

**11****BACTERIAL IDENTIFICATION TESTS****11.1 INTRODUCTION**

In the previous chapter we have discussed various methods of isolation of bacteria. The bacteria thus isolated needs to be further identified to genus and species level. The identification is required so as to cure the illness or the infection caused due to the bacteria by using appropriate antibiotics. Identification also holds significance for epidemiological purposes.

**OBJECTIVES**

After reading this chapter, you will be able to :

- describe the processes involved in the identification of bacteria.
- explain the significance of microscopy in the process of identification of bacteria.
- explain the significance of biochemical test in the process of identification of bacteria.
- describe the significance of serology in the process of identification of bacteria.
- describe the significance of phage typing in the process of identification of bacteria.
- explain the significance of antimicrobial susceptibility testing in the process of identification of bacteria

11.2 BACTERIAL IDENTIFICATION

The isolated bacteria are further processed through one or few of the procedures mentioned below so as to identify the bacteria

- Staining of the isolated bacteria
- Motility testing

Bacterial Identification Tests

- Biochemical testing
- Serological tests
- Phage typing
- Identification disc testing
- Semiautomated and Automated identification systems
- Molecular techniques

(i) Staining of the isolated bacteria

Staining of the bacteria forms the foremost and the most important step in the identification of bacteria. The isolated bacteria are stained by various methods depending upon the bacteria in focus. Various staining techniques are as follow

1. Gram staining: differentiates bacteria into two types
Gram positive and Gram negative bacteria
Gram positive bacteria can be either cocci or bacilli or vibrios. Gram positive pathogenic bacteria are staphylococci, streptococci, pneumococci, etc
Gram negative bacteria can be either cocci or bacilli. Gram negative pathogenic bacteria commonly encountered are E.coli, Klebsiella, Salmonella spp, shigella, etc
2. Albert staining: is performed in case if one suspects a Corynebacterium spp.
3. Acid fast staining: is performed in cases suspected of Mycobacterial infection. Eg. Tuberculosis, leprosy, etc.
4. Special staining is necessary in case of spirochetes and other organisms.



INTEXT QUESTIONS 11.1

1. of bacteria is the important step in identification of bacteria
2. Gram stain differentiates bacteria as &
3. staining is used in Identification of Corynebacterium spp
4. staining is used in identification of Mycobacterial infection
5., & are examples of Gram Positive Bacteria
6., & are examples of Gram Negative Bacteria

MODULE

Microbiology



Notes



Notes

(ii) Motility testing

Motility testing is performed by preparing a wet mount and is then observed under the microscope. Motility of bacteria can also be tested by inoculating the bacteria in the semisolid motility medium.

(iii) Biochemical tests

The staining is followed by use of various biochemical reagents and tests to get closer to the identification of bacteria. There are many biochemical tests available for bacterial identification. Few of them are required to be carried out depending upon the bacteria. The commonly used biochemical tests are as mentioned below

- (a) Catalase test
- (b) Coagulase test
- (c) Oxidase test
- (d) Sugar fermentation test
- (e) Indole test
- (f) Citrate test
- (g) Urease test

(a) Catalase test**Purpose**

The catalase test facilitates the detection of the enzyme catalase in bacteria. It is essential for differentiating catalase-positive *Micrococcaceae* from catalase-negative *Streptococcaceae*. While it is primarily useful in differentiating between genera, it is also valuable in speciation of certain gram positives such as *Aerococcus urinae* (positive) from *Aerococcus viridians* (negative) and gram-negative organisms such as *Campylobacter fetus*, *Campylobacter jejuni*, and *Campylobacter coli* (all positive) from other *Campylobacter* species.

Procedure:

Place a microscope slide inside a petri dish. Keep the petri dish cover available. Using a sterile inoculating loop or wooden applicator stick, collect a small amount of organism from a well-isolated 18- to 24-hour colony and place it onto the microscope slide. Be careful not to pick up any agar. This is particularly important if the colony isolate was grown on agar containing red blood cells. Carryover of red blood cells into the test may result in a false-positive reaction. Using a dropper or Pasteur pipette, place 1 drop of 3% H₂O₂ onto the organism on the microscope slide. Do not mix. Immediately cover the petri dish with a

lid to limit aerosols and observe for immediate bubble formation (O_2 + water = bubbles). Observing for the formation of bubbles against a dark background enhances readability.

