Methods of Laboratory Diagnosis of Bacterial Infectious

Scope

Bacterial infections affect

- the skin; the eye; the ear; the mouth; the nose
- the reproductive system
- the digestive system
- the respiratory system
- the urinary system
- the nervous system
- the circulatory system
- the locomotion organs

Types of Bacteria

- **Cocci:**
- Round spherical shaped bacteria
- Some forms of pneumonia and sepsis are caused by this bacteria
- **Bacilli:**
- Rod shaped
- Single, pairs, or arranged in chains
- Cause many serious diseases in animals
- **Spirila**
- Shaped like spirals or corkscrews
- Very motile
- Require moist atmosphere to live
- Live very well in the reproductive tracts of animals
- Leptospirosis
- Vibrosis and spirochetosis

Why diagnosis is needed?

- To administer the treatment
- For prognosis
- To initiate appropriate control measures
- To take suitable preventive steps
- To understand epidemiology
- To know the disease history
- For certification in International trade
- To export
- For import
- To know who is at risk

What is needed for diagnosis

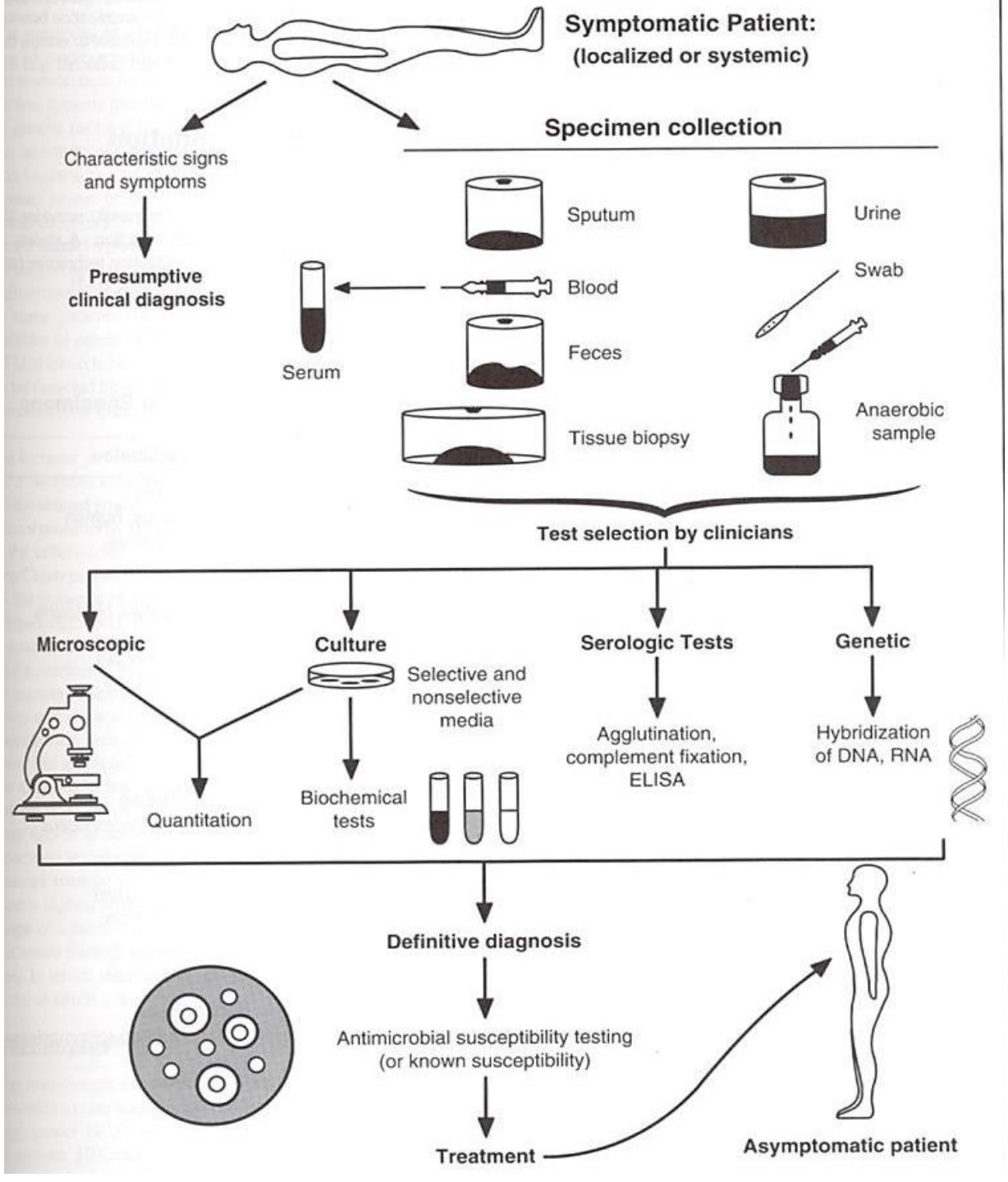
- knowledge about the diseases
- Knowledge about the host
- Knowledge about the environment
- clinical experience
- Right material (Sample)
- Diagnostic facilities
- Laboratory expert

Steps in Diagnosis of Bacterial diseases

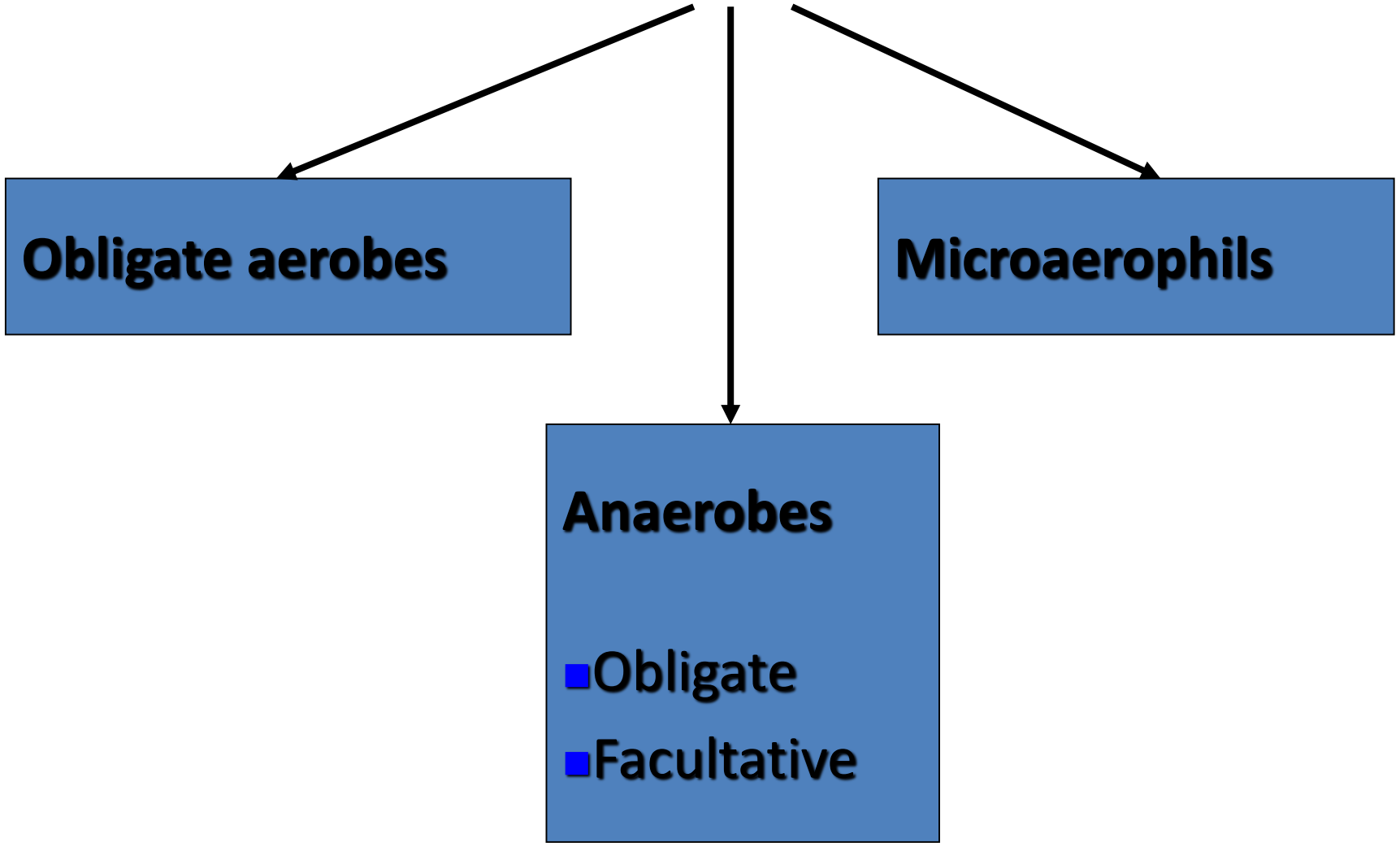
- Clinical Signs
- Laboratory examination
- 1 Microscopy
- 2 Bacteriological methods
- 2.1- Culture techniques
- 2.2- Biochemical reactions
- 3- Serological identification
- 4- Molecular biology techniques
- 5- Bacteriophage typing
- 6 -Allergological methods
- 7- biological methods

Site of sampling

- **Sterile sites**
- □ Blood
- □ Cerebrospinal fluid (CSF)
- □ Body fluids (Peritoneal and pleural)
- **Non-sterile (normal flora)**
- □ Respiratory tract
- □ Ear, eye and mouth
- □ Skin (wound and abscess)
- □ Urine (mid-stream)
- □ Feces


Symptomatic Patient:
(localized or systemic)
Specimen collection
Characteristic signs and symptoms
Presumptive clinical diagnosis
Sputum
Urine
Blood
Swab
Serum
Feces
Anaerobic sample
Tissue biopsy
Test selection by clinicians
Microscopic
Culture
Serologic Tests
Genetic
Selective and nonselective media
Agglutination, complement fixation, ELISA
Hybridization of DNA, RNA
Quantitation
Biochemical tests
Definitive diagnosis
Antimicrobial susceptibility testing (or known susceptibility)
Treatment
Asymptomatic patient

Classification of bacteria according to type of respiration.



