Biochemical Reaction Tests

ENZYMATIC AND BIOCHEMICAL ACTIVITIES OF BACTERIA

Laboratory Objectives

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Biochemical tests

To identify bacteria, we must rely heavily on biochemical testing. The types of biochemical reactions each organism undergoes act as a "thumbprint" for its identification.

1. **Catalase Test**
2. **Oxidase Test**
3. **Coagulase Test**
4. **Urease Test**
5. **IMViC:**
   - **Indole Test**
   - **Methyl Red Test**
   - **Voges-Proskauer Test**
   - **Citrate Utilization Test**
6. **Triple Sugar Iron (TSI) Agar Test**
7. **Hydrogen Sulphide Test**
8. **Motility Test**

Catalase Test

INTRODUCTION

Catalase is an enzyme produced by many bacteria. The enzyme splits hydrogen peroxide into water and oxygen. Hydrogen peroxide is a by product of aerobic respiration and is lethal if it accumulates in the bacterial cell. Catalase degrades the hydrogen peroxide in the bacterial cell before it can do any damage to the bacterial cell.

PRINCIPLE

- Chemically, catalase is…… ??
- The enzyme converts hydrogen peroxide into water and oxygen.

2H202 —— 2H2 0+02 (gas bubbles)

PROCEDURE

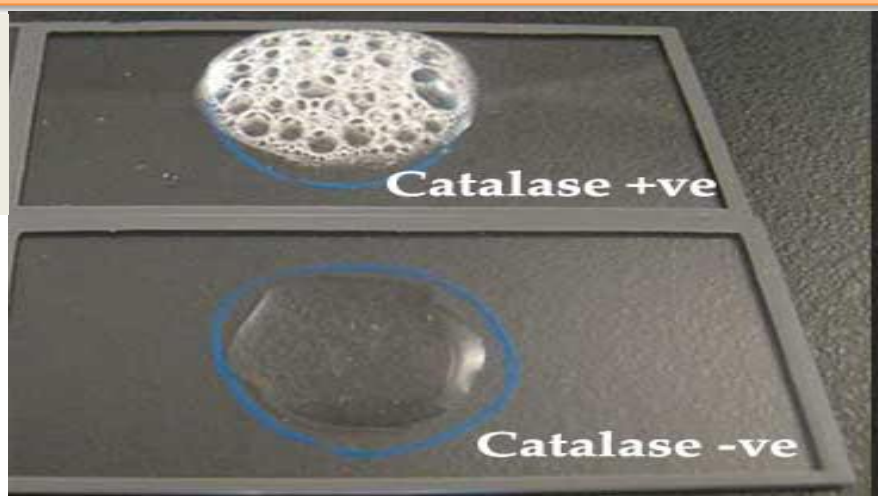
Test can be done by 2 methods as follows:

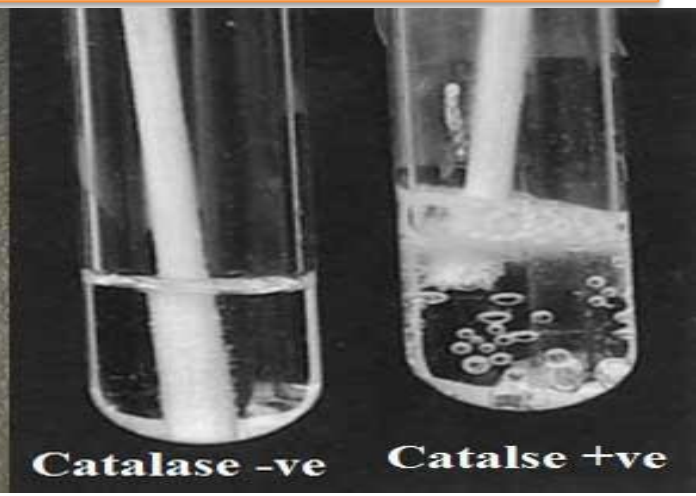
1- Slide method

2- Tube method

-----Observe the release of bubbles.

Reagent:
3% hydrogen peroxide.





QUALITY CONTROL

Positive control: *Staphylococcus aureus* (catalase positive bacteria).

Negative control: *Streptococcus* species (catalase negative bacteria).