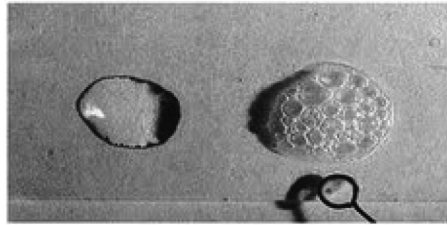


Fig. 11.1

Catalase positive bacteria: *Staphylococcus* spp

Catalase negative bacteria: *Streptococcus* spp

b. Coagulase test

Purpose

The coagulase test differentiates strains of *Staphylococcus aureus* from other coagulase-negative species. *S. aureus* strains are capable of coagulating plasma in the tube test and will produce clumps of cells in the slide test.

The coagulase test can be performed using two different procedures - Slide test and tube test. The slide test is simple, giving results within 10 seconds, but it can give false negatives. The tube test is the definitive test, however, it can take up to 24 hours to complete. For both tests, clumping or clots of any size indicate a positive response. While *S. aureus* is the most commonly isolated coagulase-positive organism, there are several other species of *Staphylococcus* which are positive for coagulase activity. *S. schleiferi* and *S. lugdunensis* may give positive results in the slide test for bound coagulase, and *S. schleiferi* and *S. intermedius* may give positive results in the tube coagulase test .

Procedure:

The slide test is performed by preparing a suspension of bacterial cells mixed into a drop of rabbit plasma on a microscope slide. If bound coagulase is present on the bacterial cells, then the presence of plasma will cause the bacterial cells to clump. The clumping will occur because the clumping factor is an adhesin, which causes the cells to bind to fibrinogen in the plasma. This will result in visible clumping of bacterial cells on the microscope slide. Figure given below illustrates the visible clumping of cells on the microscope slide.



Notes



Notes

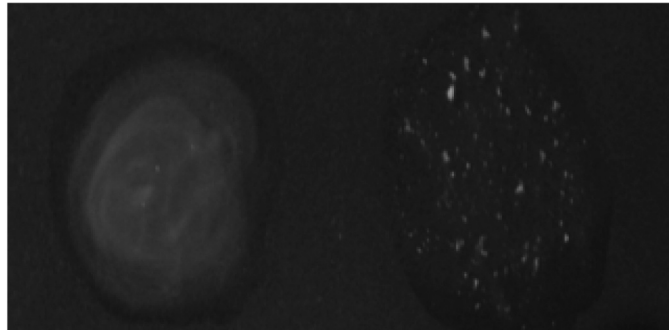


Fig. 11.2: Slide coagulase test.

The tube coagulase test is performed by mixing bacterial cells into a larger volume of plasma in a small test tube. As the bacteria multiply in the plasma, they secrete staphylocoagulase. Staphylocoagulase initiates blood coagulation by activating prothrombin. Staphylocoagulase adheres to fibrinogen, forming a complex that cleaves fibrinogen into fibrin, bypassing the blood clotting cascade and directly causing a clot of fibrin to form. Formation of a clot will be noted within 24 hours for a positive response. Figure shows a negative reaction and a positive reaction.

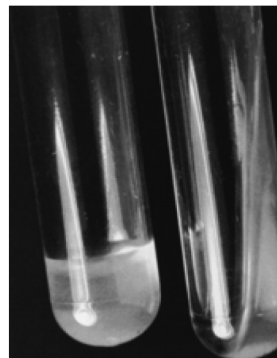


Fig. 11.3: Tube coagulase test.