Laboratory Diagnosis of Infection

microscopy

unstained or stained with e.g. Gram stain

Stain Decolorise Counterstain

culture

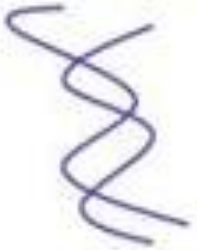
identification by biochemical or serological tests on pure growth from single colony

on plates or in broth

sensitivities

by disc diffusion methods, breakpoints or MICs

Serodiagnosis



DNA technologies

Sample for Bacterial Isolation

Prevent drying of the sample or swab.

 Culture container must contain fluid/ semisolid transport medium to keep bacteria alive for 24 hrs.

 Some media for swab transportation:

 Liquid

 Liquid transport medium

 Campylobacter transport medium

 Brucella transport medium

 Semisolid

 Stuart transport medium

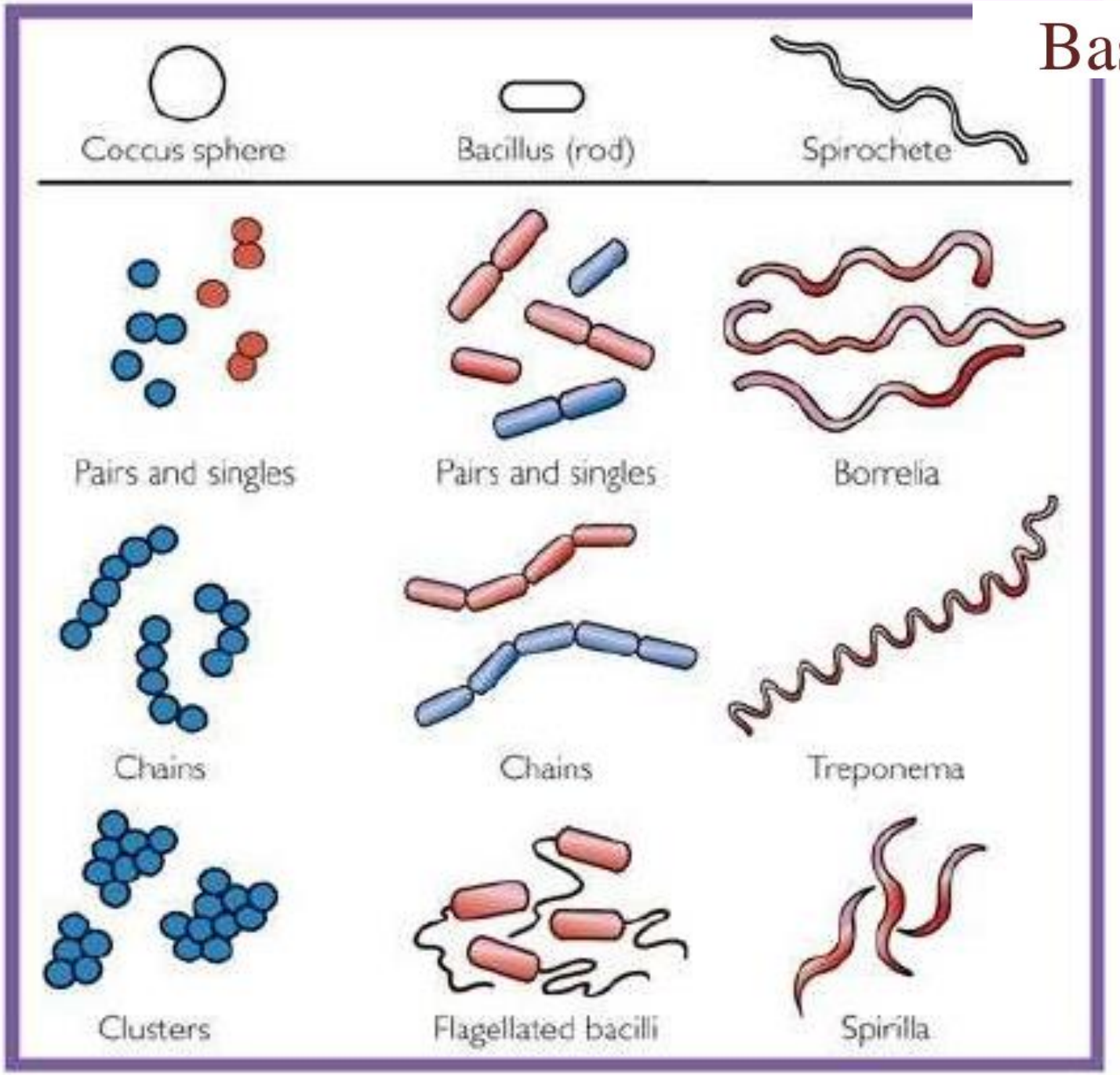
 Carry and Blair transport Medium with and without charcoal

 Amies transport medium

Bacteria are of many types

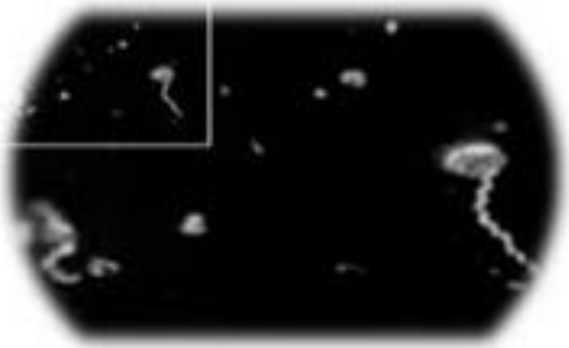
- **With Cell Wall**
- Gram +
- Staphylococcus, Streptococcus, Clostridium, Bacillus
- Gram -
- Enteric, respiratory and others
- **Acid-fast**
- Mycobacterium
- **Wall-less**
- Mycoplasma
- **Unusual**
- Obligate intracellular
- Rickettsia, Chlamydia

Basic shapes

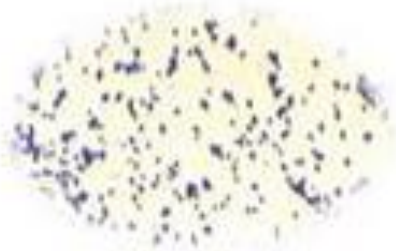


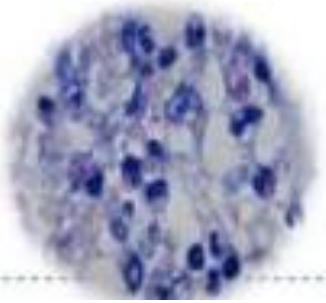
Microscopy

- Unstained preparations
  - "Wet prep"
  - Dark-ground illumination for syphilis



