Practical Pathogenic Bacteria

LAB: 1

Bacterial Identification (Gram Positive Bacteria)

Methods of Bacterial Identification

1-Microscopic examination.

2-Cultural appearance.

3-Biochemical reactions.

4-Serological identification.

5-Molecular methods.

1- Microbial Causes of Infection:

- Bacteria, viruses, fungi, and parasites.
- The pathogen may be exogenous (acquired from environmental or animal sources or from other people) or endogenous (from the microbiota).

2- Specimen Selection, Collection, and Processing:

- The quantity of specimens must be adequate. Specimens are selected on the basis of symptoms, should be representative of the disease.

- Contamination of the specimen must be avoided by using a sterile equipment and aseptic precautions.

- The specimen must be taken to the laboratory and examined promptly. Special transport media may be helpful. Reliable specimens must be secured for diagnosis of the bacterial infection before antimicrobial drugs are administered.

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3- Microscopic Examination:

- Gram staining is a differential staining technique that differentiates bacteria on their ability to retain color.

- Gram stain procedure. This staining method will differentiate gram-positive (purple) bacteria from gram-negative (red) bacteria.

- It is based on the composition of the bacterial cell wall of both Gram-negative and Gram-positive bacteria. The mechanism of Gram staining in the decolorizing step acts differently in both bacteria resulting in two different colors for identification.

- Gram-positive bacteria: *Staphylococcus* and *Streptococcus*.

Gram-negative bacteria: *E. coli* and *Klebsiella*.

- Microbial morphology and arrangement can be observed. For example, Staphylococci are Gram-positive cocci which are arranged in clusters (grapes)

4- Microbiological Culture Examination (Media Selection):

- It is based on the specimen source (urine, blood, etc.).

- The appropriate bacteriological media are selected to grow the organism for further work-up.

- Selection of the appropriate temperature and incubation conditions (aerobic versus anaerobic) allow the optimal bacterial growth.

-Colony morphological characteristics on growth media. For example, size, color, odor and the ability to lyse (break apart) blood cells are important for bacterial identification.

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- Nonselective media permit the growth of many microorganisms.

- Selective media contain inhibitory substances that permit the isolation of specific types of microorganisms.

5- Biochemical Identification:

- The ability of a bacterial species to use a sugar, an amino acid or an enzymatic substrate is very useful for bacterial identification.

- These tests can be used individually (coagulase for *Staphylococcus aureus*) to identify an organism, or in a set of tests to identify Gram-negative bacilli.

- Many commercially prepared kits are available to identify bacteria using biochemical and enzymatic tests.

6- Serologic Methods:

- These methods typically involve testing an unknown antibody against a known antigen bound to a latex particle or similar structure.

- After mixing the antigen and antibody together and rotating, a visible agglutination (clumping) will appear if positive for the organism tested.

7- Molecular Methods

- These methods are the latest and most specific methods available in the laboratory.

- These tests are based on the ability to detect, identify and characterize microorganisms based on their DNA or RNA.

- Polymerase chain reaction (PCR) is a common molecular method used to identify bacteria.

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8- Antimicrobial Susceptibility Examination:

- **Microorganisms, particularly bacteria, are tested *in vitro* to determine whether they are susceptible to antimicrobial agents.**

Biochemical tests for the identification of Gram-positive bacterial species

1. **Catalase test**
2. **Oxidative/Fermentative**
3. **Bacitracin susceptibility test**
4. **Bile Esculin test**
5. **Hippocrates hydrolysis test**
6. **Coagulase test**

1- Catalase Test

- It determines the ability of bacteria to produce the catalase enzyme which forms gas bubbles when reacting with 3% H2O2.

- Catalase mediates the breakdown of hydrogen peroxide (H_2O_2) into oxygen and water.

Principle:

$2H_2O_2 \rightarrow 2H_2O + O_2$ (gas bubbles)

- A small inoculum of a bacterial isolate is mixed into hydrogen peroxide solution (3%). It is observed for the rapid elaboration of

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oxygen bubbles. The lack of catalase is evident by a lack of or weak bubble production.