Coagulase positive bacteria: *Staphylococcus aureus*

Coagulase negative bacteria: *Staphylococcus epidermis*, *Staphylococcus saprophyticus*



INTEXT QUESTIONS 11.2

1. Motility of bacteria can be tested by inoculating the bacteria in medium
2. Catalast test is primarily useful in differentiating between
3. Example of catalase positive bacteria is

4. In coagulast test is formed in slide test and is produced in tube test.
5. is the most common coagulase positive organism

(c) Oxidase test**Purpose**

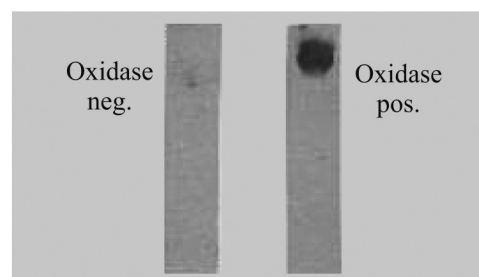
The oxidase test is a biochemical reaction that assays for the presence of cytochrome oxidase, an enzyme sometimes called indophenol oxidase. In the presence of an organism that contains the cytochrome oxidase enzyme, the reduced colorless reagent becomes an oxidized colored product.

Procedure

There are many method variations to the oxidase test. These include, but are not limited to, the filter paper test, filter paper spot test, direct plate method, and test tube method.

Filter Paper Test Method

1. Soak a small piece of filter paper in 1% Kovács oxidase reagent and let dry.
2. Use a loop and pick a well-isolated colony from a fresh (18- to 24-hour culture) bacterial plate and rub onto treated filter paper.
3. Observe for color changes.
4. Microorganisms are oxidase positive when the color changes to dark purple within 5 to 10 seconds. Microorganisms are delayed oxidase positive when the color changes to purple within 60 to 90 seconds. Microorganisms are oxidase negative if the color does not change or it takes longer than 2 minutes.

**Fig. 11.4**

Oxidase positive bacteria : Pseudomonas, Vibrio cholera

Oxidase negative bacteria: E. coli, Klebsiell, Salmonella.

**Notes**



Notes

(d) Indole test**Purpose**

The indole test screens for the ability of an organism to degrade the amino acid tryptophan and produce indole. It is used as part of the IMViC (indole, MR-Vp Citrate) procedures, a battery of tests designed to distinguish among members of the family Enterobacteriaceae.

Procedure

Inoculate the tube of tryptone broth with a small amount of a pure culture. Incubate at 37°C for 24 to 48 hours.

To test for indole production, add 5 drops of Kovác's reagent directly to the tube.

A positive indole test is indicated by the formation of a pink to red color (“cherry-red ring”) in the reagent layer on top of the medium within seconds of adding the reagent.

If a culture is indole negative, the reagent layer will remain yellow or be slightly cloudy.

Indole positive bacteria : *E. coli*, *Vibrio cholera*

Indole negative bacteria : *Klebsiella*, *Salmonella*, *Shigella spp.*

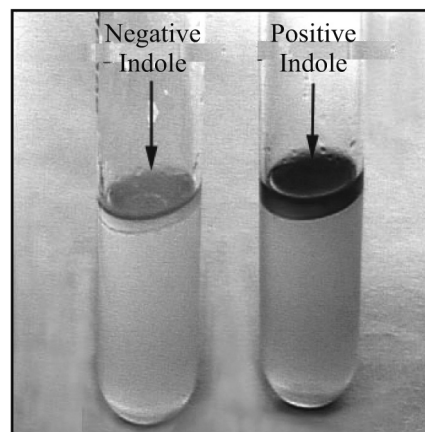


Fig. 11.5

(e) Citrate Test**Purpose**

The citrate test screens a bacterial isolate for the ability to utilize citrate as its carbon and energy source. A positive diagnostic test rests on the generation of alkaline by-products of citrate metabolism. The subsequent increase in the pH of the medium is demonstrated by the color change of a pH indicator.

The citrate test is often part of a battery of tests used to identify gram-negative pathogens and environmental isolates.

Procedure

Use a fresh (16- to 18-hour) pure culture as an inoculation source. Pick a single isolated colony and lightly streak the surface of the slant. A needle is the preferred sampling tool in order to limit the amount of cell material transferred to the agar slant. Avoid using liquid cultures as the inoculum source. Citrate utilization requires oxygen and thus screw caps, if used, should be placed loosely on the tube. Incubate at 35°C (+/- 2°C) for 18 to 48 hours. Some organisms may require up to 7 days of incubation due to their limited rate of growth on citrate medium.

