ISOLATION OF BACTERIA IN PURE CULTURE:

Pure cultures are essential to the accurate determination of colony characteristics, biochemical properties, morphology, staining reaction, immunologic reactions, and suseptibility to antimicrobial agents.

Microorganisms are ubiquitous; therefore, aseptic techniques must be used during collection of specimens and work withculture media etc.

The streak – plate method, if properly performed, is probably the most practical and most useful for obtaining discrete colonies and pure cultures. The streak-plate method consists of the spreading of a bacterial suspensions over an agar surface in a definite pattern to separate single cells or small clumps of cells from the culture so that isolated colonies will grow during incubation

MATERIALS

A mixture of broth cultures of Staph.albus and Esch.coli
1 CLED agar plate

PROCEDURE:

Watch how culture aseptic techniques and streak-plate technique will be illustrated in the lab

1st day:

A) CLED agar plate will be used in the first period. Streak the plate and incubate it.

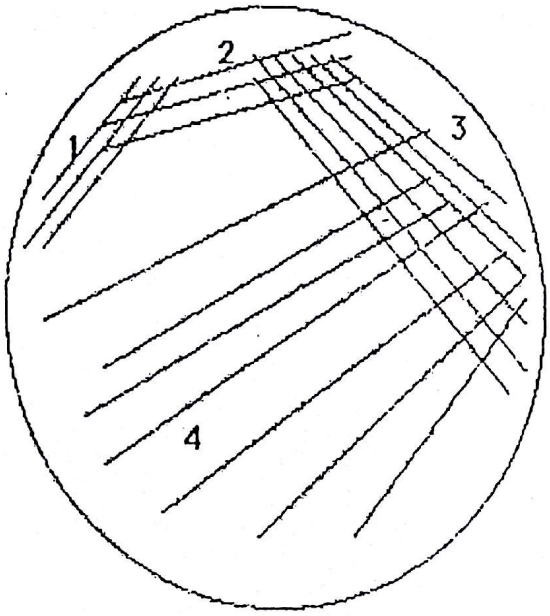
B) At the next lab period, examine your streak plate and look for well isolated colonies of both species.

C) At the next lab. period prepare a gram-stained smear for microscopic examination. Did you isolate a pure culture? (Staph. or E.coli).

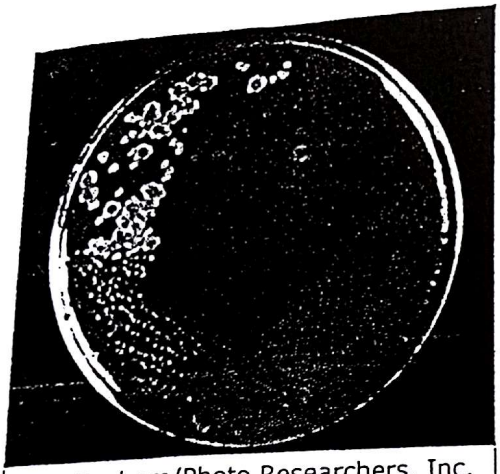
Nahe

. Transfer to Agar Plates (Quadrant streak Plate)

1. Spend the organisms in the broth culture or use directly from a slant. Flame the loop and wire until it is red hot. Remove the cap from the culture tube and flame the mouth of the tube. Do not contaminate the cap or the loop during this procedure. Remove a loopful of organisms. Flame the mouth again and replace the cap on the tube.
2. Spread the organism over a small region on the edge of the plate as in 1 in the diagram below.



3. Flame the loop and let it cool for a few seconds.
4. Streak from the end of region 1 across the edge of the plate forming region 2.
5. Flame the loop and let it cool for a few seconds.
6. Streak from the end of region 2 across a quarter of the plate forming region 3.
7. Flame the loop and let it cool for a few seconds.
8. Streak from region 3 across the remaining portion of the plate forming region 4.
9. Flame the loop before setting it down.
10. Incubate the plate for 24-48 hours in an inverted position.



John Durham/Photo Researchers, Inc.

Specimen, Collection, Processing, and Testing

Proper Collection of Specimens:

1. All specimens should be collected in a sterile manner. This requires that they be put into a sterile container to prevent contamination by normal flora and airborne organisms.
2. The material should be collected from the site where the suspected organism is most likely to be found and where the least contamination is likely to occur.
3. Specimens should be obtained before antibiotic therapy has begun. If this is not possible, the laboratory should be informed of which antibiotic is being used.
4. The acute stage of the disease is the appropriate time for the collection of most specimens. Some viruses, however, are more easily isolated during the onset stage of the disease.
5. Specimen collection should be performed with care and tact to avoid harming the patient, causing discomfort , or causing undue embarrassment . If the specimen , such as sputum or urine , is to be collected by the patient , clear and detailed instructions should be given.
6. A sufficient quantity of the specimen should be obtained to allow enough for all diagnostic tests that need to be performed. The amount should be indicated by the physician or laboratory microbiologist.
7. Specimens should be protected from heat and cold, and promptly delivered to the laboratory so the results of the analysis will be a valid representation of the organisms present at the time of collection. If delivery is delayed, some delicate pathogens will die. Anaerobes will die when exposed to the air. Also , the normal flora may overgrow the pathogens, which will inhibit or kill them. Delay of delivery considerably decreases the chances of isolating the pathogen.
8. Dangerous specimens must be handled with even geater care to avoid contamination of the ward messenger, patients , nurses, and other hospital personnel. Such dangerous specimens are usually placed in a sealed plastic bag for immediate transport to the laboratory.
9. All specimens containers must be cleaned , sterilized, and properly stored to avoid contamination of the specimen by microbes and harmful chemicals from the container.

