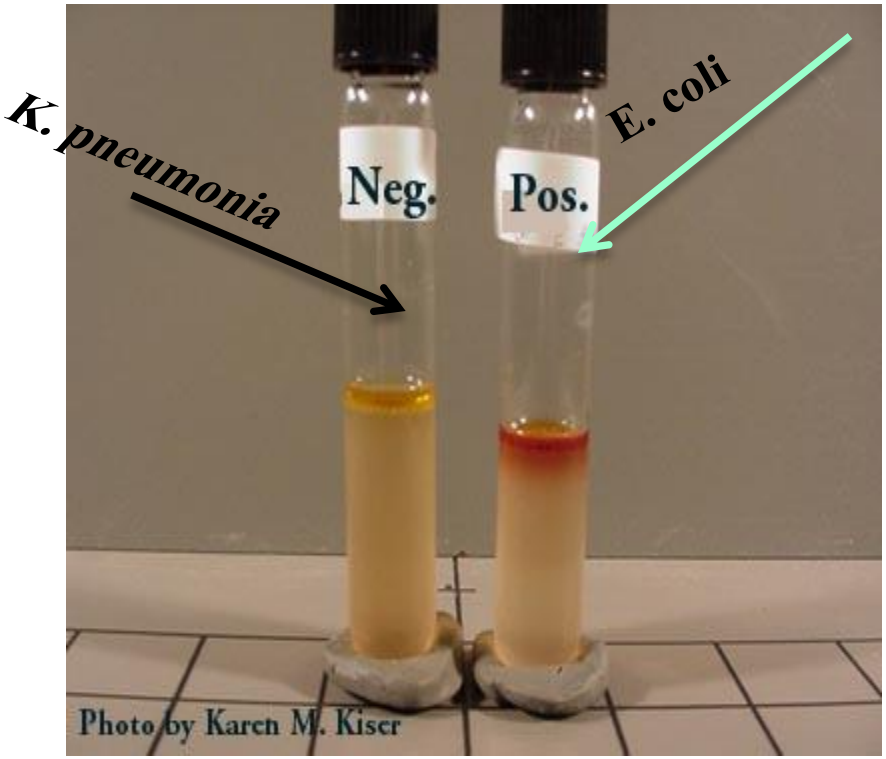
❖ The indicator is Kovac`s reagent (positive results→ red color).

- Motility test (semi-sold media)