Citrate positive: growth will be visible on the slant surface and the medium will be an intense Prussian blue. The alkaline carbonates and bicarbonates produced as by-products of citrate catabolism raise the pH of the medium to above 7.6, causing the bromothymol blue to change from the original green color to blue.

Citrate negative: trace or no growth will be visible. No color change will occur; the medium will remain the deep forest green color of the uninoculated agar. Only bacteria that can utilize citrate as the sole carbon and energy source will be able to grow on the Simmons citrate medium, thus a citrate-negative test culture will be virtually indistinguishable from an uninoculated slant.

Citrate positive bacteria: *Klebsiella* spp.

Citrate negative bacteria: *E. coli*.

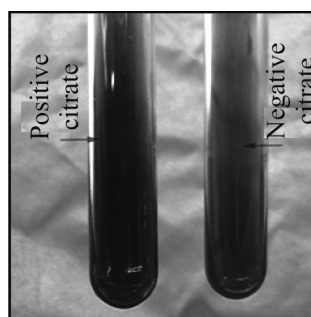


Fig. 11.6

(f) Urease test**Purpose**

The urease test identifies those organisms that are capable of hydrolyzing urea to produce ammonia and carbon dioxide. It is primarily used to distinguish urease-positive bacteria from other *Enterobacteriaceae*.

**Notes**



Notes

Procedure

Christensen's Urea Agar (4, 5)

Use a heavy inoculum from an 18- to 24-hour pure culture to streak the entire slant surface. Do not stab the butt as it will serve as a color control. Incubate tubes with loosened caps at 35°C. Observe the slant for a color change at 6 hours, 24 hours, and every day for up to 6 days. Urease production is indicated by a bright pink (fuchsia) color on the slant that may extend into the butt. Note that any degree of pink is considered a positive reaction. Prolonged incubation may result in a false-positive test due to hydrolysis of proteins in the medium. To eliminate protein hydrolysis as the cause of a positive test, a control medium lacking urea should be used.

Rapidly urease-positive *Proteeae* (*Proteus* spp., *Morganella morganii*, and some *Providencia stuartii* strains) will produce a strong positive reaction within 1 to 6 hours of incubation. Delayed-positive organisms (e.g., *Klebsiella* or *Enterobacter*) will typically produce a weak positive reaction on the slant after 6 hours, but the reaction will intensify and spread to the butt on prolonged incubation (up to 6 days). The culture medium will remain a yellowish color if the organism is urease negative.

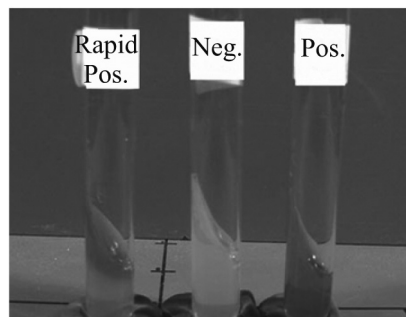


Fig. 11.7

Urease positive bacteria : *Proteus* spp., *Morganella morganii*

Urease negative bacteria : *E. coli*,



INTEXT QUESTIONS 11.3

1. Example of oxidase negative bacteria
2. Positive indole test is indicated by formation of in the reagent layer
3. Indole test is used to distinguish among members of the family
4. Citrate test is commonly used to identify pathogens
5. Example of Urease positive bacteria is

Serology

It forms an important step in bacterial identification. It usually involves detection of antigens by enzyme or fluorescence immunoassays. Serology is also used to confirm identification obtained by other methods. For example, salmonella species identified by biochemical tests is processed for serotyping by slide agglutination. Another example being *Vibrio cholera*.

Phage typing

Phage typing is a method used for detecting single strains of bacteria. It is used to trace the source of outbreaks of infections. The viruses that infect bacteria are called bacteriophages (“phages” for short) and some of these can only infect a single strain of bacteria. These phages are used to identify different strains of bacteria within a single species.

A culture of the strain is grown in the agar and dried. A grid is drawn on the base of the petri dish to mark out different regions. Inoculation of each square of the grid is done by a different phage. The phage drops are allowed to dry and are incubated: The susceptible phage regions will show a circular clearing where the bacteria have been lysed, and this is used in differentiation.