10. After the specimen is collected, the container must be properly labeled and accompanied by the appropriate laboratory instructions written on the requisition .The label must identify the patient and the source of the specimen (*e.g.*, throat , wound).The requisition must give the date, time of collection , doctors name and address, and laboratory tests requested .The laboratory also should be given any additional clinical information that will aid in performing the appropriate analysis.

11. Specimens should be collected and delivered to the laboratory as early in the day as possible to allow the technicians time to process the material, especially if the hospital or clinic does not have 24-hour laboratory service.

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THROAT SWAB FOR CULTURE

METHOD:

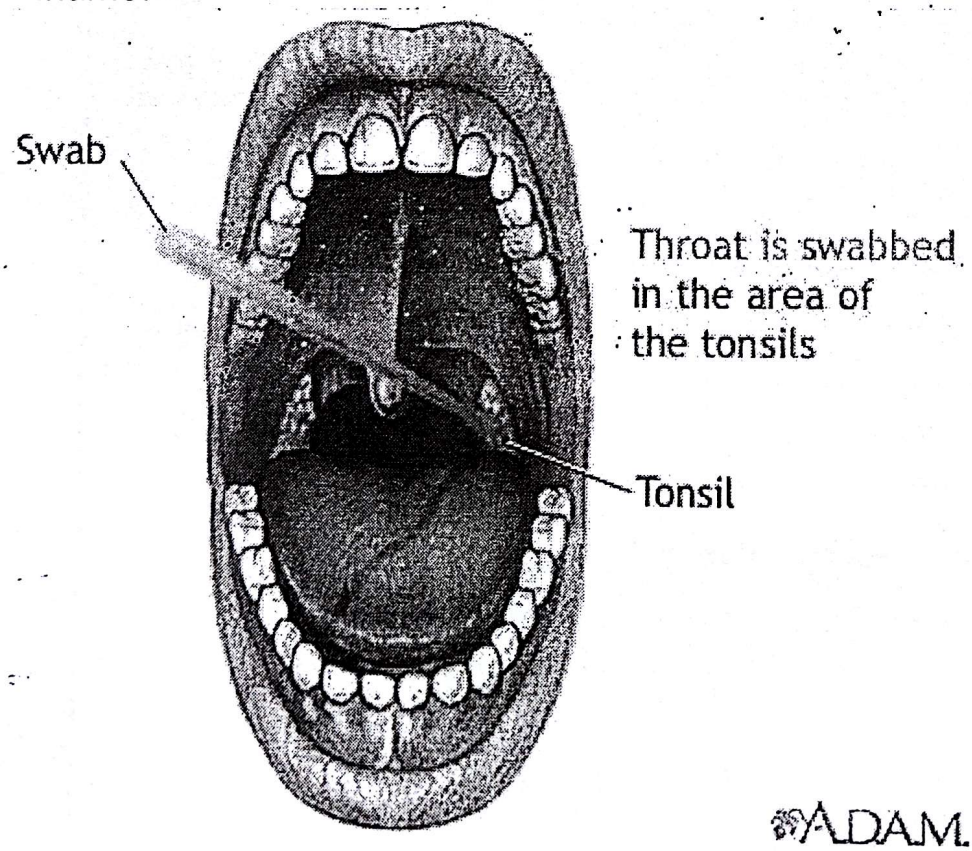
1. With the patient's head tilted back and the throat well illuminated, depress the tongue so that the back of the throat can be seen.