RESULTS AND INTERPRETATION

1- The rapid and sustained appearance of bubbles or effervescence constitutes a positive test. It means bacteria possesses the enzyme catalase, hence is catalase positive.

2- Some bacteria possess enzymes other than catalase that can decompose hydrogen peroxide. Hence, forming a few tiny bubbles after 20-30 seconds is not considered a positive test.

Oxidase Test

INTRODUCTION

- The enzyme oxidase plays a vital role in the operation of the electron transport system during aerobic respiration.
- Aerobic bacteria, as well as some facultative anaerobes and microaerophiles…? exhibit oxidase activity.
- Cytochromes in aerobic respiration transfer electrons (H) to oxygen to form water.
- The reagent used is a dye **tetra methyl-para-phenylene diamine di hydrochloride** acts as an artificial electron accepter substituting the oxygen.

Purpose

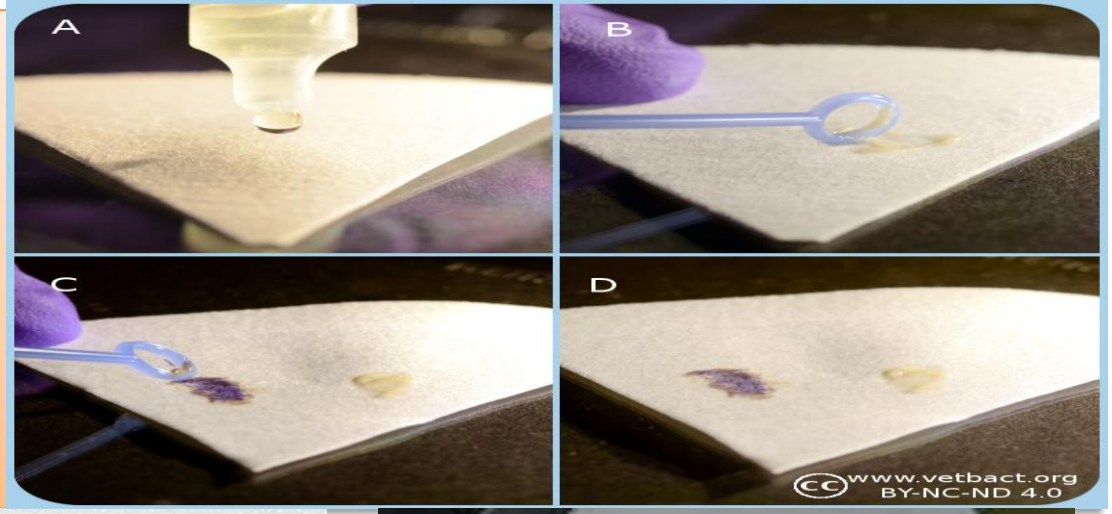
This test determines the presence of cytochrome oxidase activity in microorganisms for the identification of oxidase-negative Enterobacteriaceae, differentiating them from other oxidase-positive gram-negative bacilli.