- Catalase producing Gram-positive bacteria:

Staphylococcus* spp *and Micrococcus spp

- Catalase-positive bacteria include strict aerobes as well as facultative anaerobes.

-Catalase-negative bacteria may be anaerobes or facultative anaerobes (i.e., Streptococci).

Percentage of H2O2 used in catalase test

Percentage	Purpose
3% H_2O_2	Routine testing of aerobes
15% H_2O_2	Identification of anaerobic bacteria
30% H_2O_2	*Neisseria* spp

Procedure:

-Slide Test

1. **Transfer a small amount of bacterial colony to a surface of a clean, dry glass slide using a loop or sterile wooden stick (be sure the colony is visible to the naked eye on the slide).**
2. **Place a drop of 3% H_2O_2 onto the slide and mix.**
3. **A positive result is the rapid evolution of oxygen (within 5-10 seconds), as evidenced by bubbling.**

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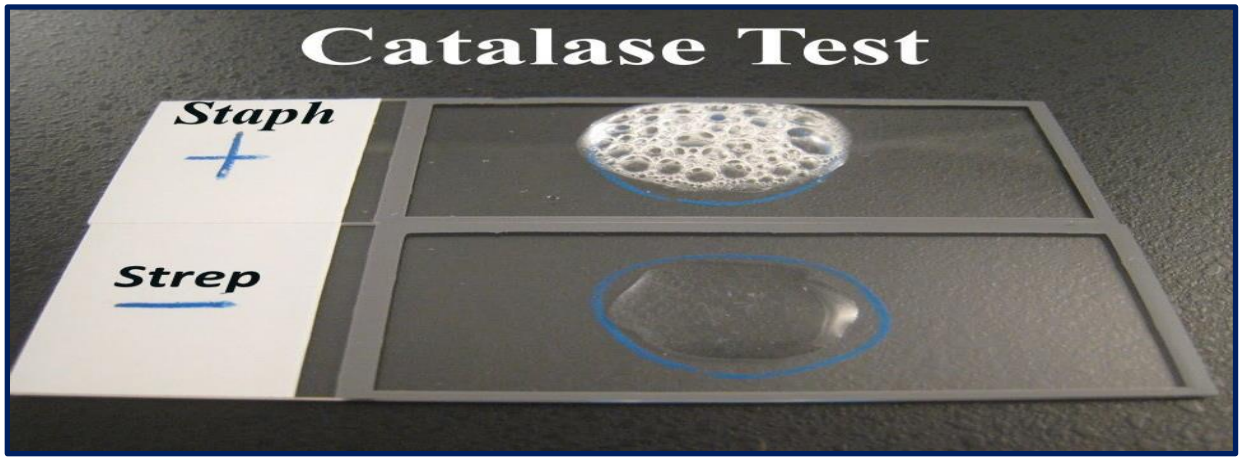
4. **A negative result is no bubbles or only a few scattered bubbles.**
5. **Dispose of your slide in the biohazard glass disposal container.**



-Tube Test

1. **Add 4 to 5 drops of 3% H_2O_2 to a test tube**
2. **Using a wooden applicator stick, collect a small amount of organism from a well-isolated 18 to 24-hour colony and place it into the test tube *(Note: Be careful not to pick up any agar* (especially if using Blood Agar).**
3. **Place the tube against a dark background and observe for immediate bubble formation (O2 + water = bubbles) at the end of the wooden applicator stick.**

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2-Oxidase Test

Principle:

-This test is performed to determine or identify the presence of an enzyme cytochrome oxidase (of the electron transport chain) in bacterial cells. This enzyme sometimes called indophenol oxidase.

-The reagent used is tetramethyl-p-phenylene diamine dihydrochloride, which is oxidized to a purple-colored end product called indophenol by the enzyme oxidase.

- The development of a dark purple color is a positive test that indicates the presence of oxidase, whereas if the enzyme is not present, the reagent remains reduced and is colorless.