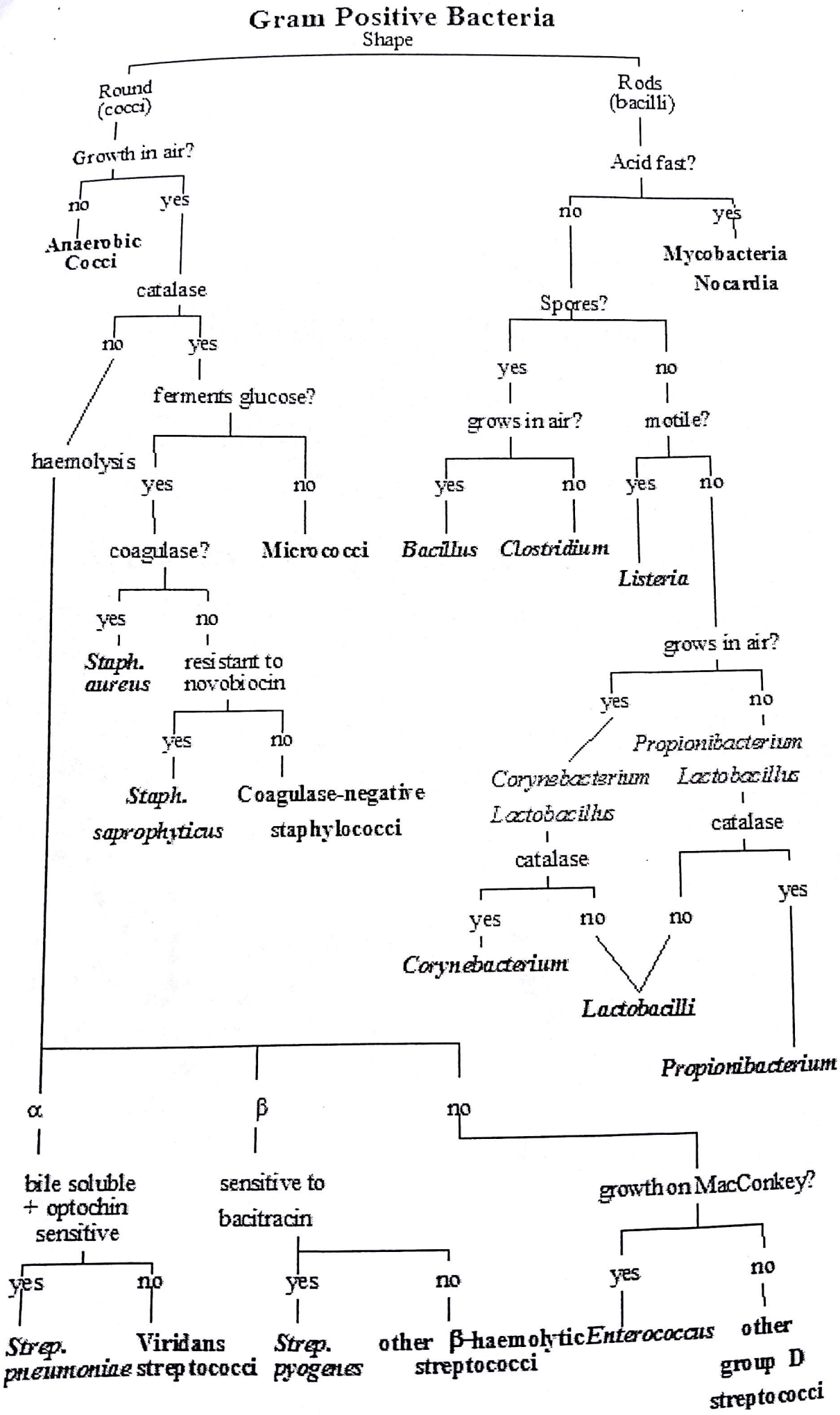
2. Rub the swab up and down the back of the throat and against any white patches in the tonsillar area. Avoid the tongue and the cheeks.

3. Replace the swab in the transport tube. If using the Virus/Chlamydia/ Mycoplasma Collection Kit swab, break off or bend swab shaft, leaving tip in tube.

4. Seal tube tightly and label with patient name, date and initials of collector.

5. Send the specimen to the laboratory with a completed on-line test order or with a test request form that indicates patient name, account number, medical record number, source, collection date and time, tests ordered, ordering physician's name.