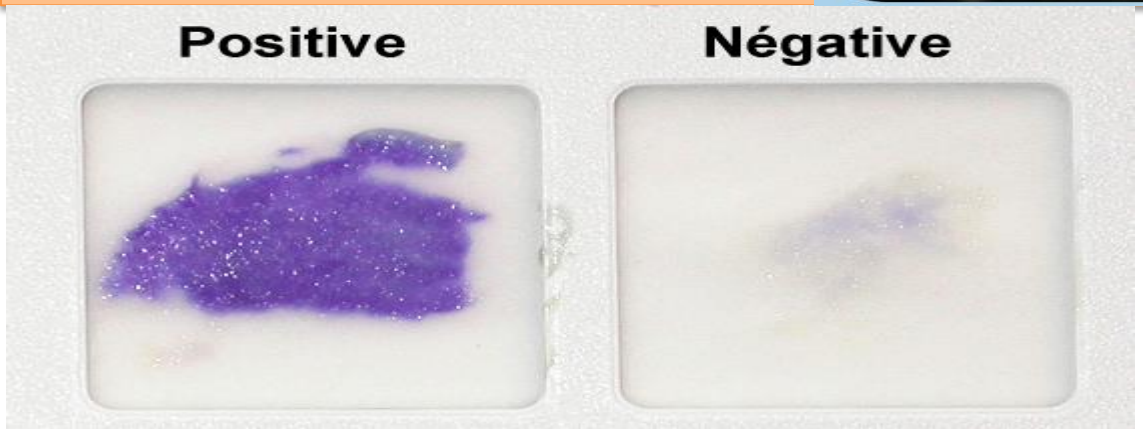
PRINCIPLE

The Principle test

To determine the presence of bacterial cytochrome oxidase using the oxidation of the substrate **tetra methyl-para-phenylene diamine di hydrochloride** to indophenol, a dark purple colored end product. A positive test (presence of oxidase) is indicated by the development of a dark purple color. No color development indicates a negative test and the absence of the enzyme.

PROCEDURE





in the presence of enzyme cytochrome oxidase dye is oxidized to indophenol blue which is a dark purple colored end products.

QUALITY CONTROL

Positive control: *Pseudomonas aeruginosa* (oxidase positive bacteria).
Negative control: *Escherichia coli* (oxidase negative bacteria).

RESULTS AND INTERPRETATION

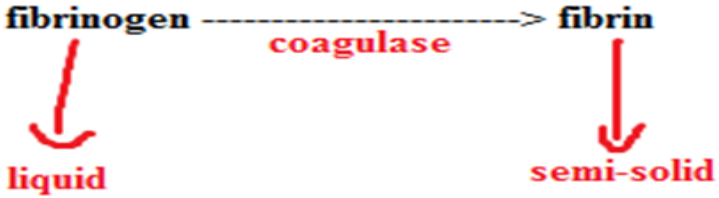
Bacterial colonies having cytochrome oxidase activity develop a deep blue colour at the inoculation site within 10-30 seconds. In filter paper test, deep blue colour develops at the site of smear within 10-30 seconds. It means bacteria possesses the enzyme oxidase, hence is oxidase positive.

Coagulase Test

INTRODUCTION

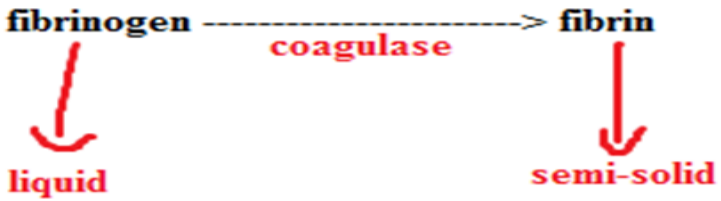
The enzyme, coagulase, produced by a few *Staphylococcus* species, is a key feature of pathogenic *Staphylococcus*. The enzyme causes coagulation of blood, allowing the organism to "wall" its infection off from the host's protective mechanisms rather effectively.

Coagulase is a protein having a prothrombin-like activity capable of converting fibrinogen into fibrin, which results in the formation of visible clot. In the laboratory, the coagulase test is used to identify *S. aureus* and differentiate it from the other species of coagulase-negative *Staphylococcus*.



PRINCIPLE

- *S. aureus* produces the enzyme coagulase in 2 forms:
- **Bound coagulase**
- **Free coagulase**