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Procedure:

1- Take a filter paper and moisturize it with the substrate i.e. 1% tetramethyl-p-phenylene diamine dihydrochloride or select a commercially available paper disk that has been saturated with the same substrate.

2- Remove a small portion of a bacterial colony (preferably not more than 24 hours old) from the agar surface with a sterile platinum wire or wooden stick.

3- Rub the sample on the filter paper or commercial disks.

4- Observe the inoculated area of the paper of disks for the color change to deep blue or purple within 10 seconds because timing is very critical.

***Positive result: Development of a dark purple color within 10 seconds of inoculation.**

***Neisseria gonorrhoeae, Vibrio cholera, Pseudomonas* are oxidase positive.**

***Negative result: No change in color (no blue color seen)**

Members of family Enterobacteriaceae like *E. coli* are oxidase negative.

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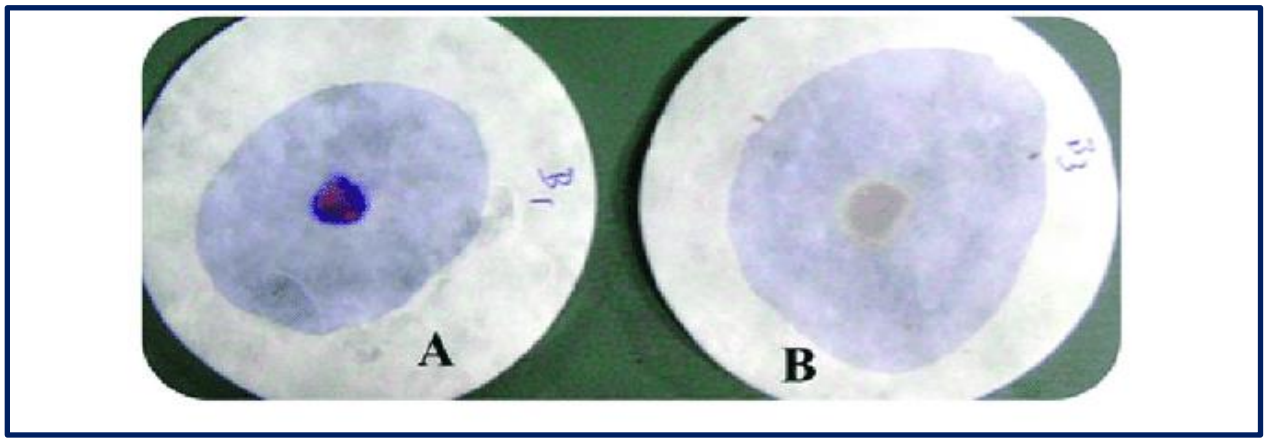

3- Coagulase Test

- The coagulase test is one way to differentiate the highly pathogenic *S. aureus* (coagulase-positive) from the other less pathogenic staphylococcal species on the human body (coagulase-negative Staphylococcus (CONS)).

- *S. aureus* produces a bound and free form of coagulase that converts soluble fibrinogen into insoluble fibrin.

- Coagulase test is done either in a slide or in a tube which is determined by the form of coagulase produced.

- Cell bound coagulase is detected by the slide coagulase test which forms agglutination in case of positive results.