Identification Flow Chart

Gram stain

↓

Gram-positive cocci

↓

Catalase Test

\+ ← → -

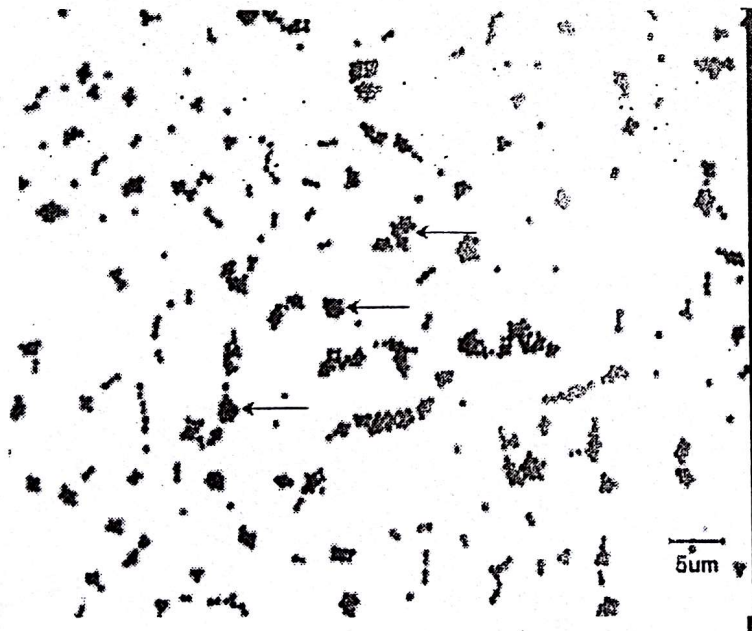
Staphylococcus Streptococcus

↓

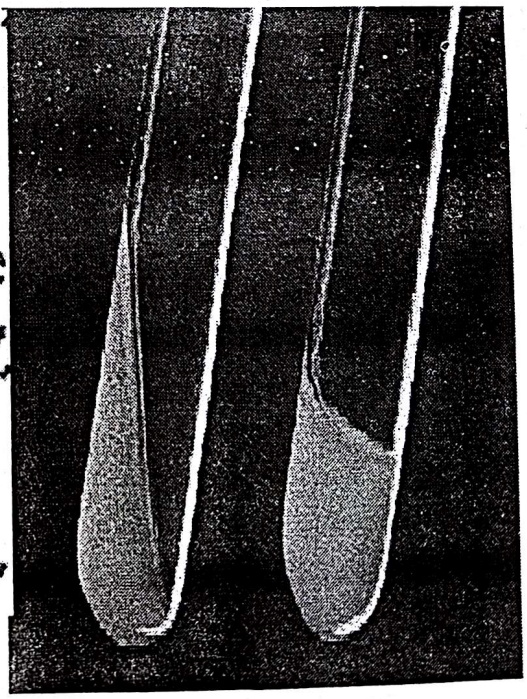
Coagulase test