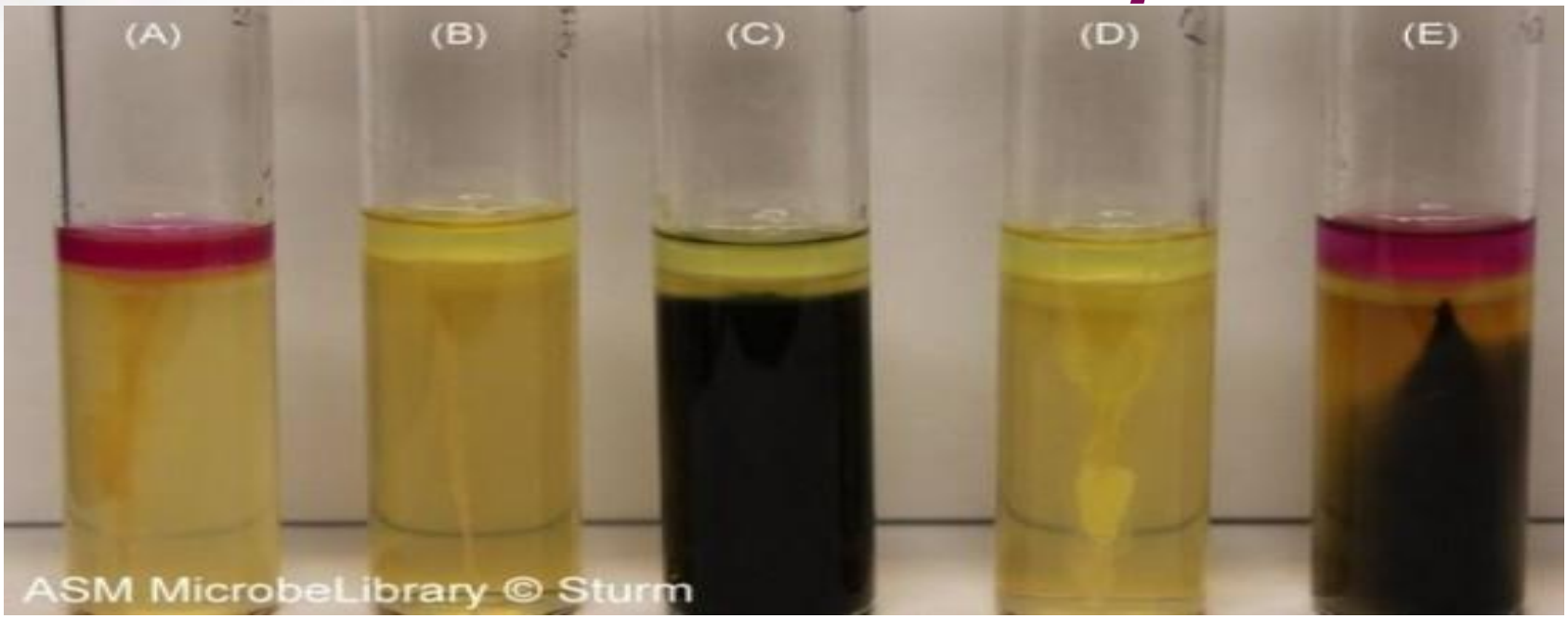
SIM

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.



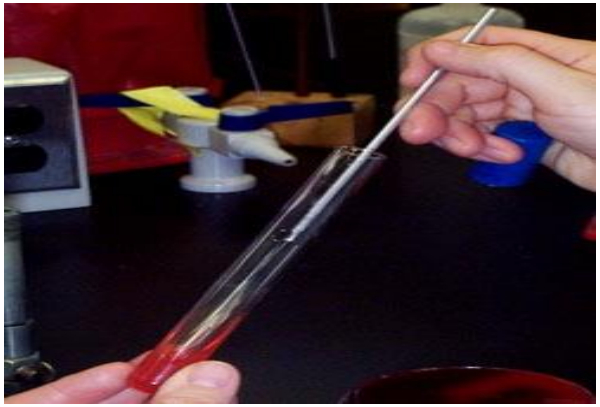
SIM: Sulfur, Indole and Motility test



- ***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

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.