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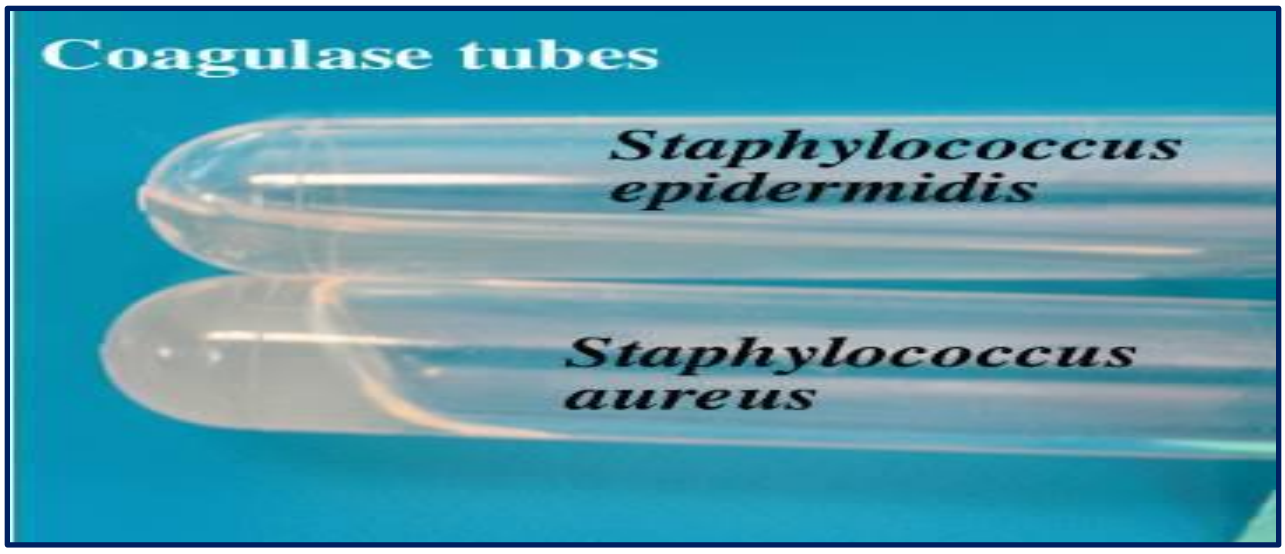
- **Free coagulase is detected in a tube which forms a clot if the organism is tested positive.**
- **Coagulase producing Gram-positive organism: *Staphylococcus aureus.***



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4- Bile Esculin Test:

- The bile-esculin test is widely used to differentiate enterococci and group D streptococci (which are bile tolerant and can hydrolyze esculin to esculetin) from non-group D viridans group streptococci (which grow poorly on bile).

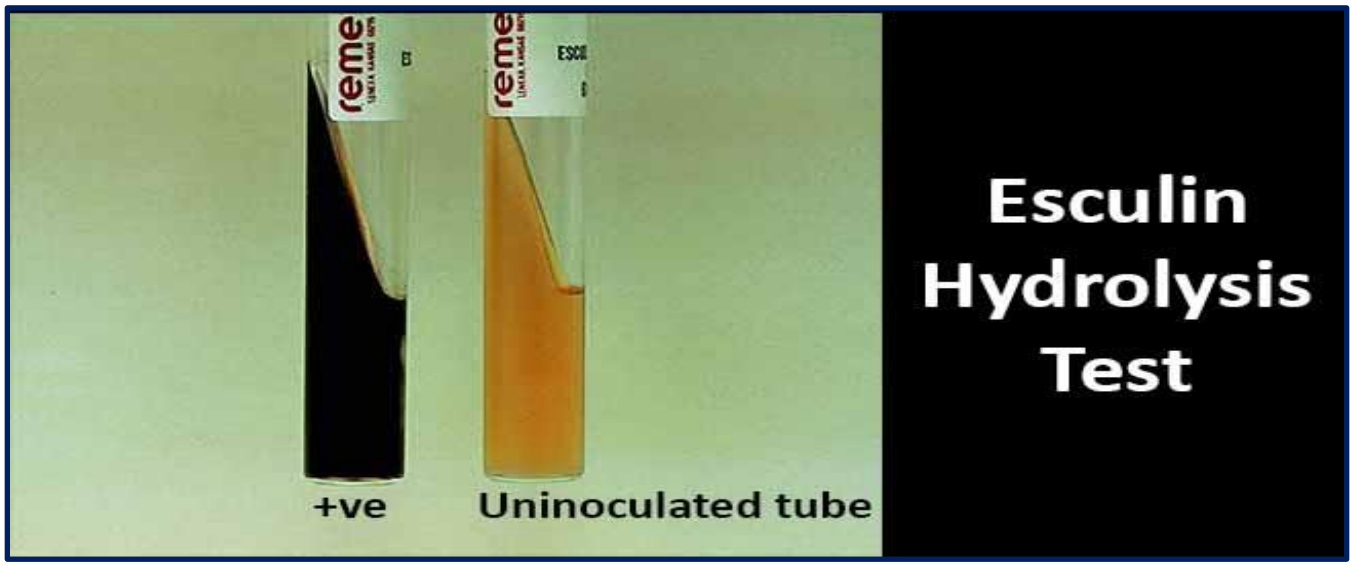
- Bile esculin agar medium is both selective as well as the differential medium in which its selective ingredient is bile that inhibits the growth of other Gram-positive bacteria except enterococci and some streptococci species.