\+ ← → -

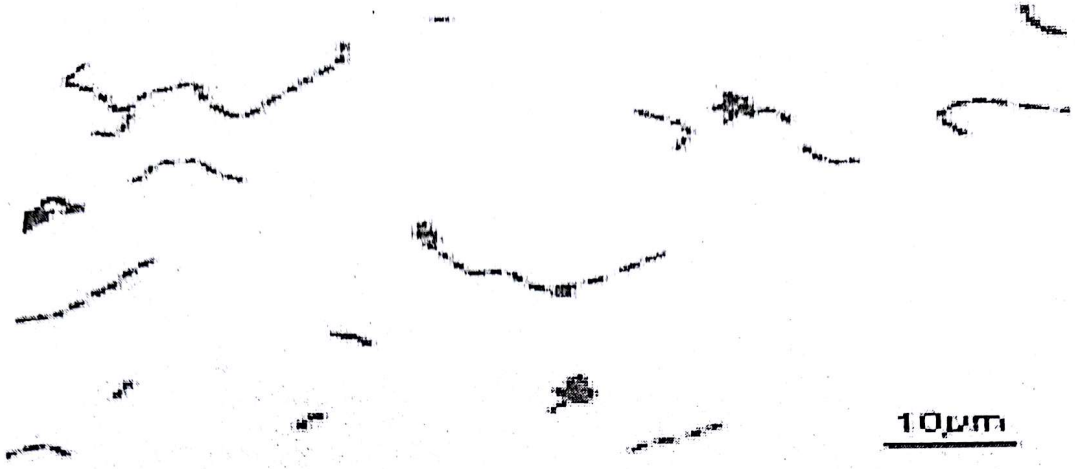
S. aureus ***S. epidermidis***



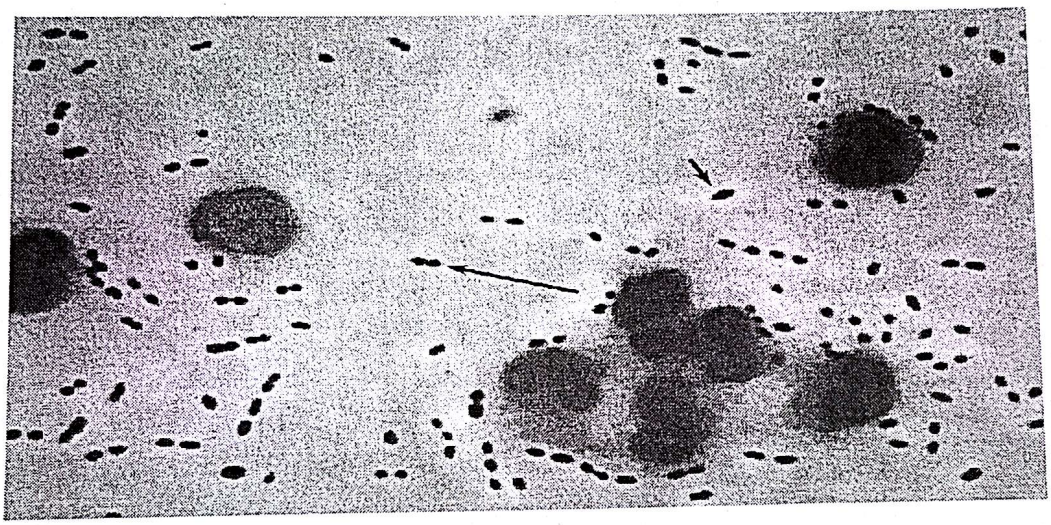
Staphylococci gram stain



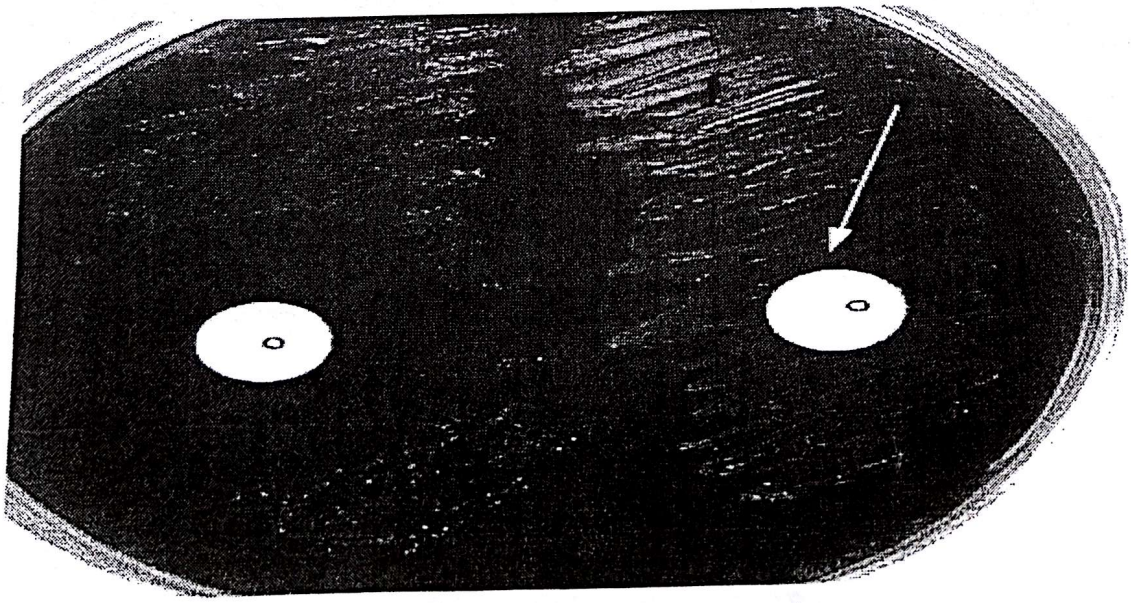
coagulase test