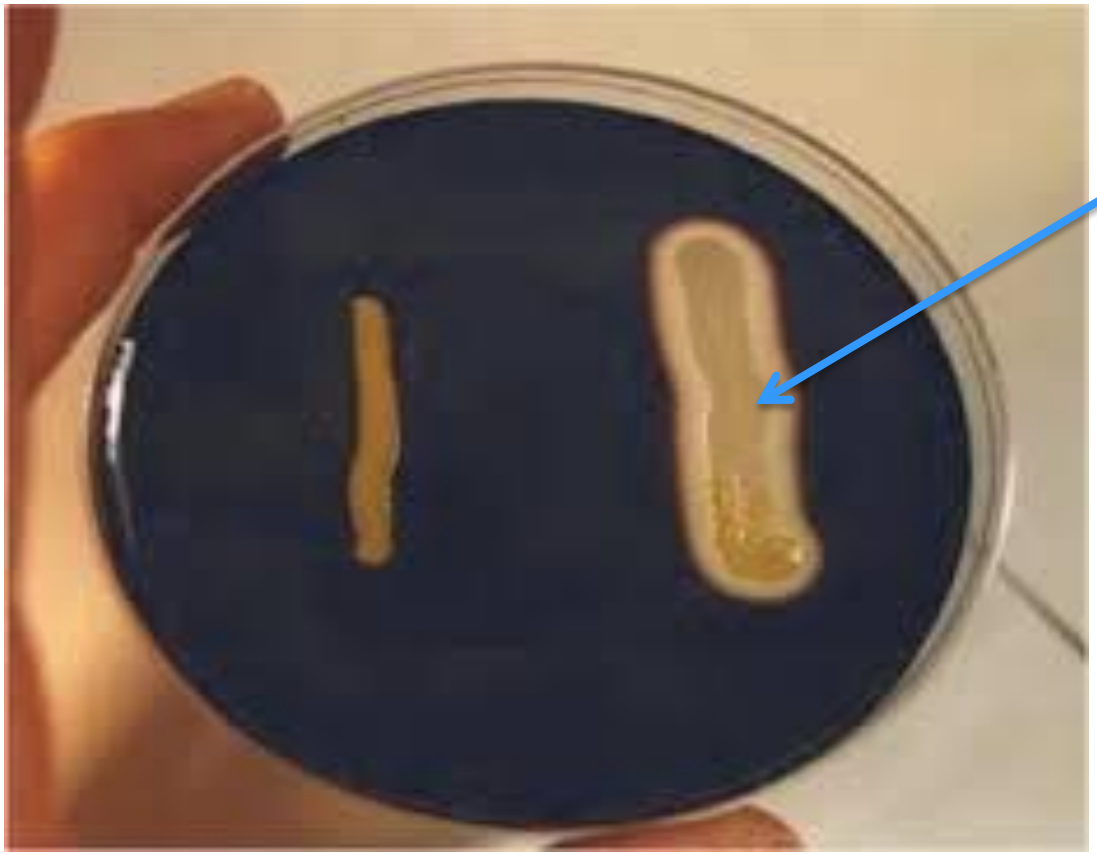


TSI: Triple Sugar Iron Test.

Starch Hydrolysis:

- Test the ability of an organism to produce certain coenzymes such as
- Starch addition make the media nutritive.
- NO color change occur in the medium when starch is hydrolyzed.
- IODINE (IKI) should be added to the plate AFTER incubation.
- If the color change to black or blue→ no hydrolysis.
- If there was no change in medium color or a clear zone appeared → hydrolysis occurred

Starch Hydrolysis:



Positive for starch hydrolysis

Biochemical Tests:

Test	Test for	Indicator
SIM	Sulfur reduction / tryptophanase enzyme/ motility.	Iron for sulfur reduction / Kovac`s reagent for Indole.
TSI	Fermentation of three sugars / sulfur reduction/ gas production	Iron for sulfur reduction / phenol red → yellow (acid).
Starch hydrolysis	Starch hydrolysis by Amylase	Iodine
Lipid hydrolysis	Lipids hydrolysis by Lipases	Neutral red → red (acid)
Gelatin liquefaction	The presence of Proteases	----------
Citrate utilization	If the bacteria can use citrate as a source of carbon.	Bromothymo blue → blue (basic).
Urea utilization	The presence of Urease.	Phenol red→ Pink (basic)
Oxidation test	The presence of Cytochrome c oxidase.	Tetramethyl-p-phenyliamine → purple
Nitrate reduction	The presence of Nitroreductase	Alpha-naphtyl amine and sulfanic acid
Catalase test	Catalase enzyme	----------
Coagulase test	Coagulase enzyme	----------

Lab #10 Biochemical activities of bacteria (part 2)

Objectives

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

Tests to know

1- Citrate
2- Urea utilization
3- Oxidase
4- Nitrate reduction
5- Catalase
6- Coagulase
7-PAD

SIM

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

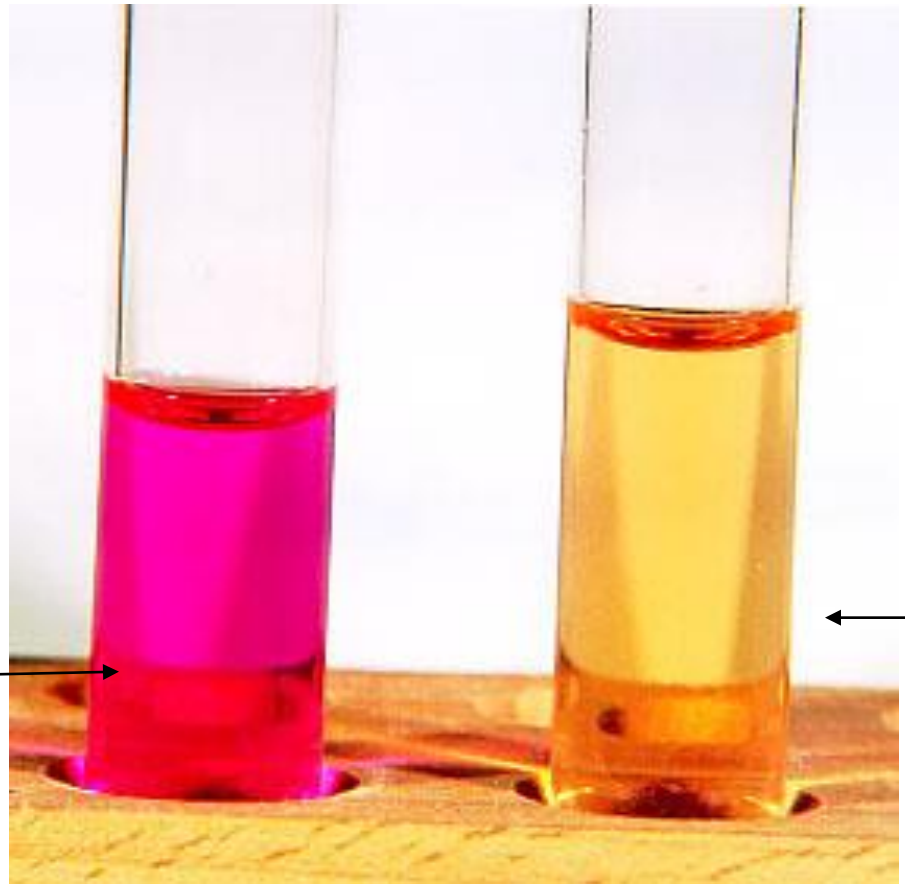
Negative test: No growth and color remains green