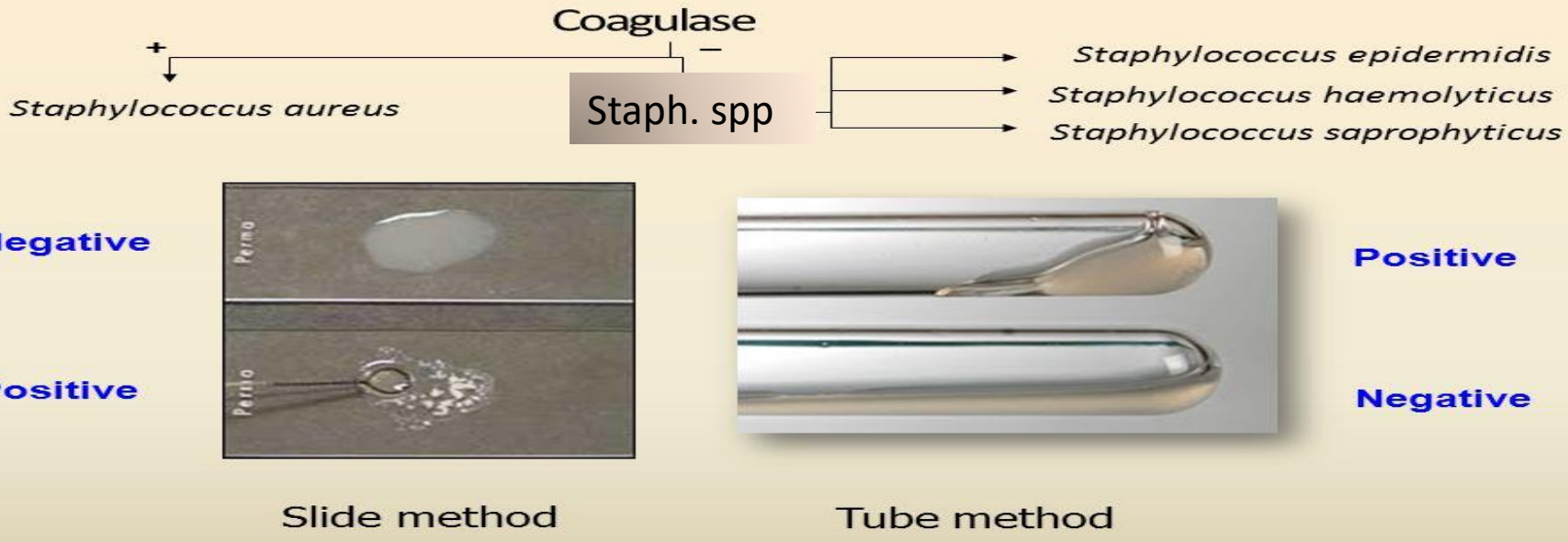


PROCEDURE

1- Slide test

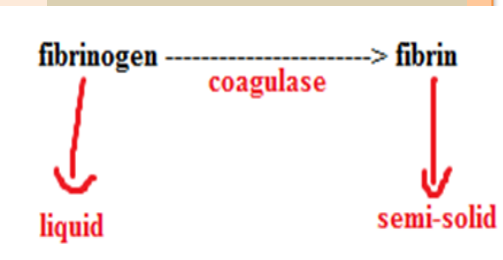
2- Tube test

Rabbit plasma, with EDTA anticoagulant, saline, Pure growth of *S. aureus* from solid media



OBSERVATION

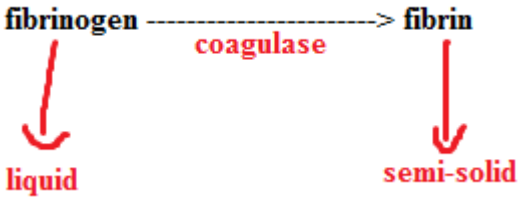
- In a positive slide test, prompt clumping of the organism shows the presence of the bound coagulase.
- In a positive tube test, the plasma in the tube clots.



QUALITY CONTROL

Positive control: *S. aureus* (Coagulase positive bacteria).

Negative control: *S. epidermidis (sp.)* (Coagulase negative bacteria).



RESULTS AND INTERPRETATION

In slide test, Positive reaction will be detected within 10–15 seconds of mixing the plasma with the suspension by the formation of a white precipitate and agglutination of the organisms. The test is considered negative if no agglutination is observed after 2 minutes. All strains that are coagulase positive can be reported as *S. aureus.* All strains producing negative slide tests must be tested with the tube coagulase test. The tube coagulase test is considered positive if any degree of clotting is noted.

Urease Test

INTRODUCTION

Certain bacteria and fungi possess the enzyme urease that hydrolyzes urea releasing ammonia into the medium. This produces a change in the pH of the medium that can be detected by the color change in the indicator dye. This test can be used to differentiate different groups of bacteria and fungi.

Principles

- Some bacteria are able to produce an enzyme called **urease** that attacks the nitrogen and carbon bond in amide compounds such as urea, forming the end products ammonia, CO2, and water. Urease activity (the **urease test**) is detected by growing bacteria in a medium containing urea and using a pH indicator such as phenol red, When urea is hydrolyzed, ammonia accumulates in the medium and makes it alkaline. This increase in Ph causes the indicator to change from orange-red to deep pink or purplish red (cerise) and is a positive test for urea hydrolysis.