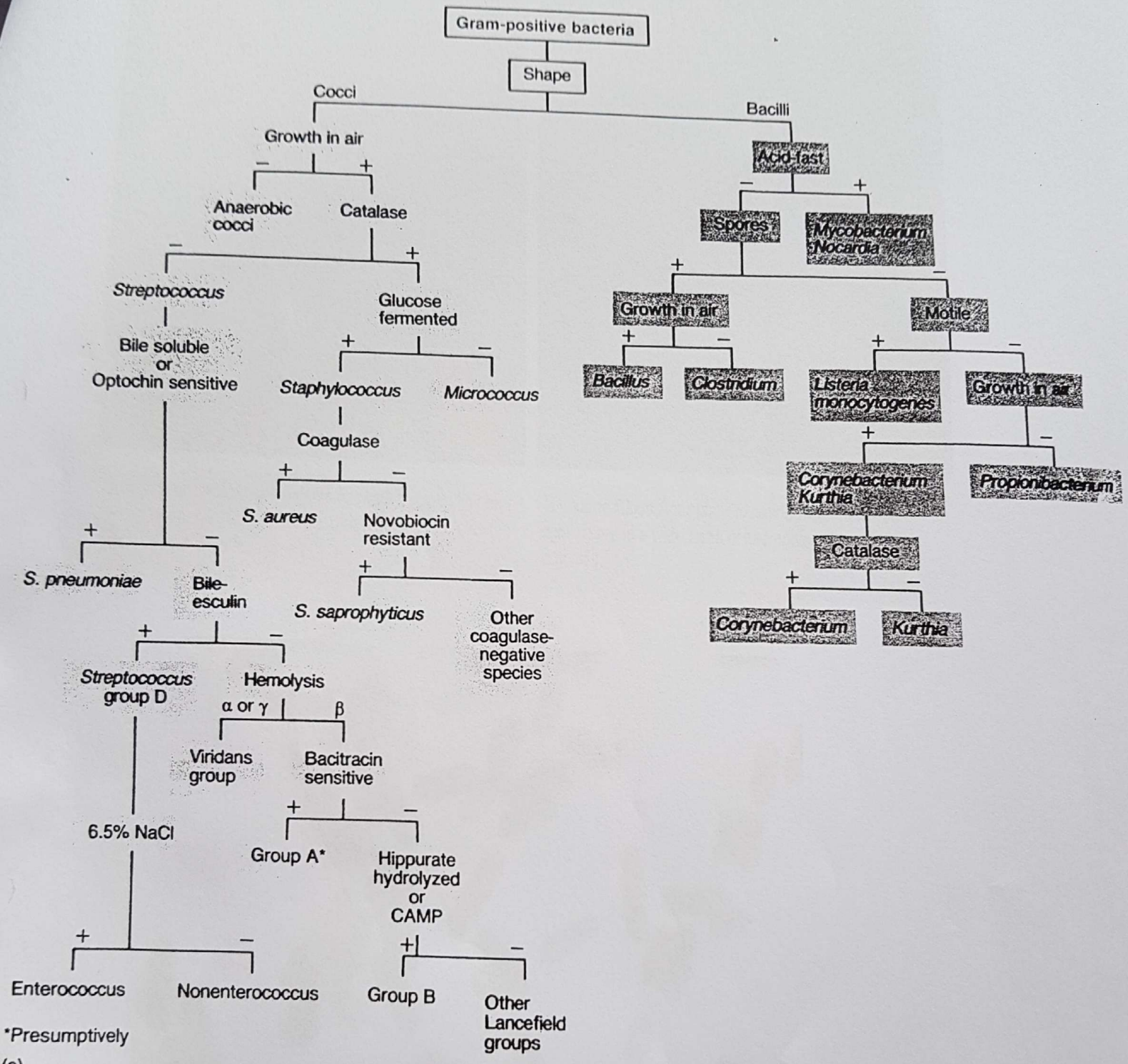
β-hemolytic streptococci



Streptococcus pneumonia



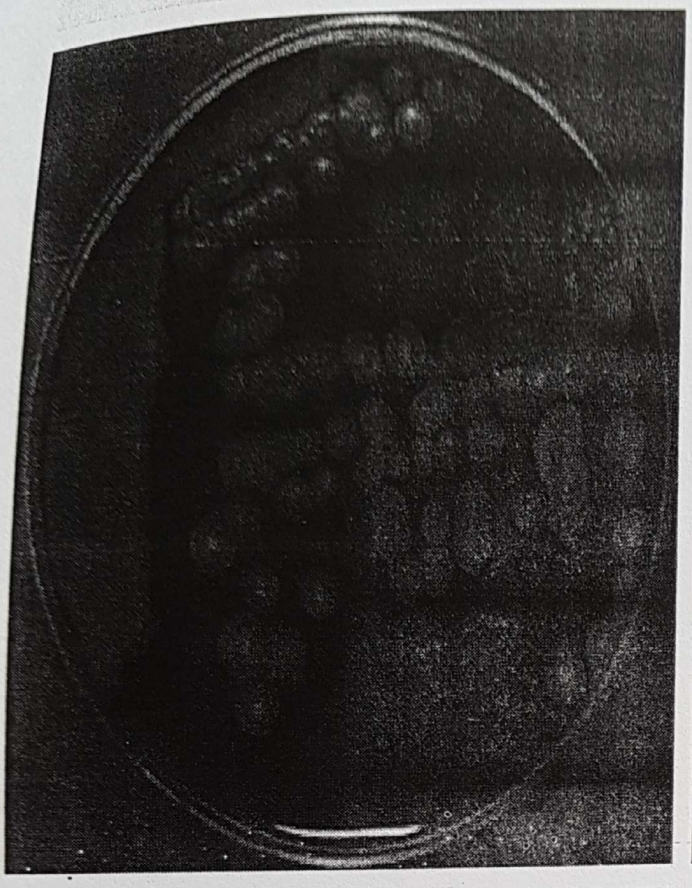
Optochin susceptibility test for *Streptococcus pneumonia*



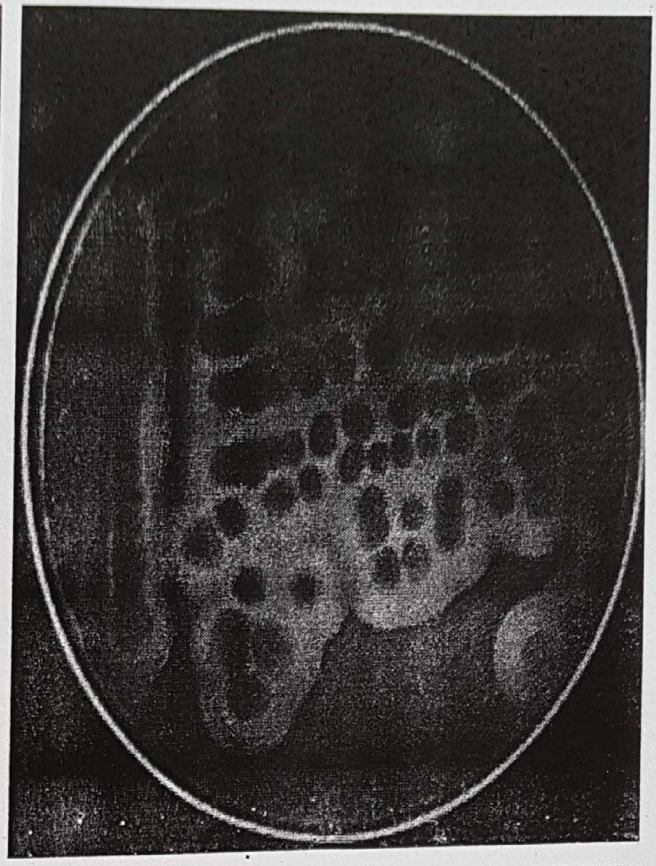
(a)