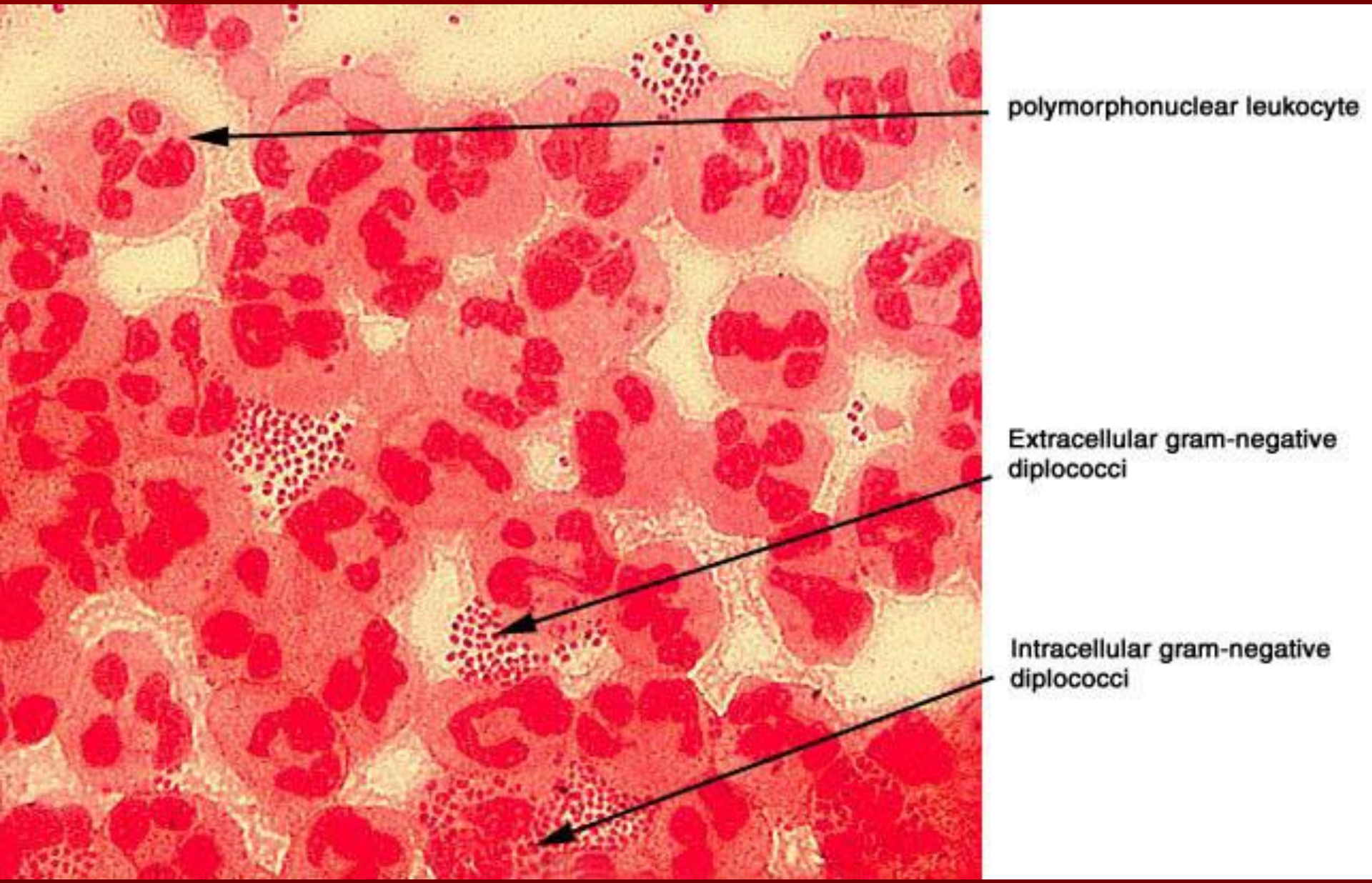
- Stained preparations
  - Gram-stain
  - Acid-fast stain
    - Ziehl-Neelsen
  - Fluorescence
    - Direct, e.g. auramine
    - Immunofluorescence

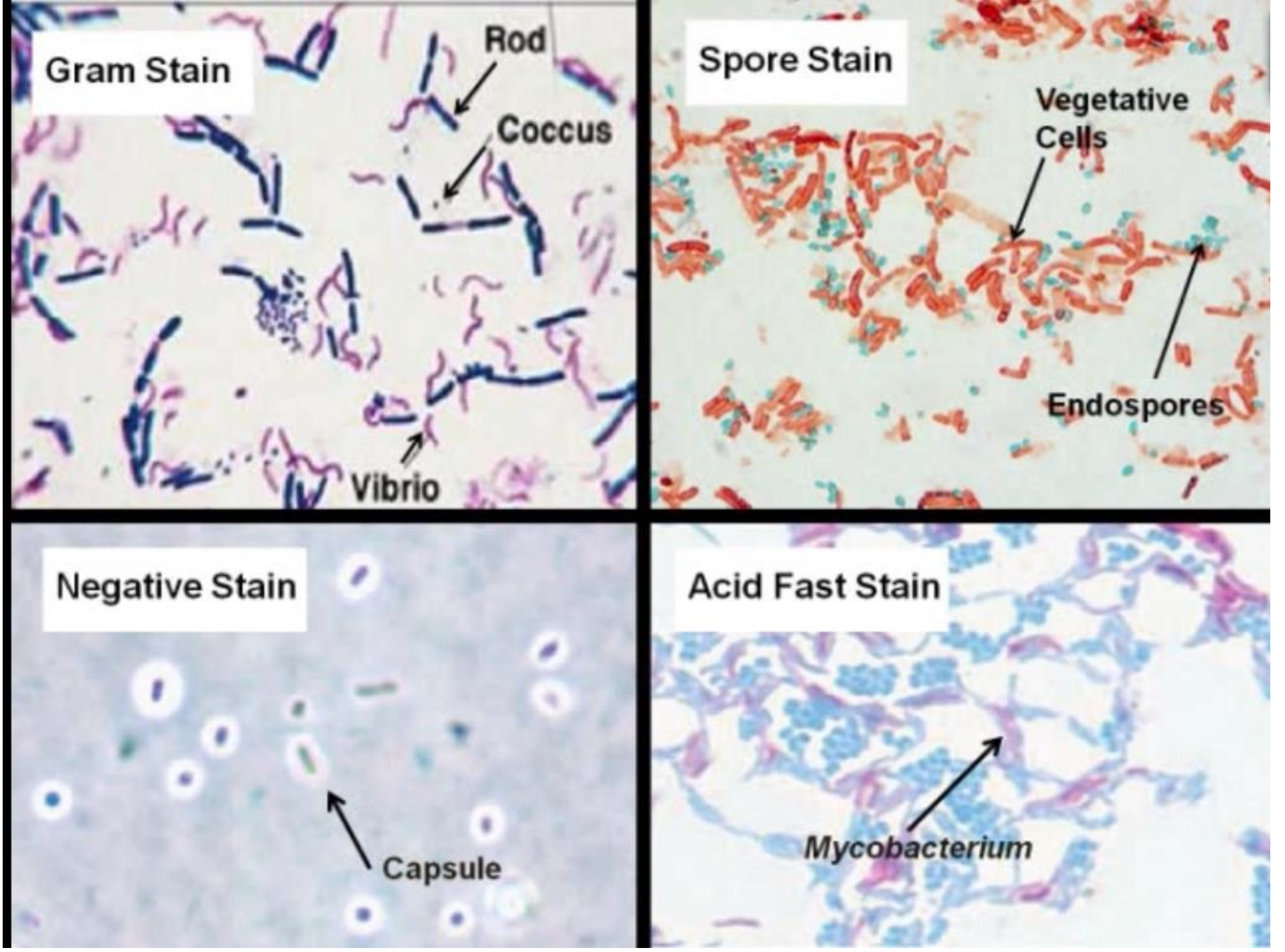




Types of Stains

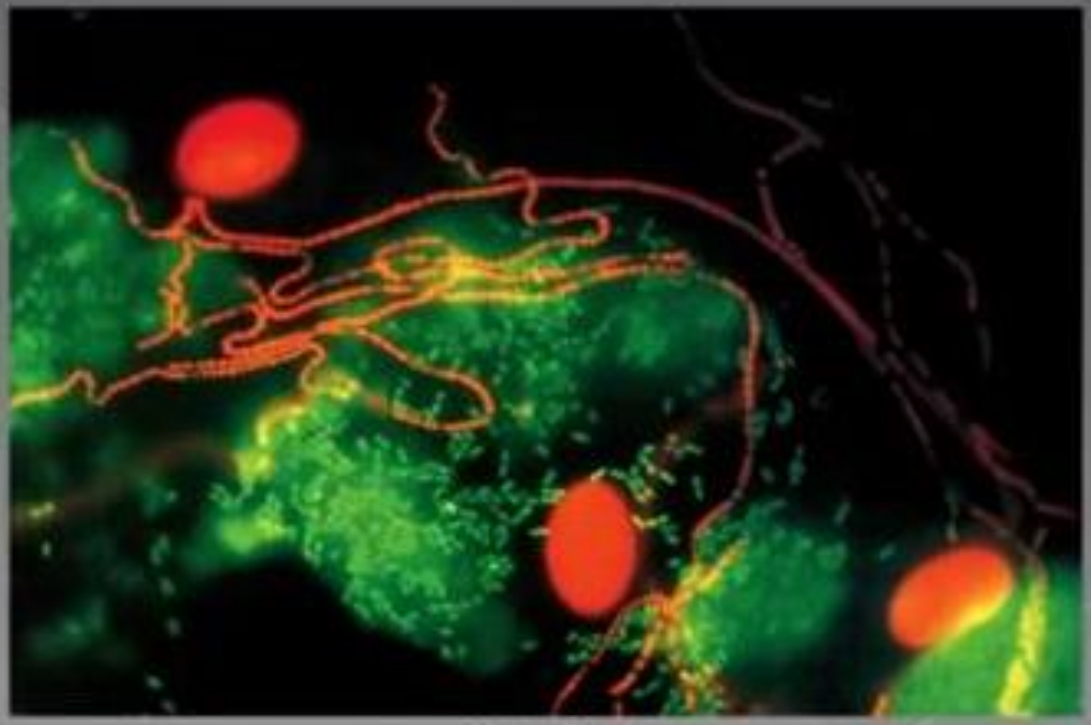
- **Simple stains** – one dye is used; reveals shape, size, and arrangement
- **Differential stains** – use a primary stain and a counterstain to distinguish cell types or parts (examples: Gram stain, acid-fast stain, and endospore stain)
- **Structural stains** – reveal certain cell parts not revealed by conventional methods: capsule and flagellar stains


polymorphonuclear leukocyte
Extracellular gram-negative diplococci
Intracellular gram-negative diplococci