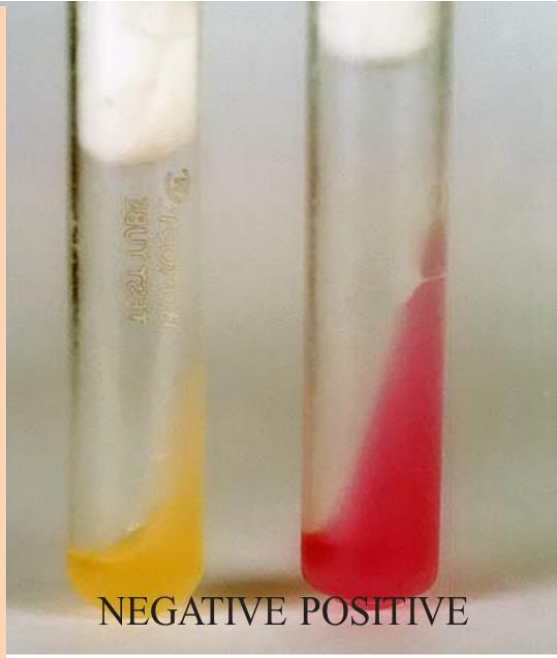
PROCEDURE

1 Pick up the colonies of *P. mirabilis* from the culture on nutrient agar.

2 Inoculate Christensen's urea agar slope with these bacterial colonies.

3 Incubate the tube at 37°C for 18 hours.

4 Observe any change of colour in the inoculated medium. The uninoculated medium is colour less. In a positive test, after incubation, the colour of the medium changes to purple pink.



QUALITY CONTROL

Positive control: *P. mirabilis* (urease positive bacteria).

Negative control: *Escherichia coli* (urease negative bacteria).

It means:

P. mirabilis is a urease producing bacteria. *E. coli* does not produce the enzyme urease.

- **IMViC tests**
- **IMViC: it's a group of tests used mainly to identify Enterobacteriaceae**
- **members which include:**
- 1. **I**ndole test
- 2. **M**ethyl red test
- 3. **V**oges-Proskauer test
- 4. **C**itrate test

1. Indole test

Indole is a component of the amino acid tryptophan. Some bacteria have the ability to break down tryptophan for nutritional needs using the enzyme **tryptophanase**. When tryptophan is broken down, the presence of indole can be detected through the use of **Kovacs' reagent**. Kovac's reagent, which is yellow, reacts with indole and produces **a red color** on the surface of the test tube.

Results: Indole-Positive reaction: red color ex. *E.coli;* Negative reaction: yellow color ex*. Klebsiella.*

PROCEDURE

- Take 0.5 ml of 24 hours to 48 hours peptone water cultures of *E. coli* in a small test tube. Add 0.2 ml of Kovac's reagent to the peptone water and shake. Allow it to stand for few minutes and read the result.

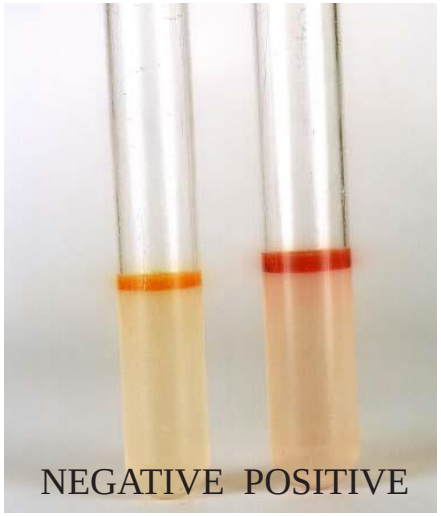
QUALITY CONTROL

Positive control: *E. coli* (indole positive bacteria).

Negative control: *Klebsiella pneumoniae* (indole negative bacteria).

OBSERVATION

In a positive test, a red-violet ring develops within minutes on addition of Kovac's reagent. In a negative test a yellow- brown ring appears..



MR-VP test

MR test:

Principle to test the ability of the organism to produce **acid end product** from glucose fermentation, this is a qualitative test for acid production.