Identification of Gram-Positive and Gram-Negative Bacteria
Figure 33.4a

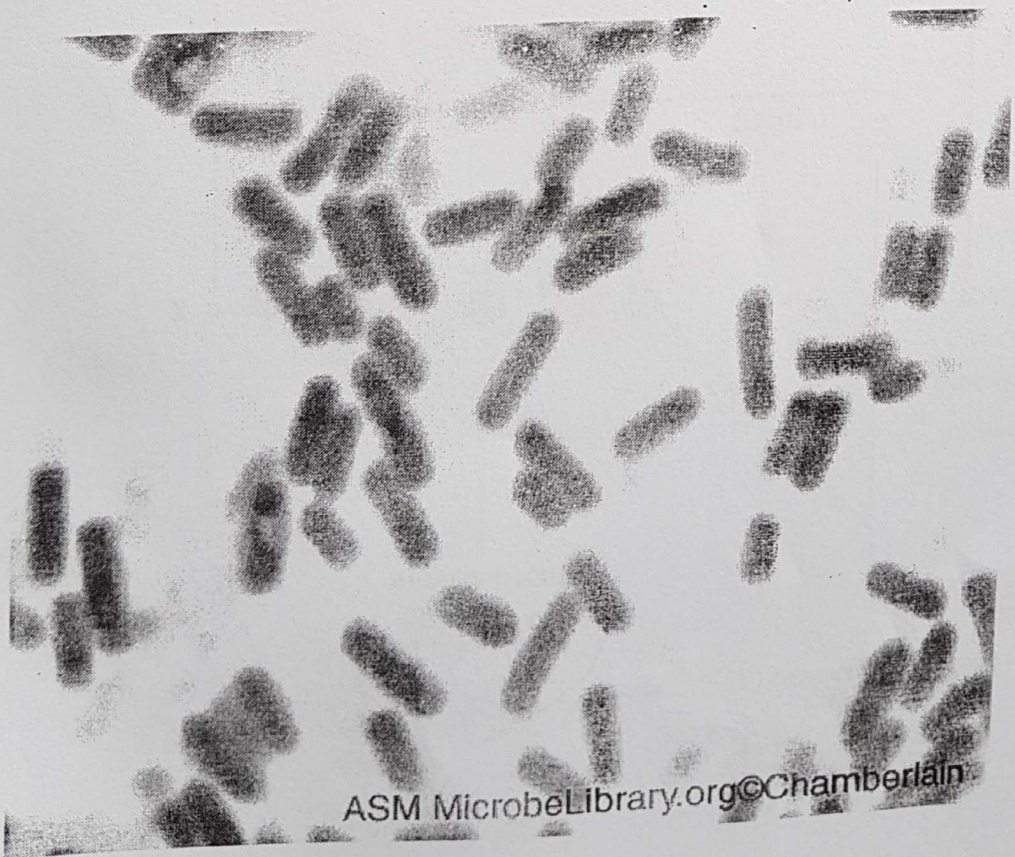
Bacillus cereus



The form of *Bacillus cereus* colonies varies depending on strain. Generally large colonies with a dull or frost-glass surface and undulate margin.



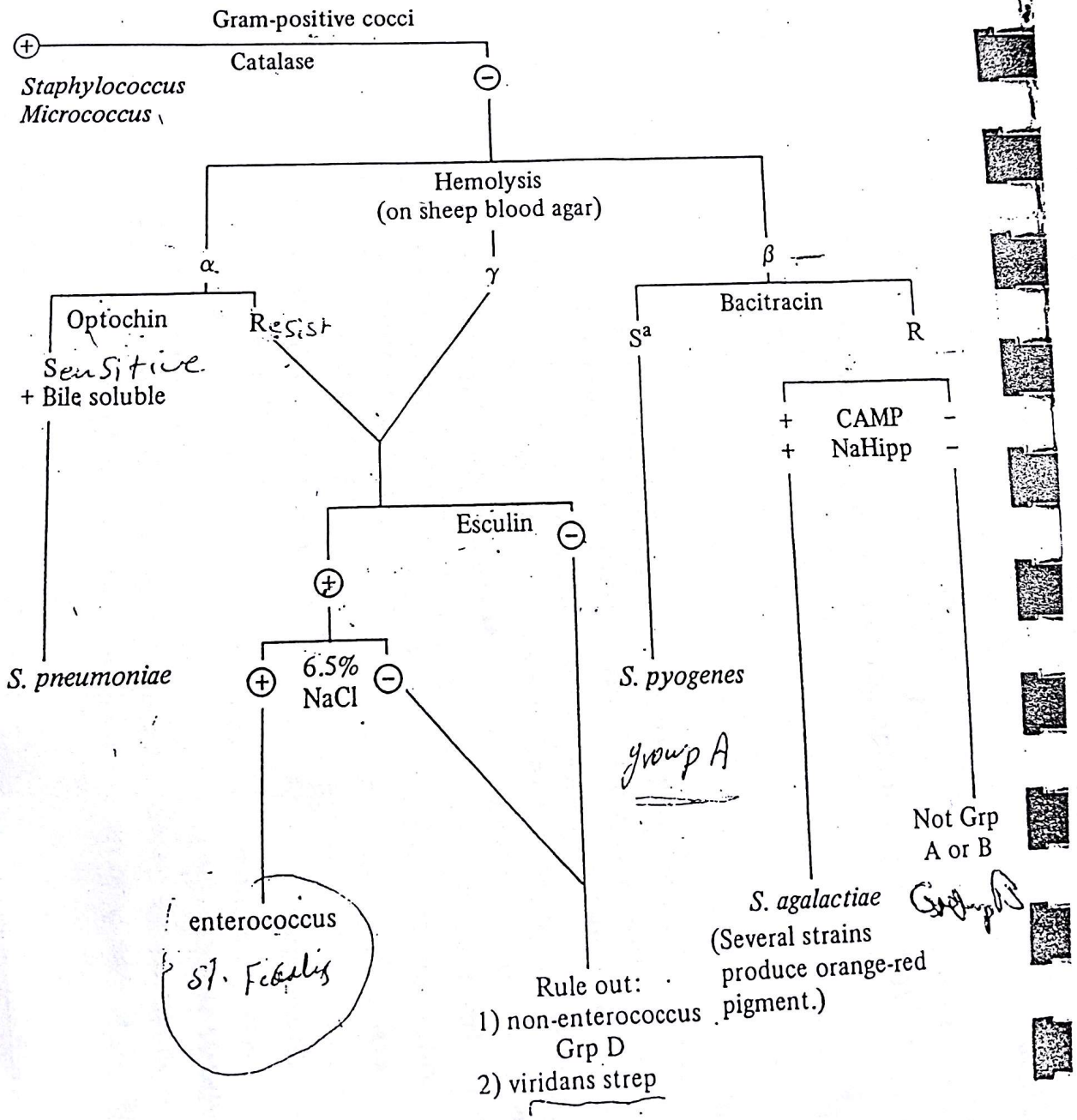
The same Blood Agar plate examined with transmitted light. There is a wide zone of haemolysis around the colonies.