Gram Stain
Rod
Coccus
Vibrio
Spore Stain
Vegetative Cells
Endospores
Negative Stain
Capsule
Acid Fast Stain
Mycobacterium

Fluorescence Microscope

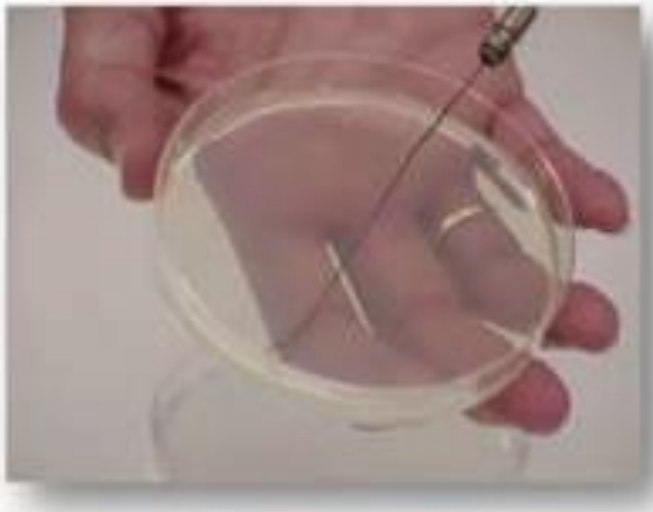
- Modified microscope with an ultraviolet radiation source and filter.
- Uses dyes that emit visible light when bombarded with shorter UV rays - fluorescence
- Useful in diagnosing infections



2 Bacteriological methods

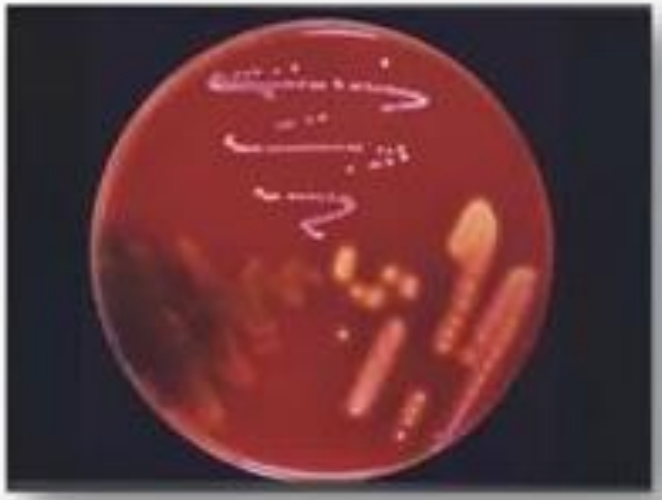
Culture of Pathogenic Microbes

- Solid media
  - Agar plates
    - For Identification
    - For Enumeration
  - Slopes
    - For safe long-term culture, e.g. Lowenstein-Jensen media for TB
- Liquid media (broth)
  - For enrichment or maximum sensitivity
  - E.g. blood cultures



Identification of Bacteria

- Morphology
- Growth requirements
- Biochemistry
- Enzymes
- Antigens



Inoculation – introduction of a sample into a container of media to produce a **culture** of observable growth

Isolation – separating one species from another

Incubation – under conditions that allow growth

Inspection

Information gathering

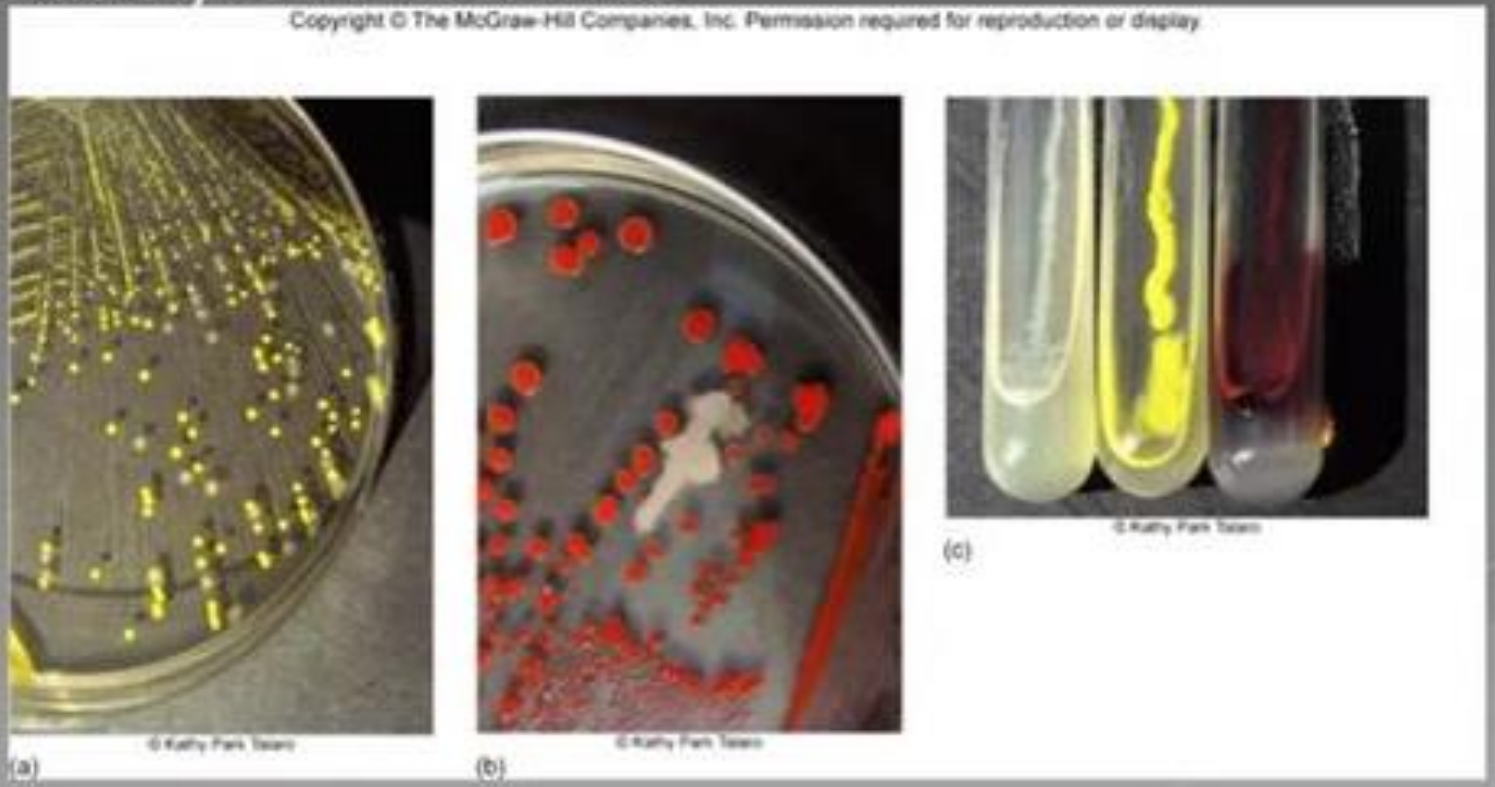
Identification

Isolation

- If an individual bacterial cell is separated from other cells and has space on a nutrient surface, it will grow into a mound of cells— a **colony**. A colony consists of one species.

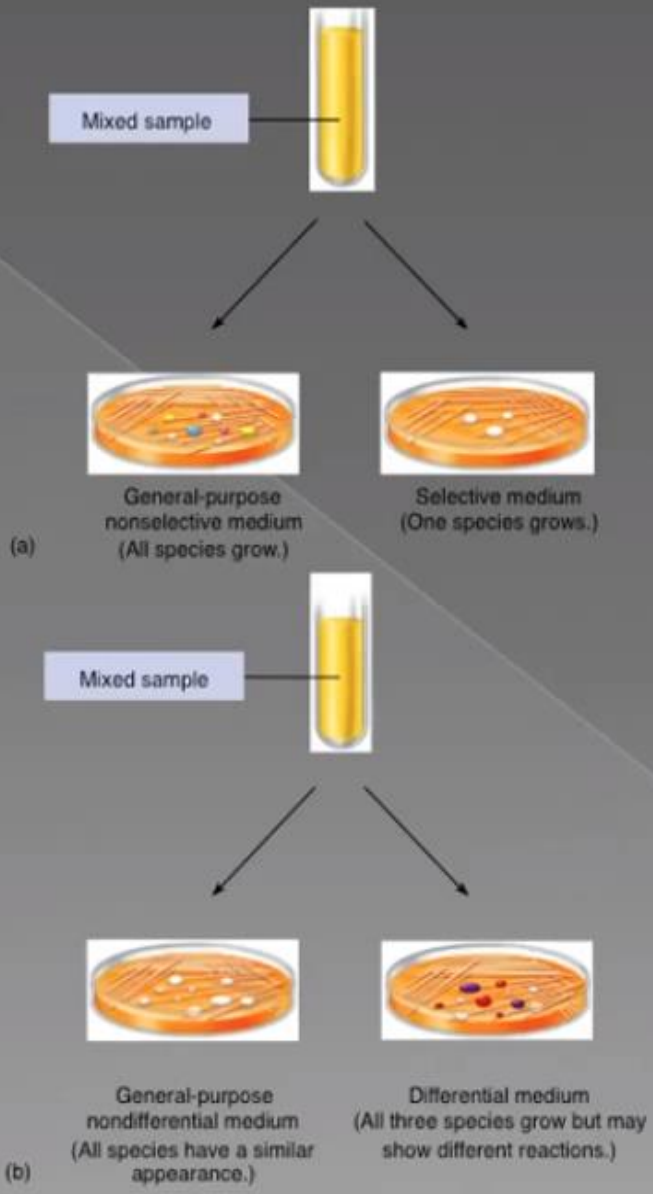
Inspection

- If a single species is growing in the container, you have a **pure culture** but if there are multiple species than you have a **mixed culture**.
- Check for **contaminants** (unknown or unwanted microbes) in the culture.