VP test:

To determine the ability of the organisms to produce **neutral end product** (acetoin) from glucose fermentation.

procedure

1. Inoculate the tested organism into 2 tubes of MR-VP broth
2. Incubate the tubes at 37°C for 24 hours
3. AFTER INCUBATION: Run the MR test in the tube 1, and the VP

test in tube 2.

– For methyl red: Add 6-8 drops of methyl red reagent.

– For Voges-Proskauer: Add 12 drops of **Barritt's A** (**α-naphthol**),

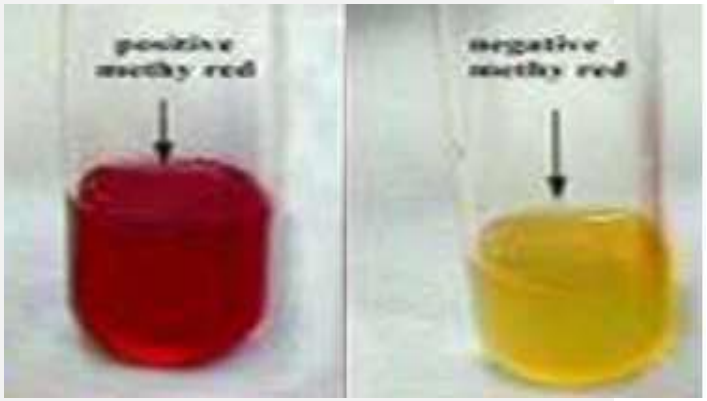
mix, 4 drops of **Barritt's B** (40% KOH), mix

– Let sit, for at least 1hour

Results

MR results: Red: Positive MR (*E. coli*); Yellow: Negative MR (*Klebsiella*)

Voges-Proskauer results Pink: Positive VP (*Klebsiella*), yellow: Negative VP (*E. coli*)

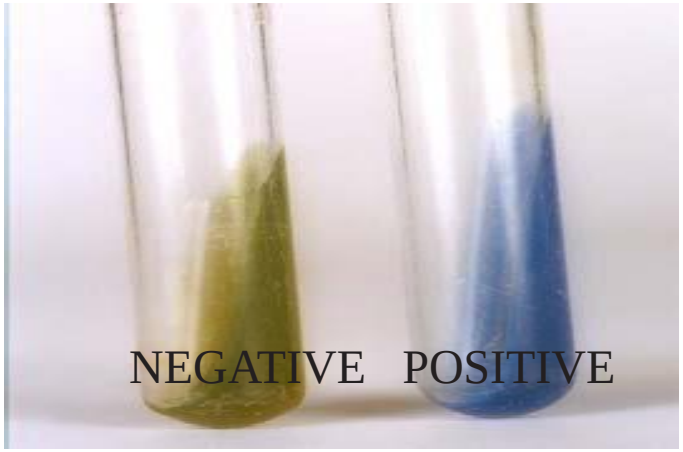


Citrate Utilization Test:

Simmons Citrate agar is a defined medium containing sodium citrate as the sole carbon source. **The pH indicator, bromthymol blue, will turn from green at neutral pH (6.9) to blue when a pH higher than 7.6 is reached (alkaline)**. If the citrate is utilized, the resulting growth will produce alkaline products changing the color of the medium from green to blue. (Blue color= positive reaction eg; *Klebsiella)*; (green color=negative reaction eg; *E.coli*).

QUALITY CONTROL

- (Blue color= positive reaction eg; *Klebsiella)*
- (green color=negative reaction eg; *E.coli*)



Triple Sugar Iron (TSI)

- TSI contains
- Three different types of sugars
- Glucose
- Lactose
- Sucrose
- Phenol red (acidic: Yellow)
- TSI dispensed in tubes with equal butt & slant

➢ Reaction on Triple Sugar Iron (TSI) Agar

Principle

To determine the ability of an organism to attack a specific carbohydrate incorporated into a basal growth medium, with or without the production of gas, along with the determination of possible hydrogen sulphide production.

- Reaction on TSI

Procedure:

•Inoculate TSI medium with an organism by inoculating needle by stabbing the butt and streaking the slant Incubate at 37°C for 18-24 hours.