Bacillus spores

is referred to as gamma (γ) hemolysis. Streptococci produce two hemolysins, streptolysin O, which is antigenic and oxygen-labile, and streptolysin S, which is nonantigenic and oxygen-stable. These hemolysins produce complete clearing of the blood agar around the colonies. Streptolysin O is formed by the streptococci belonging to group A, and by some B, C, F and G strains.[3] Strains of all groups generally produce streptolysin S; however, about 2 per cent of group A streptococci fail to produce this hemolysin and may be missed in cultures incubated aerobically.[4]

TABLE 2–3 FLOW CHART FOR IDENTIFICATION OF STREPTOCOCCI



[a]Some strains of *S. agalactiae* and "viridans" streptococci are bacitracin sensitive.

Exercise: THE NEISSERIAE

The species of genus *Neissiae* are Gram-negative diplococci, aerobic to facultatively anaerobic, some species encapsulated, and very fastidious in growth requirements. Best growth occurs on media enriched with blood or hemoglobin-chocolate agar, with incubation under increased CO2. The two species known pathogenic for man occurs intracellularly, with man as the only host. They are *N. gonorrhoeae* and *N. meninigitidis* . The other species recognized as Neisseriae are part of the normal flora of the human upper respiratory tract and oral cavity. All Neisseriae are oxidase positive. *N. gonorrhoeae* ferments only glucose. *N. meningitidis* is classified on the basis of the antigenic nature of the capsular polysaccharides into one of four groups-A,B,C and D. Identification is made also by positive oxidase reaction and fermentation of glucose and maltose, or fluorescent-antibody staining.

Materials:

a) Demonstration of *Neisseriae* spp. grow on blood agar, biochemical tests.

Procedure:

Prepare Gram stain and oxidase tests.

GRAM – POSITIVE BACILLI;

Exercise : CORYNEBACTERIA- C. diphteriae

C. diphteriae is a species of aerobic, non-motile, highly pleomorphic Gram-positive rods that tend to form club-shaped appearances-metachromatic granules – in old cultures. The genus Corynebacteria contain many saprophytic species- Diphteroids. The isolation of pathogenic *C. diphteriae* may perform by using blood – potassium tellurite plate-Tellurite medium, and loefflers serum slant. Pathogenicity is ascertained by the Elek test, which demonstratestoxin production in an agar medium contain anti-diphteriae toxin, Or by animal pathogenicity with guinea pigs. Staining of C.diphteria could be performed by applying 4-5 minutes of methylene blue , using Albert stain 1 and 2 or Neisser stain.

Materials:

Demonstration of C. diphteria and diphtheroids in direct throat smear, Tellurite medium .

Procedure:

prepare gram stain from provided culture of diphteroids.

Exercise : Clostridia and Bacillus

The genera *Bacillus and clostridum* develop a highly resistant resting-phases or endospore whereby the organism can survive in a dormant state through a long period of starvation or other adverse enviromental condition.

Ordinarily spores resist penetration of dyes, but some basic (E.G., malachite green) when applied to a smear and heated will penetrate the spore wall. The dye is then removed from the vegetative cells but not from the cells to be stained a different color than the spore. Both genera are gram-positive. Culture of *clostridia* is easily accomplished in reducing broth or blood agar under anaerobic condition. The Nagler test and fermentation patterns could identify the clostridia spp. The genus *Bacillus is* spore – forming aerobic bacilli, which frequently contaminate laboratory culture media

Materials:

Demonstration of spore stain with Malachite green. Blood culture of
Bacillus spp.
Bacilli and identify the pres.

Procedure:

Prepare gram stain from a mixture of gram positive ence of spore forming bacilli.