Citrate Test results

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

Urea Test results



Positive

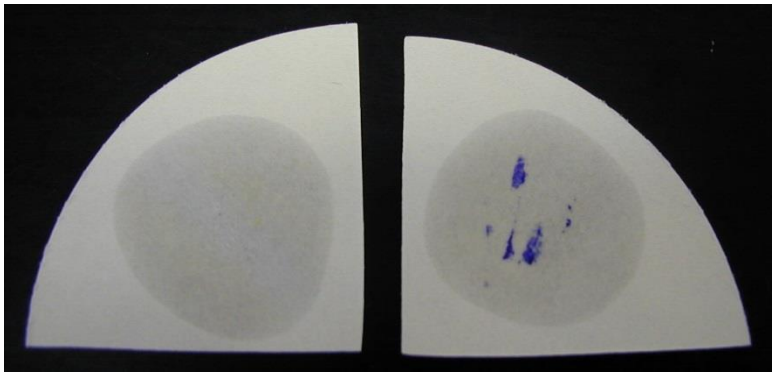
Negative

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**.



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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% KNO_3.

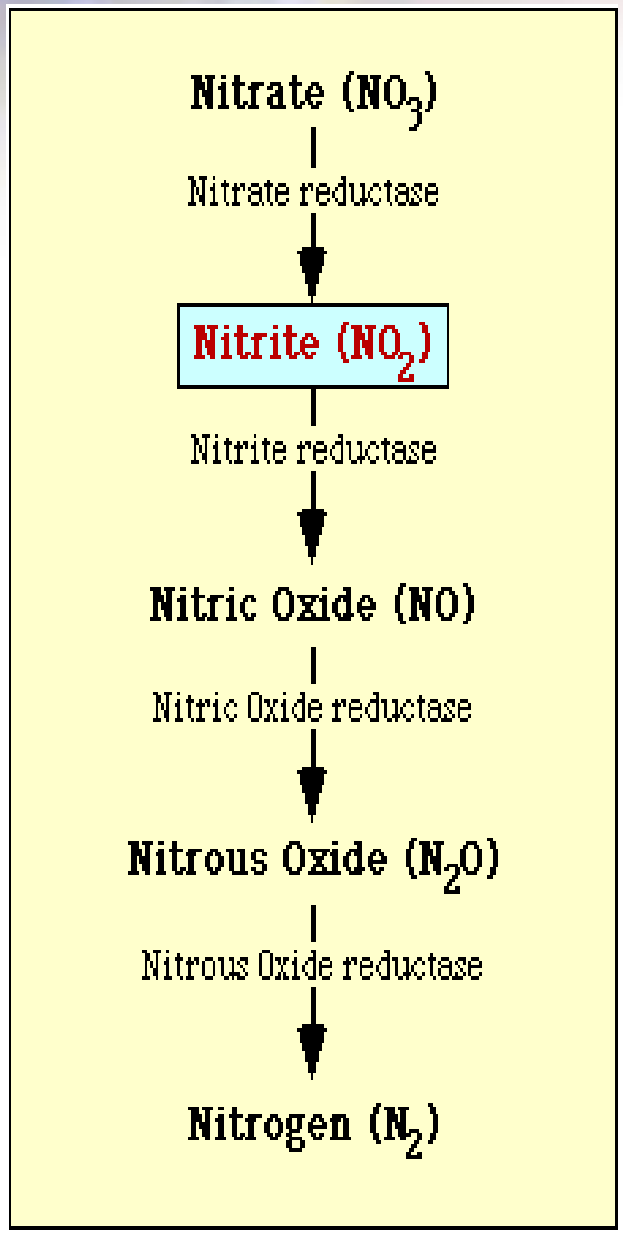
- NO_3 —(Nitrate reductase)→ $NO_2 + H_2O$