Selective & Differential Media

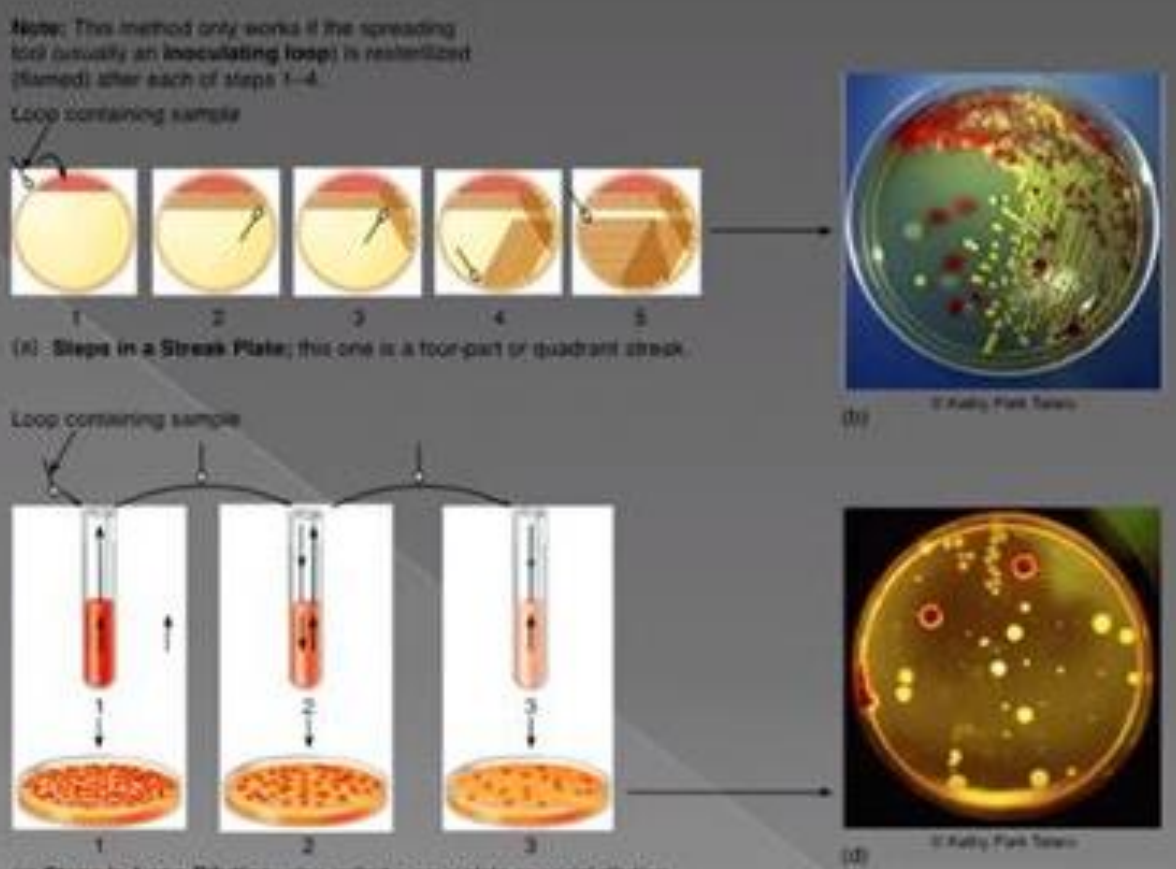
Selective media: contains one or more agents that inhibit growth of some microbes and encourage growth of the desired microbes



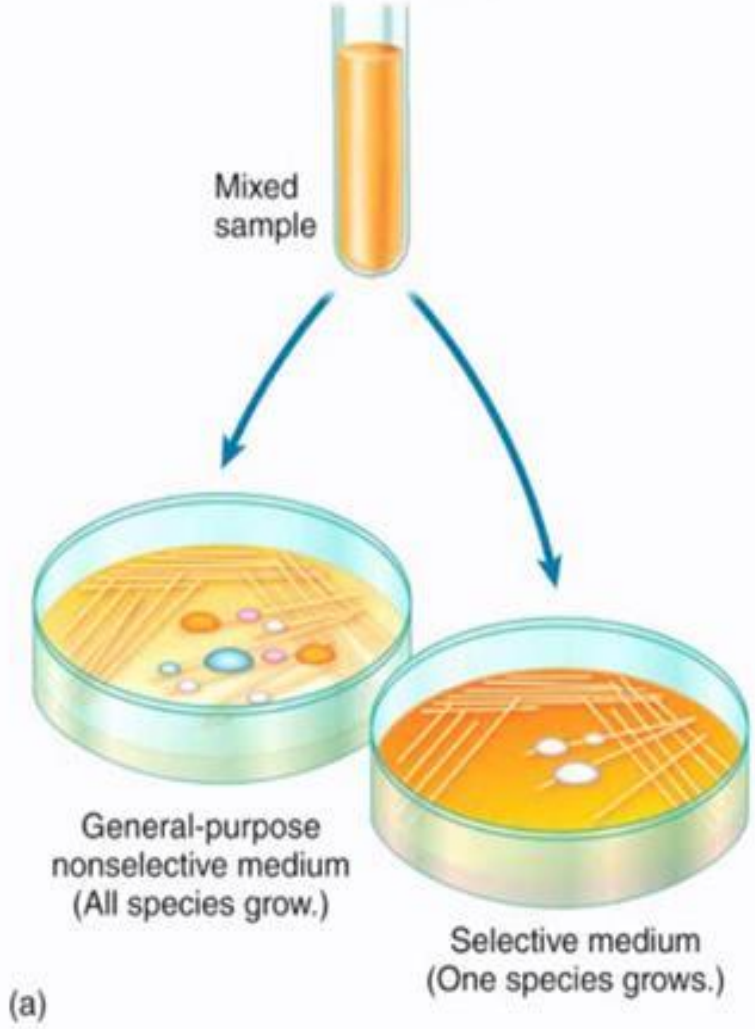
Differential media: allows growth of several types of microbes and displays visible differences among those microbes

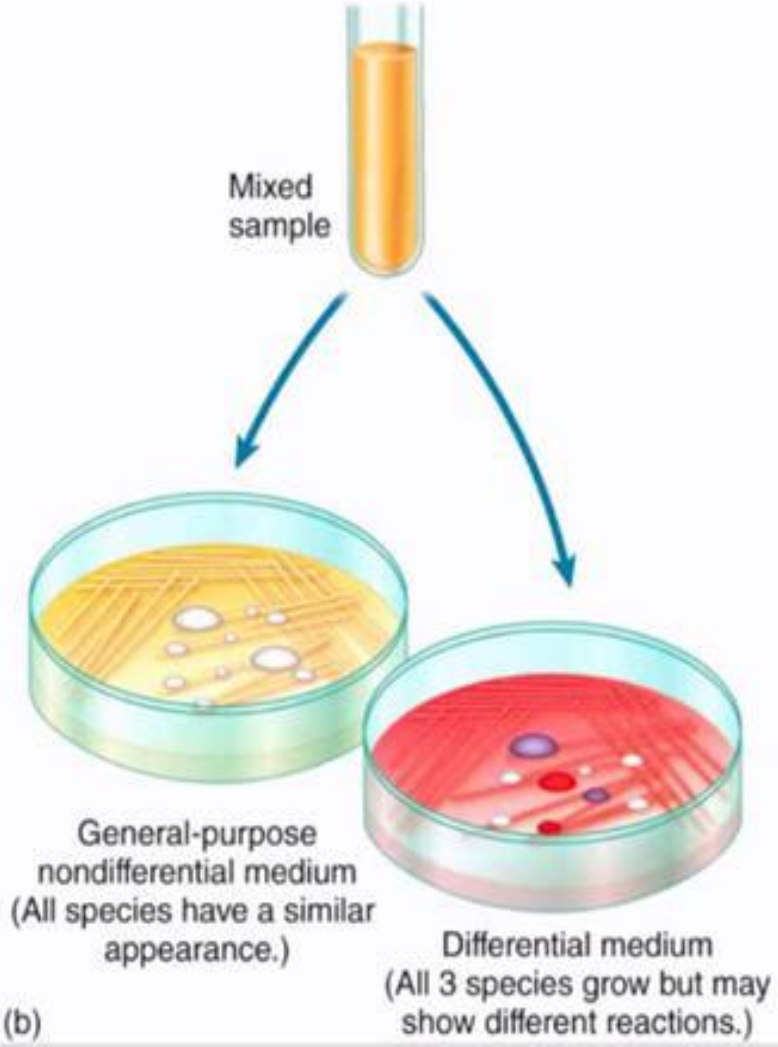
Isolation Techniques

- Streak plate technique
- Pour plate technique
- Spread plate technique



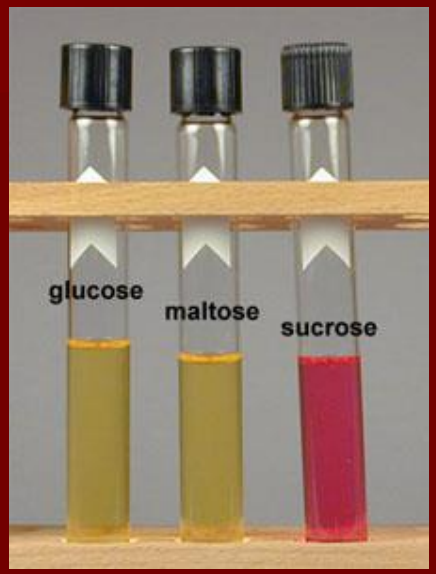
Comparison of Selective and Differential Media