- Esculin is the differential ingredient that differentiates *Enterococcus* from *Streptococcus*.

- The bile esculin test determines the ability of bacteria to hydrolyze esculin when in the presence of bile salt, esculin is formed.

- Ferric citrate is present in the medium and when it reacts with esculin, it turns the entire medium dark brown to black due to the formation of the phenolic iron complex.

- Bile esculin Gram-positive organism: *Enterococcus faecalis*



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5- Bacitracin Susceptibility Test

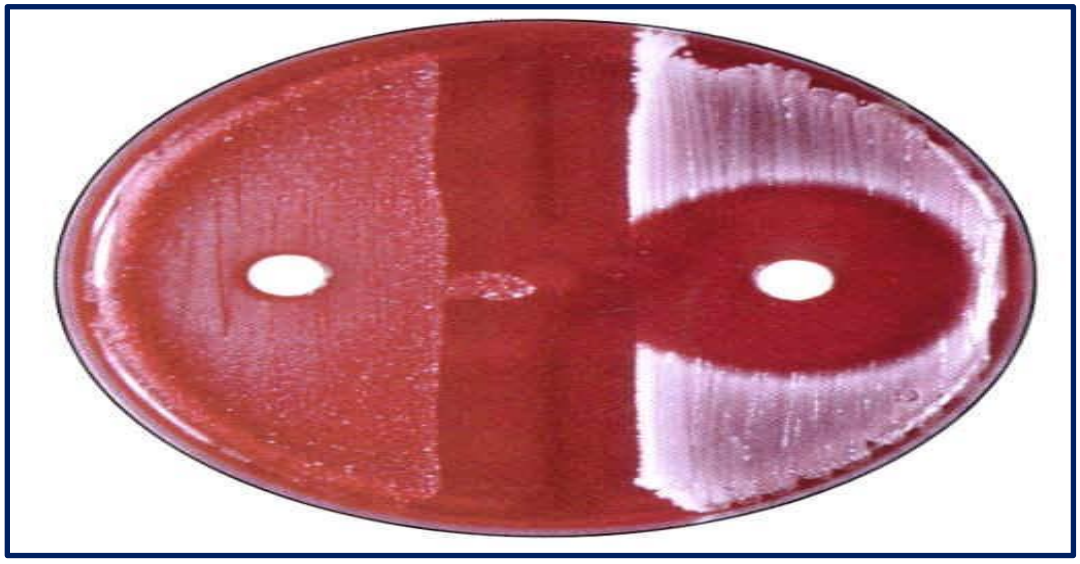
- Bacitracin is antibiotic produced by group of *Bacillus subtilis.*

- The Bacitracin test is used to distinguish β-hemolytic streptococci. It can differentiate between beta-hemolytic Group A streptococci *Streptococcus pyogenes*, which forms a zone of inhibition around the bacitracin disc (sensitive) (Positive result), and beta-hemolytic non-Group A streptococci *Streptococcus agalactiae*, which grows around the disc (resistant) (negative result).

- This test is performed on the blood agar with a streaked culture of streptococci where the bacitracin disk is impregnated with sterile forceps before incubation.