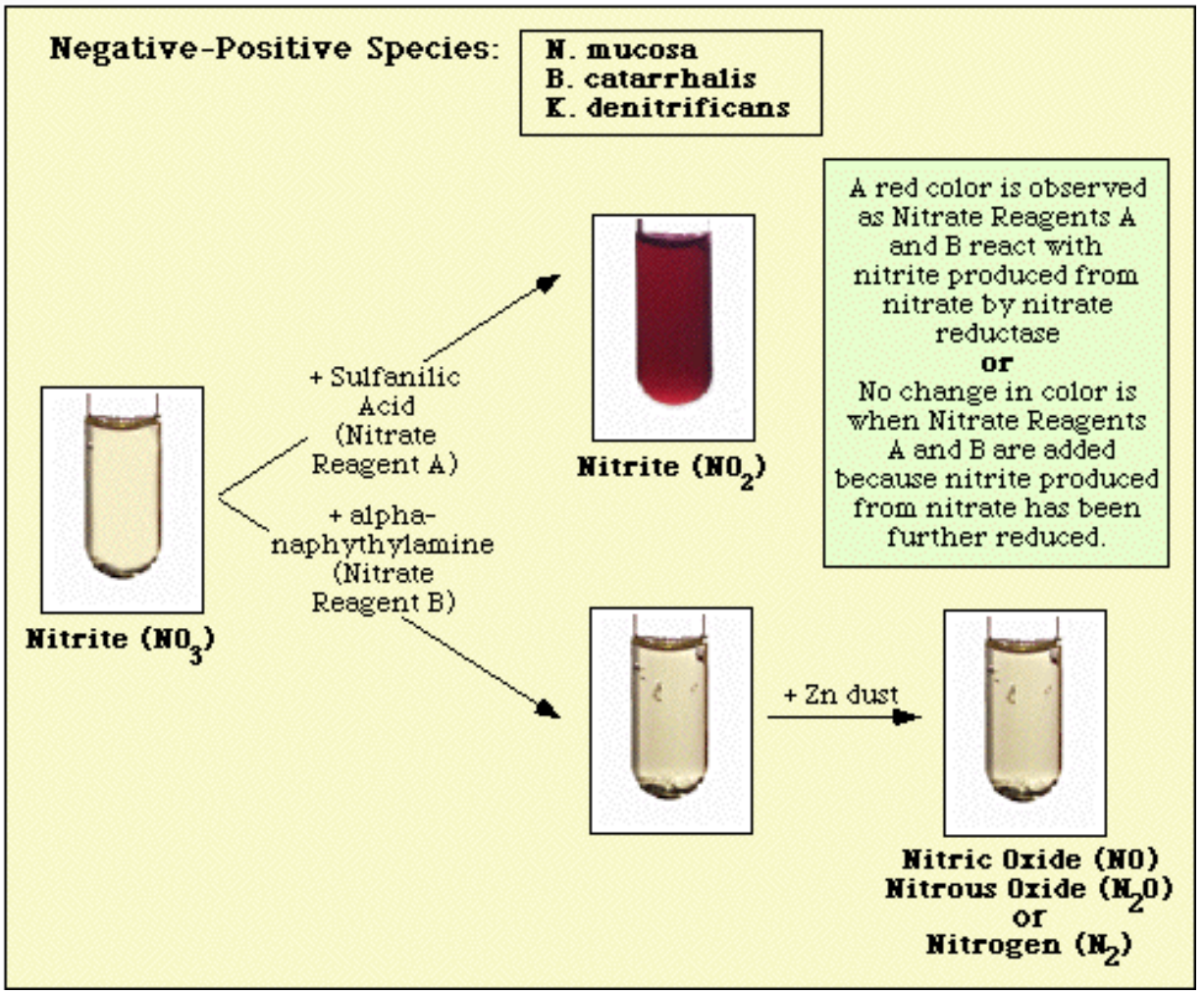
- Detection of nitrate reduction can be done by:
  - Addition of alpha-naphtyl amine and sulfanilic acid will form diazonium compound (red color).
  - If color did not develop, it means one of two possibilities:
    - NO_3 is not reduced to NO_2 or
    - NO_3 is reduced to NO_2 and further reduced to 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 NO_3 was reduced to NO_2 and further reduced to nitrogen gas