QUALITY CONTROL

1 Alkaline slant /alkaline butt (K/K reaction): *Pseudomonas aeruginosa*.

2 Alkaline slant /acidic butt (K/A reaction): *Shigella, Vibrio*.

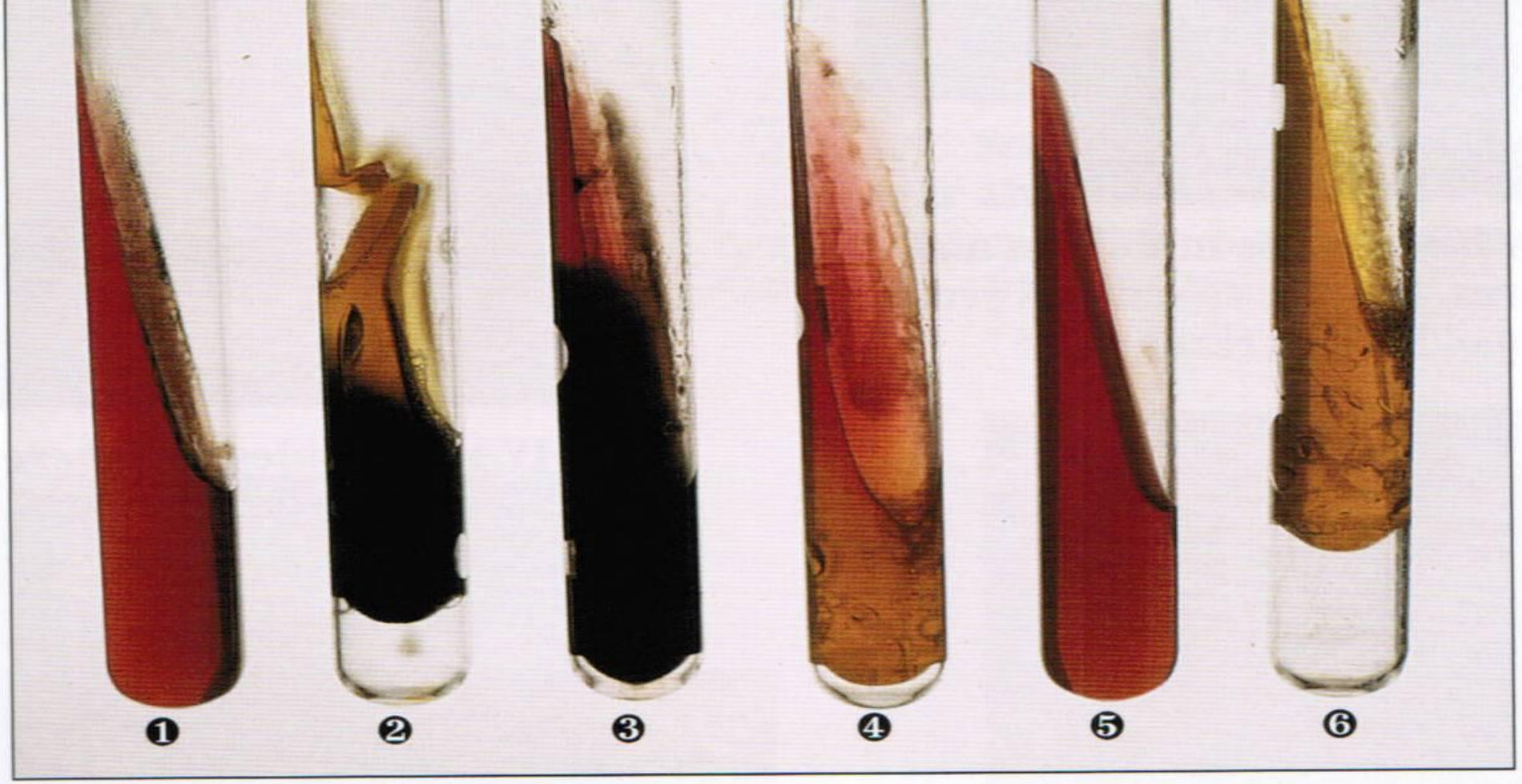
3 Alkaline slant /acidic butt /production of H_2S (K/A reaction and positive for H_2S): *Salmonella* spp, *Citrobacter* spp, *Proteus* spp.