- Bacitracin sensitive Group A streptococci: *Streptococcus pyogenes.*

Bacitracin resistant non-Group A streptococci: *Streptococcus agalactiae.*



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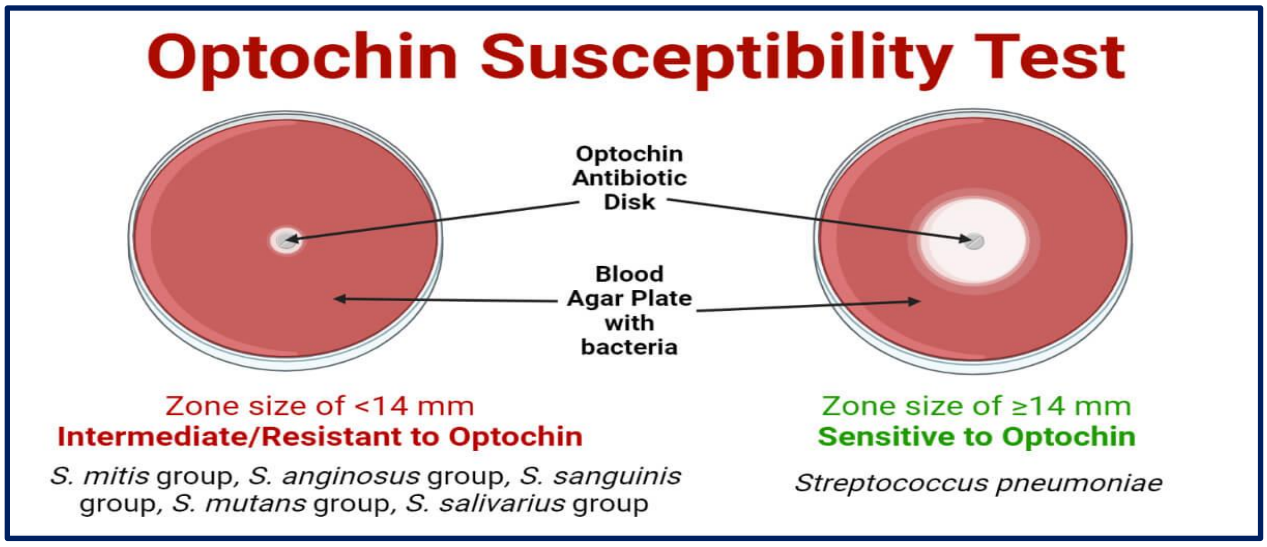
6- Optochin Susceptibility Test:

- It is a useful test for the identification of *Streptococcus pneumoniae*.

- Alpha-hemolytic *Streptococcus* is the most commonly susceptible bacteria for this test. Other alpha-hemolytic streptococcal species (viridans streptococci) are optochin-resistant and do not display this clear zone of inhibition when in the presence of optochin.

- A positive presumptive identification of *S. pneumoniae* is made when a well-defined zone of inhibition results around the saturated disk.



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Procedure:

1. **Using an inoculating loop, select three to four well-isolated colonies of the alpha-hemolytic organism to be tested. An 18–24 hour culture of isolated organism can also be used for testing.**

2. **Streak the isolate onto one-half of a TSA-5% sheep blood agar plate so as to obtain confluent growth.**
   Note: Use of media other than TSA-5% sheep blood agar is not recommended, as false identification may result.

3. **Using sterile forceps, place an optochin disk onto the inoculated surface of the agar.**

4. **Press disk gently with the sterile forceps or loop so that the disk adheres firmly to the agar surface.**

5. **Incubate the plate at 35 +/- 2.0 degrees C. for 18-24 hours in 5-10% CO2 enriched environment.**

6. **If zone of inhibition is present, measure the diameter with a millimeter ruler.**

Positive result: Zone of inhibition is 14 mm or greater in diameter with 6 mm disk.

Negative result: No zone of inhibition or a zone of inhibition of <14mm diameter.