Nitrate Reduction





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*).

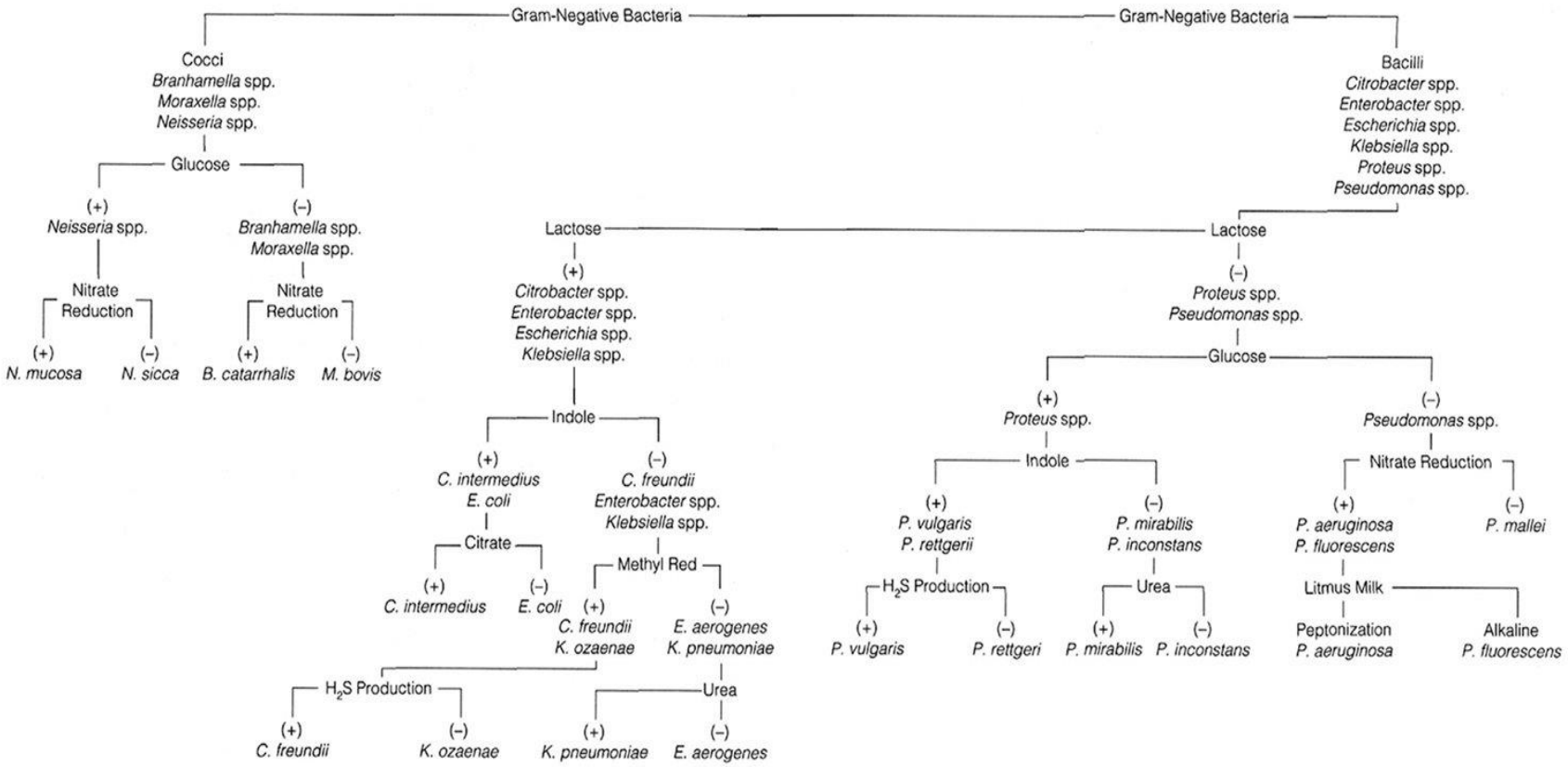
Coagulase

- 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.