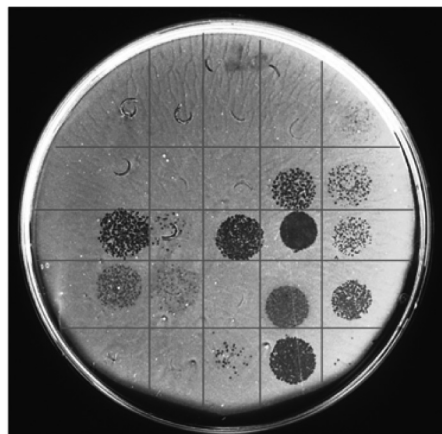


Fig. 11.8

Identification discs

Kirby Baur disc diffusion method is primarily aimed to identify the antibiotic susceptibility of the bacteria. It is also helpful in identification of some bacteria for eg *Micrococci* spp, *Streptococci* spp, *Moraxella* spp, etc

Semiautomated and Automated identification systems

The isolated colonies obtained, are processed by these system. The system identifies the bacteria and also carries out the antibiotic susceptibility testing for



Notes

MODULE

Microbiology

Bacterial Identification Tests



Notes

the same. Microscan walkaway system, Vivtek system, Sensititre Gram-Negative Auto identification system, the Phoenix system are some of the Semiautomated and Automated identification systems available for bacterial identification.

Bactec AFB system, Mycobacteria Growth Indicator Tube (MGIT), and MGIT 960 are some automated identification systems available for Mycobacterial identification.

Molecular techniques

Molecular methods includes G+C % content, DNA-DNA hybridisation and DNA base sequencing. These methods are not used routinely used in hospital laboratories. Amplification techniques like Polymerase chain reaction, ligase chain reaction, strand displacement amplification, and nucleic acid sequence based amplification are being used in clinical laboratories for direct detection of bacteria. Eg. Neisseria gonorrhoea, Leptospirosis, etc.



INTEXT QUESTIONS 11.4

1. Serology involves detection of antigens by or
2. Serology is used in confirmation of &
3. is used for detecting single strains of bacteria
4. Viruses that infect bacteria are called



WHAT HAVE YOU LEARNT

- Techniques like straining of isolated bacteria, motility testing, Biochemical testing, Serological tests, Phage typing, identification disc testing, Semiautomated and Automated identification system & Molecular techniques are used for bacterial identification.
- Various staining techniques like Gram stain, Albert stain, Acid fast Stain & Special Staining are used for bacterial identification
- Catalase test, coagulase test, Oxidase test, Sugar fermentation test, Indole test, Citrate Test, Urease test are the Biochemical tests used for bacterial identification
- Serology tests like Enzyme or Fluorescence immunoassays are used to confirm identification obtained by other methods

Bacterial Identification Tests

- Phage typing is used for detecting single strains of bacteria and also to trace the source of outbreaks of infections
- Kirby bayer disc diffusion method is used to identify antibiotic susceptibility of bacteria
- Semiautomated & automated identification systems identify bacteria and also carry out antibiotic susceptibility testing.



TERMINAL QUESTIONS

1. Describe in brief the various staining techniques
2. Enlist biochemical test performed for identification of bacteria
3. Describe in brief the role of serology in identification of bacteria
4. What do you understand by the term phage typing. Explain
5. Explain the role of antimicrobial susceptibility testing in identification of bacteria with suitable examples.
6. Name the molecular techniques used for the identification of bacteria.



ANSWERS TO INTEXT QUESTIONS

11.2

1. Staining
2. Gram Positive and Gram Negative
3. Albert
4. Acid fast
5. Staphylococci, Streptococci & Pneumococchia
6. Ecoli, Klebsiella & Salmonella

11.2

1. Semisolid motility
2. Genera
3. Staphylococci
4. Clumping & clots
5. Staphylococcus aureus

MODULE

Microbiology



Notes

MODULE

Microbiology

Bacterial Identification Tests



Notes

11.3

1. E.coli
2. Cherry-red ring
3. Enterobacteriaceae
4. Gram negative
5. Proteus spp

11.4

1. Enzyme, Fluorescence
2. Salmonella & Vibrio Cholera
3. Phage typing
4. Bacteriophages