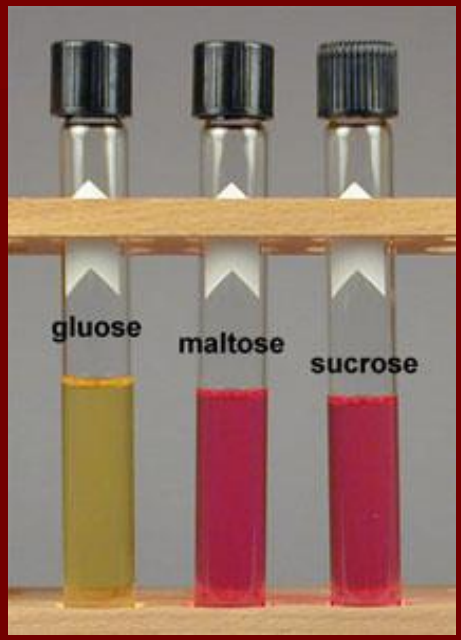
- **Carbohydrate utilization**

Neisseria meningitidis produces acid from oxidation of glucose and maltose, but not from sucrose or lactose. *Neisseria* species produce acid end products from an oxidative pathway rather than from fermentation. The acid turns the pH indicator phenol red from red to yellow. Lactose, not shown here, is not utilized by *N. meningitidis*.

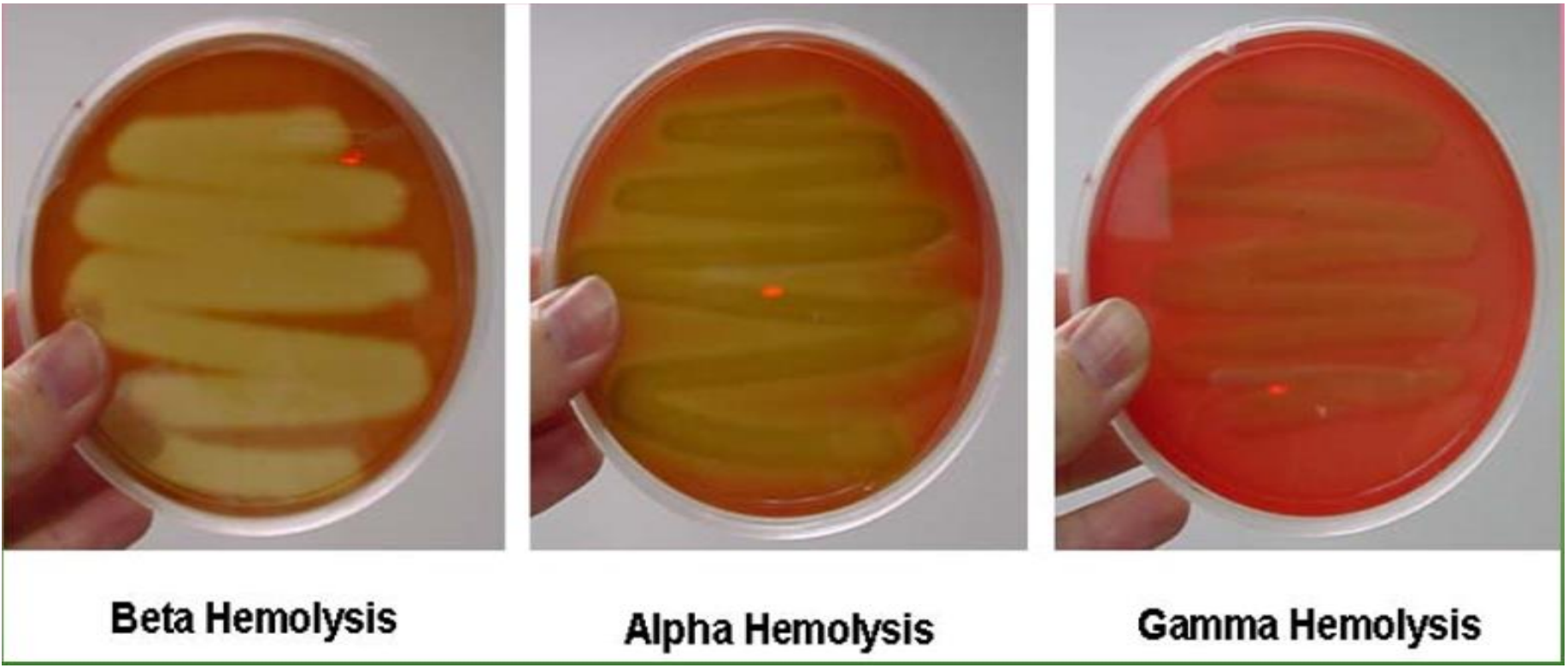


- **Carbohydrate utilization**

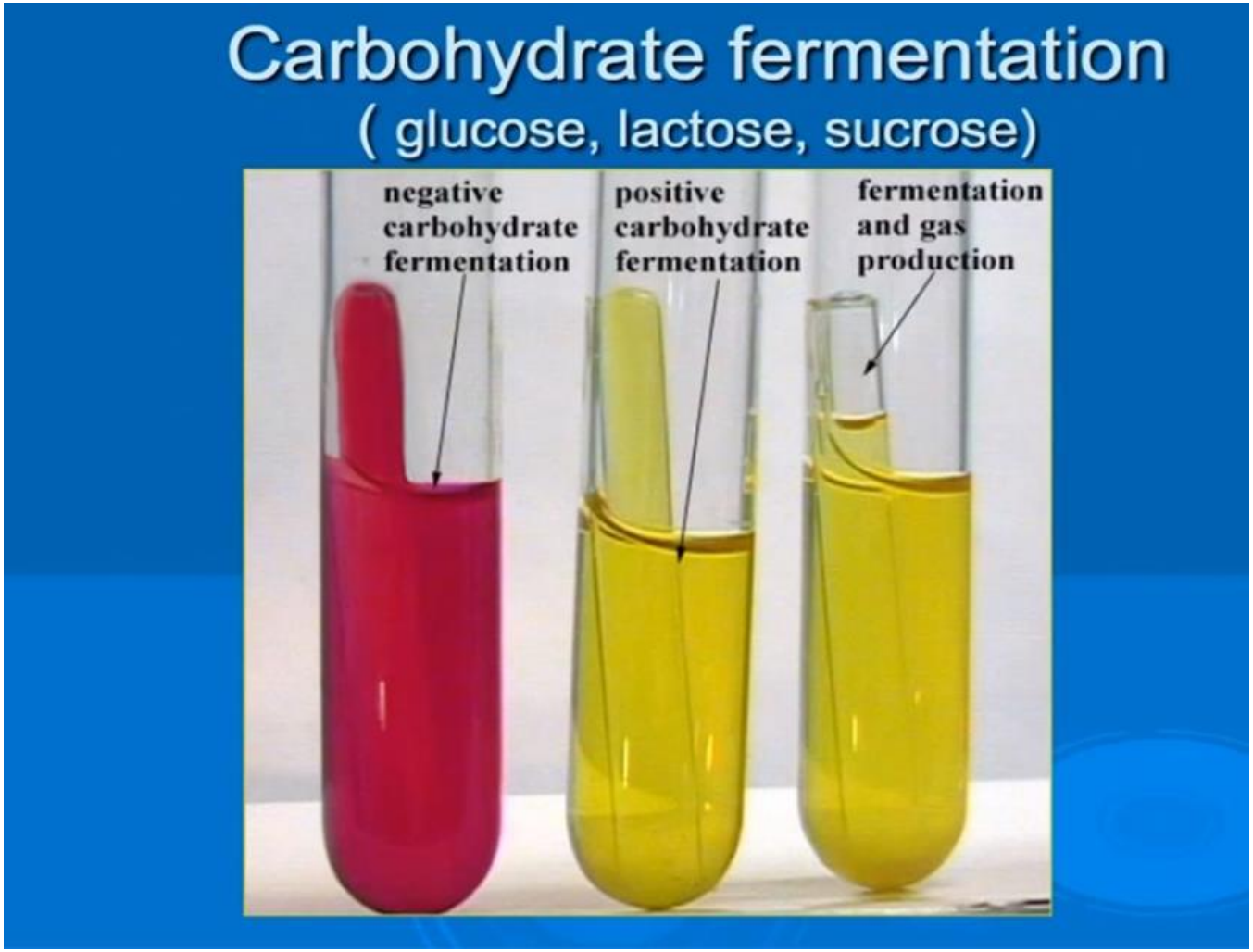
Neisseria gonorrhoeae produces acid from oxidation of glucose but not from maltose, sucrose, or lactose. *Neisseria* species produce acid end products from an oxidative pathway rather than from fermentation. The acid turns the pH indicator phenol red from red to yellow. Lactose, not shown here, is not utilized by *N. gonorrhoeae*.