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7- Motility Test

Procedure

1- Touch a straight needle to a colony of a recent culture (18- to 24 hour) growing on agar medium.

2- Stab once to a depth of only 1/3 to 1/2 inch in the middle of the tube.

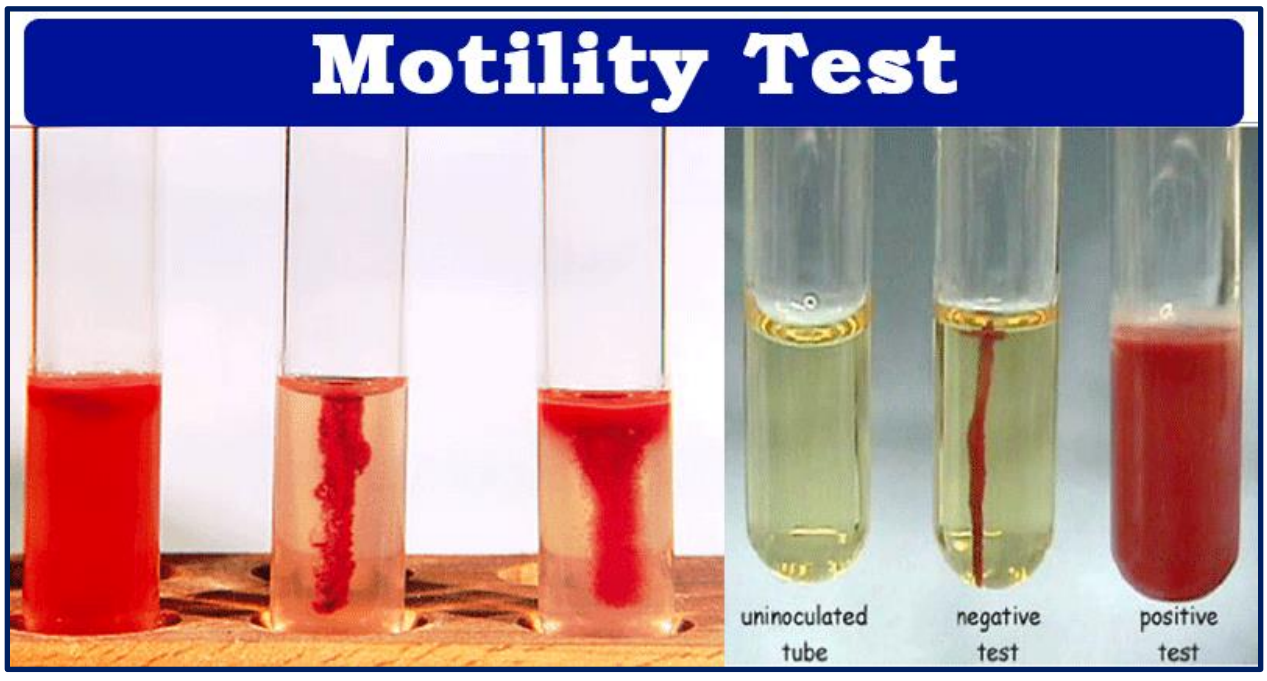
3- Incubate at 35°-37°C and examine daily for up to 7 days.

4- Observe for a diffuse zone of growth spreading out from the line of inoculation.

- Semi solid medium 0.7-0.8% agar is used to perform this test.

- Motile Bacteria: *Escherichia coli, Helicobacter pylori, Pseudomonas aeruginosa.*

- None Motile Bacteria: *Klebsiella, Shigella*, *Staphylococcus*



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Laboratory Speciemens

1- Blood : Septicemia.

2- Urine:Urinary tract infections.

3- Stool: Gastrointestinal infections.

4- Sputum: Respiratory infection.

5- Vaginal swabs: Vaginal infections.

6- Nose and ear swabs : Nose and ear infections.

7- Cerebral spinal fluid :CNS infections.

8- Food and vomit : Food poisoning.

9- Pus : Acne , burns , wounds.

10-Seminal fluid :Urethral discharge.