4 Acidic slant /acidic butt (A/A reaction): *E. coli*, *Klebsiella* spp, *Enterobacter* spp.

5 Acid butt/acid slant, H_2S positive: *Citrobacter*.

OBSERVATIONS

Look for the colour change in the slant and butt after 18–24 hours incubation and also look for the development of black precipitate to indicate H_2S production.



III.15 Reactions in triple sugar iron (TSI) agar. 1 shows growth with no fermentation or hydrogen sulfide (H_2S) production. **2** shows blackening due to H_2S and acid and gas from fermentation of glucose and sucrose and/or lactose. Sucrose and lactose were not fermented in **3**; H_2S production masks the glucose fermentation reaction although gas is produced. Acid and gas are produced from glucose in **4. 5** is uninoculated. **6** shows acid and gas production from glucose and sucrose and/or lactose (Exercise 40).

Motility Testing

Purpose

These tests are used to determine whether an enteric organism is motile. An organism must have flagella to be motile.

Principle

The inoculum is stabbed into the center of a semisolid agar deep. Bacterial motility is evident by a diffuse zone of growth extending out from the line of inoculation. Some organisms grow throughout the entire medium, whereas others show small areas or nodules that grow out from the line of inoculation.

Method

1. Touch a straight needle to a colony of a young (18- to 24-hour) culture growing on agar medium.
2. Stab once to a depth of only 1/3 to ½ inch in the middle of the tube.
3. Incubate at 35°-37°C and examine daily for up to 7 days.

Expected Results

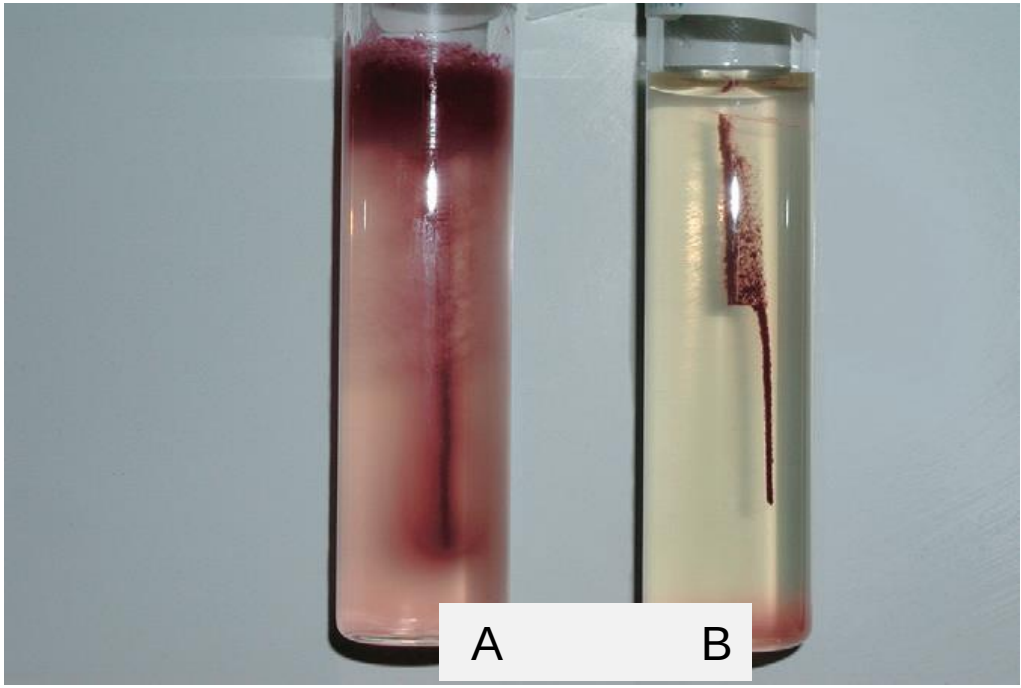
Positive: Motile organisms will spread out into the medium from the site of inoculation

Negative: Non motile organisms remain at the site of inoculation

Quality Control

Positive: *Escherichia coli*

Negative: *Staphylococcus aureus*



Motility test. **A,** Positive. **B,** Negative.

The End

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