Kingdoms

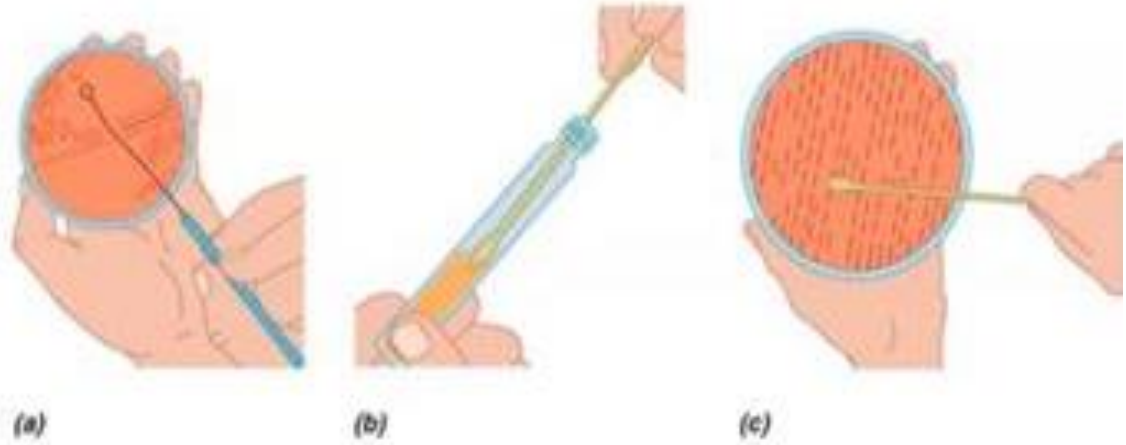


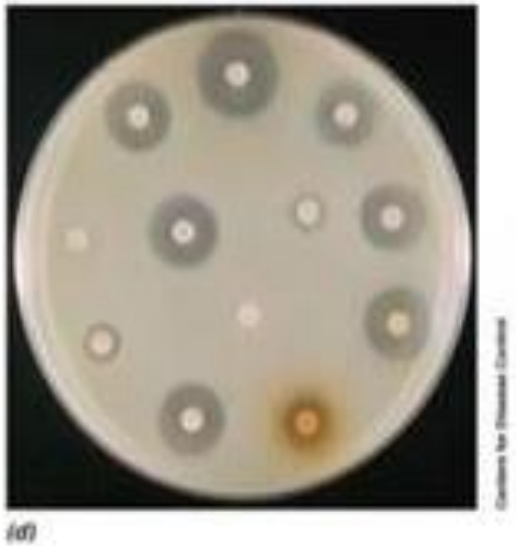
Kingdoms

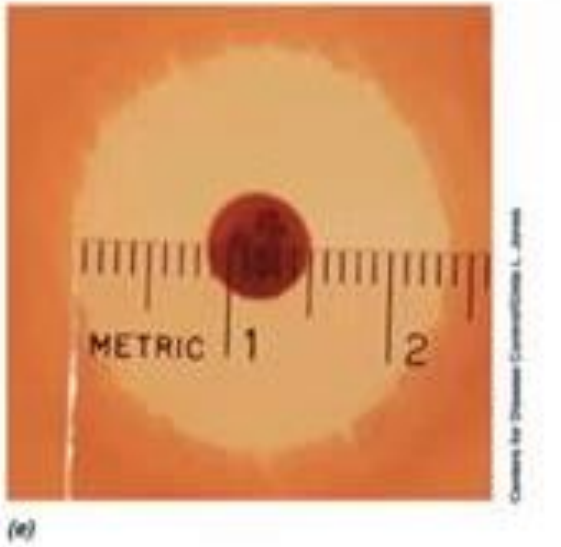

Carbohydrate fermentation
(glucose, lactose, sucrose)
negative carbohydrate fermentation
positive carbohydrate fermentation
fermentation and gas production

Antimicrobial Susceptibility Testing

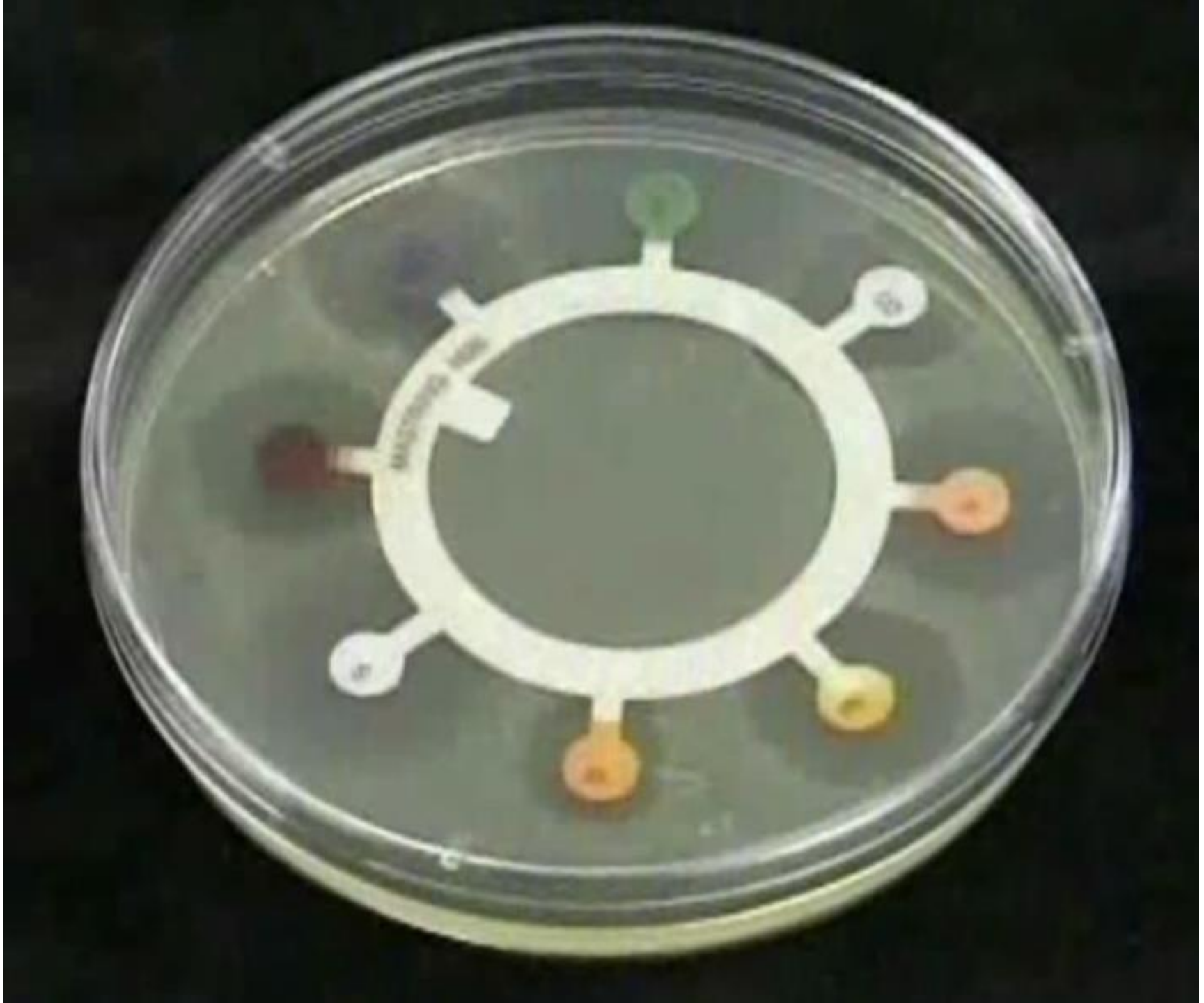
- Disk Diffusion Test
  - Standard procedure for assessing antimicrobial activity
- Inhibition Zones
  - Used to determine an organism's susceptibility to an antimicrobial agent