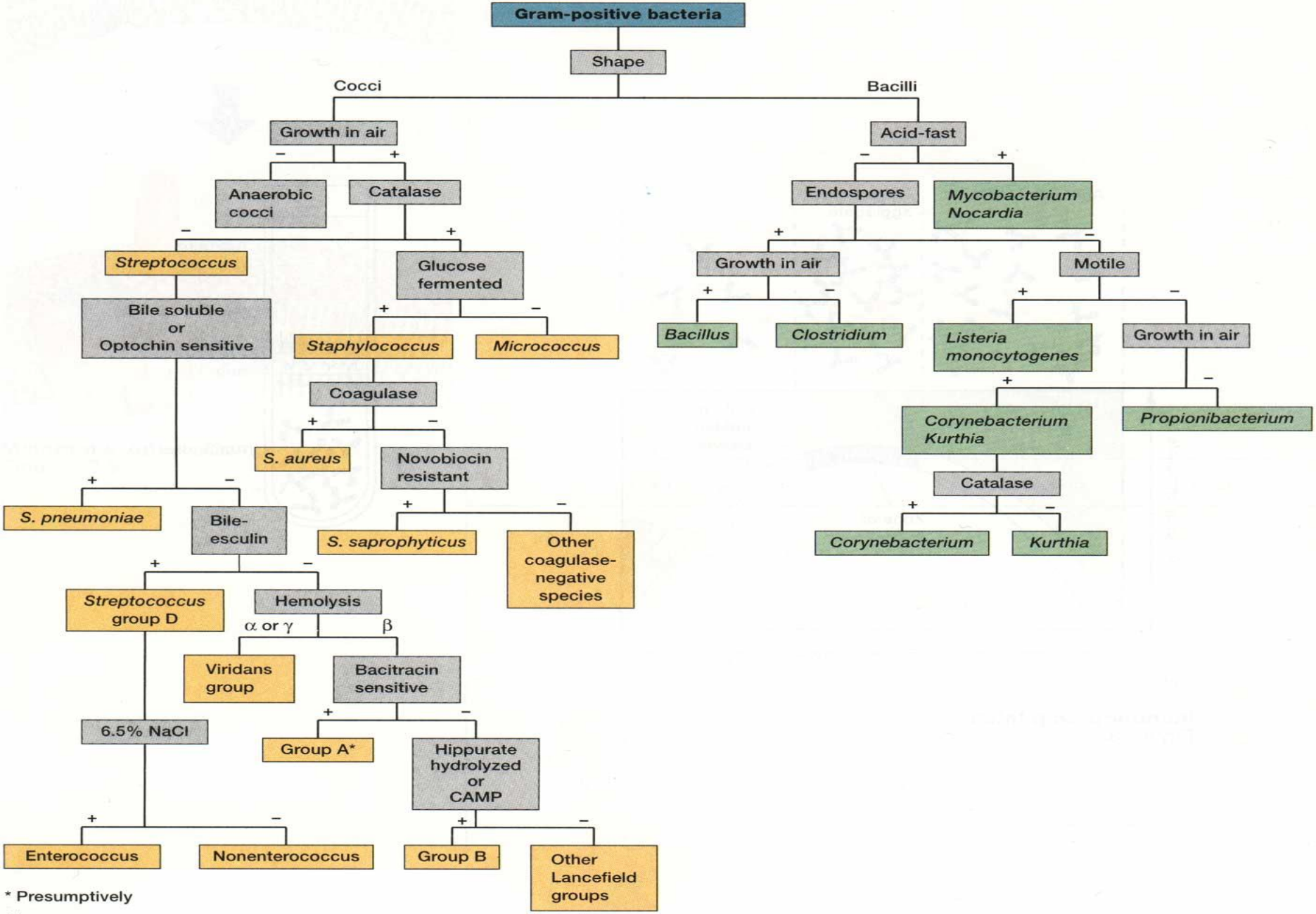
Coagulase Results

- **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.

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*