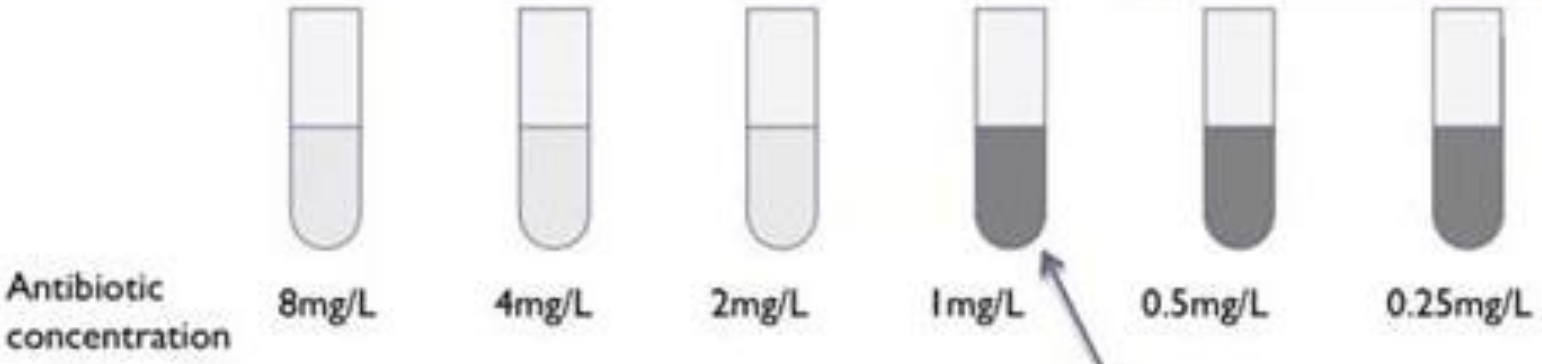



BSAC = British Society for Antimicrobial Chemotherapy



Antimicrobial Susceptibility Testing

- The MIC (minimum inhibitory concentration) procedure is used to assess antibiotic susceptibility with regard to various concentrations



Cloudiness represents growth after overnight incubation means bacteria can grow at that concentration of antibiotic
MIC=2mg/L

Serological identification

A- Direct serological tests:

- Identification of unknown organism
- Detection of microbial antigens by using specific known antibodies
- Serogrouping and serotyping of isolated organism

B- Indirect serological tests:

- Detection of specific and non specific antibodies (IgM & IgG) by using antigens or organisms

Advantages

Reduce reliance on culture

Faster

More sensitive

More definitive

More discriminating

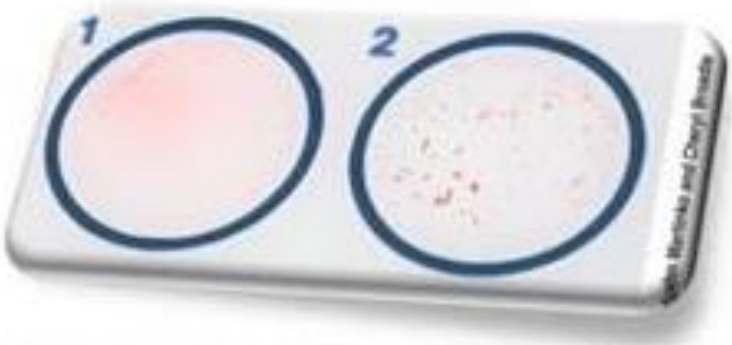
Techniques adaptable to all pathogens

Various types include

Precipitation
Agglutination
Complement fixation
immunofluorescence
Elisa
Western blot

Agglutination

- Passive Agglutination
  - The agglutination of soluble antigens or antibodies that have been adsorbed or chemically coupled to cells or insoluble particles (e.g., latex beads, charcoal)
  - Reactions can be up to five times more sensitive than direct agglutination tests



Latex Bead Agglutination Test for *Staphylococcus aureus*

Lancefield Group Test Kit

ELISA

Specific, sensitive, simple, inexpensible & reproducible
Used extensively to detect either Ag or Ab
Also detects small quantities of Ag
Used to diagnose TORCH, HIV,MEASLES,HEPATITIS(A,B,C,D,E).....

Molecular Diagnosis

Ribotyping

- Restriction fragment length polymorphism (RFLP)
- DNA hybridization
- PCR, RT-PCR and RAPD
- Nucleic acid sequence analysis
- PFGE
- Phage-GFP (TB)
- Plasmid profile analysis:

Allergological methods

Skin Testing is performed as the **tuberulin** or **Mantoux test**. **PPD (purified protein derivative)** is employed as the test antigen in the **Mantoux test**. PPD is generated by boiling a culture of M.TB, specifically Old Tuberculin (OT). 5 TU (tuberculin units), which equals 0.000lmg of PPD, in a O.1 ml volume is intracutaneously injected in the forearm. The test is read within 48-72 hours.

- Old tuberculin (O.T.) consist of filtrate of glycerol broth culture of bacilli concentrated to 1/10th of volume by evaporation on water bath.
- Purifed protein derivative (PPD) is prepared by precipitation of tubercle bacilli culture grown in synthetic medium with trichlor acetic acid. PPD is superior to O.T. because it is stable, and constant in activity. Tuberculin prepared from bovine type is as active as tuberculin prepared from human type.

Biological methods

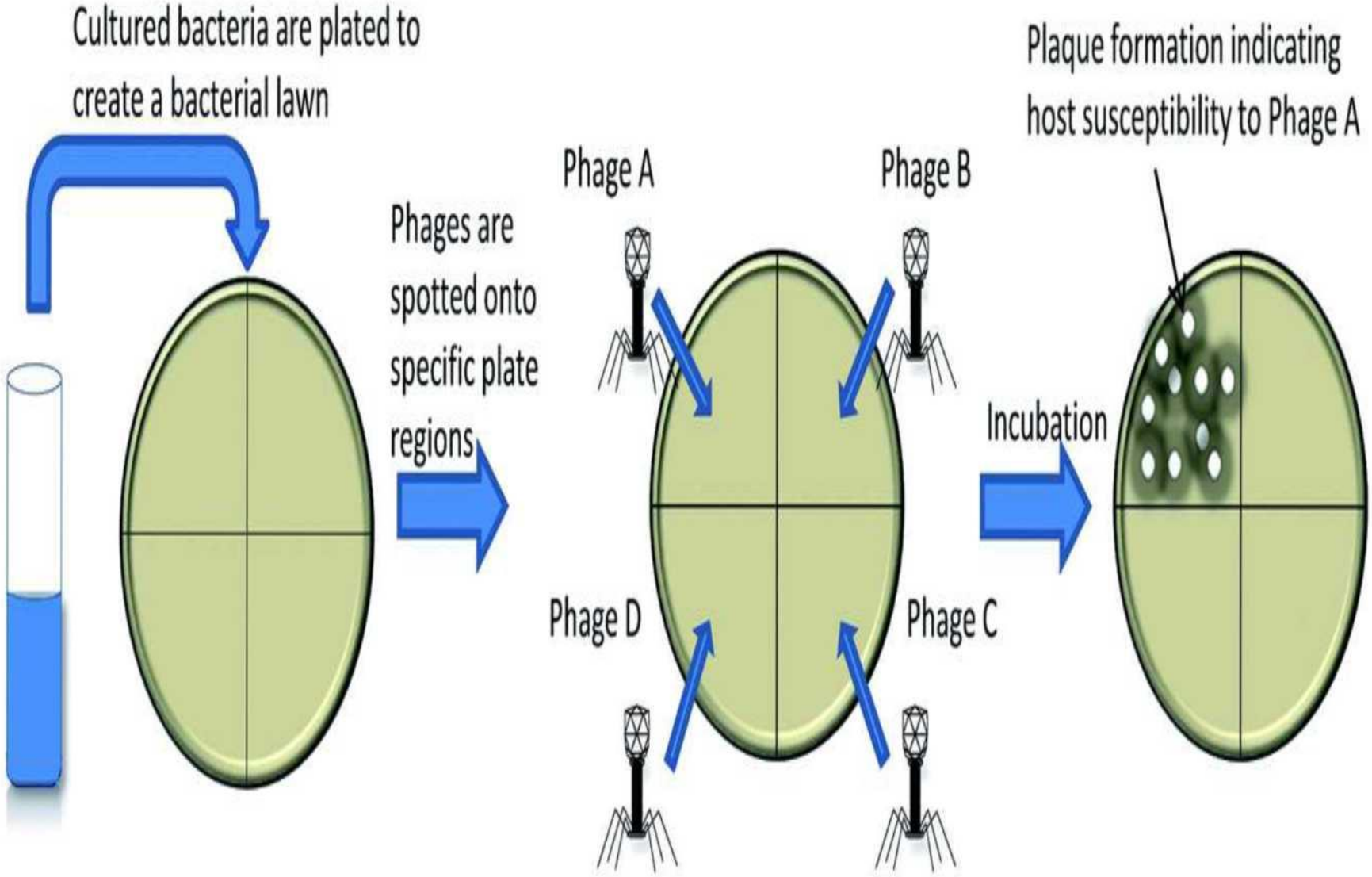
- The use of laboratory animals (mice, guinea pigs, rabbits) is now limited due to the advancement in medical microbiological techniques.

But they could be used :

- For growing the organisms that do not grow on culture such as lepra bacilli.
- To determine the virulence factor of an organism. For example if injection of diphtheria in a guinea pig caused its death, this means that the organism is toxigenic

Bacteriophage typing

- Bacteriophages are viruses which infect the bacterial cells and cause their lysis.
- Different types of a certain bacteria are lysed by different phage groups.
- If a phage is added to a plate inoculated with susceptible bacteria, a zone of lysis will appear around the phage drop.


Cultured bacteria are plated to create a bacterial lawn
Phages are spotted onto specific plate regions
Phage A
Phage B
Phage D
Phage C
Incubation
Plaque formation indicating host susceptibility to Phage A

Thanks for attention