

Practical Bacteriology II

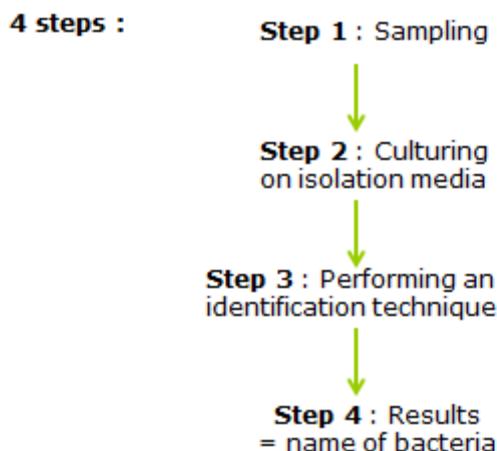
(3303-314)

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 Importance and purpose of bacterial identification

- ✓ Determining the clinical significance of particular pathogen.
- ✓ Guiding physician care of the patients.
- ✓ Determining the laboratory testing for detection of antibacterial resistance is warranted.
- ✓ Determining the type of antibacterial therapy that is appropriate.
- ✓ Determining whether infectious organisms are risk for others patients in the hospital, the public and other laboratory workers

General approach for bacterial identification

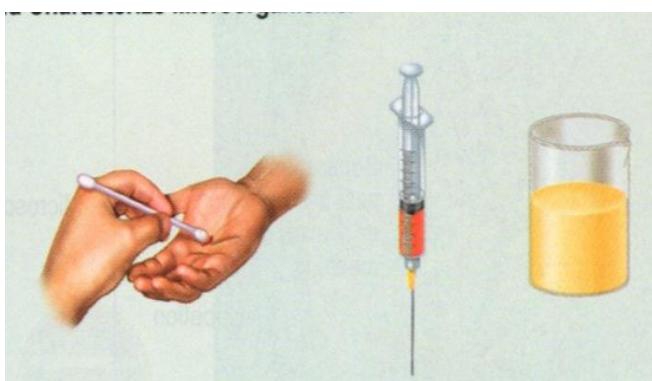


 3 major bacterial Identification methods

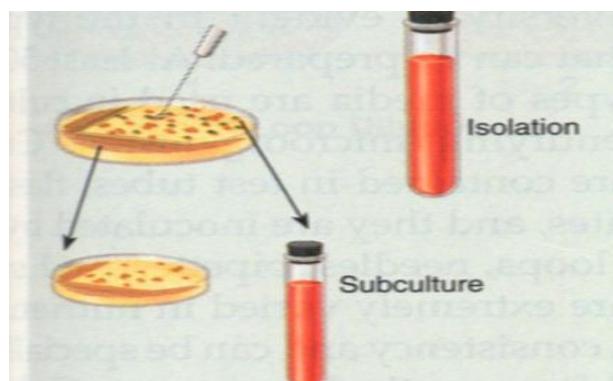
- ✓ Traditional method/**phenotypic method** : morphological (microscopic and macroscopic)
- ✓ Immunochemical method/**serological methods**
- ✓ genotypic method/**molecular method**

-  Technologies used to characterize and Identification prokaryotes (Bergey's Manual of Determinative Bacteriology)
- ✓ Microscopic examination
 - ✓ Culture characteristics

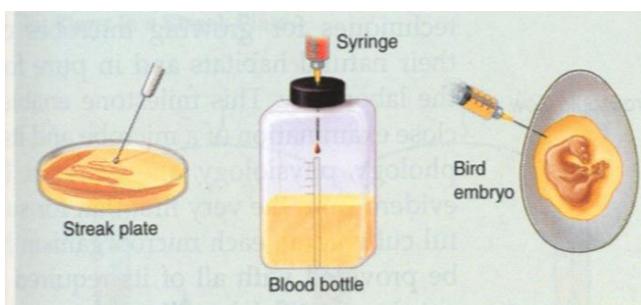
- ✓ Biochemical testing
- ✓ Serology
- ✓ Phage typing
- ✓ Nucleic acid analysis
- ✓ Combination of the above is most accurate
- ✓ After the microbe is identified for clinical samples it is used in **susceptibility tests** to find which method of control is most effective



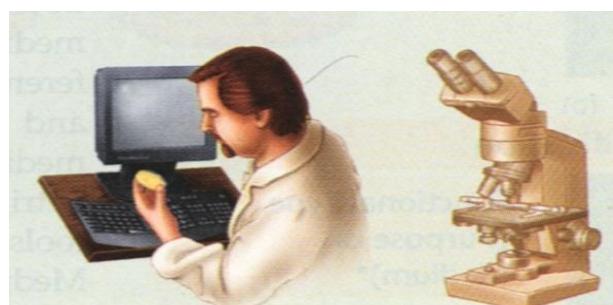
Specimen collection



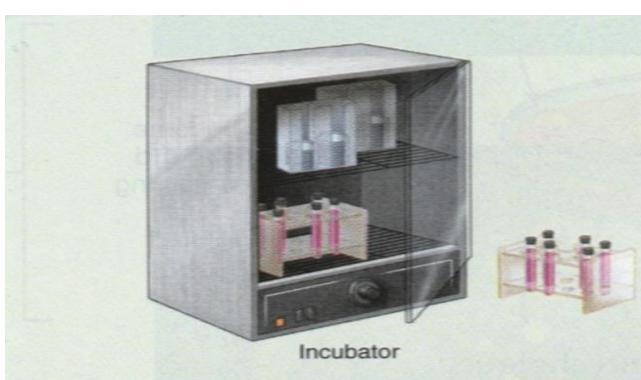
Isolation



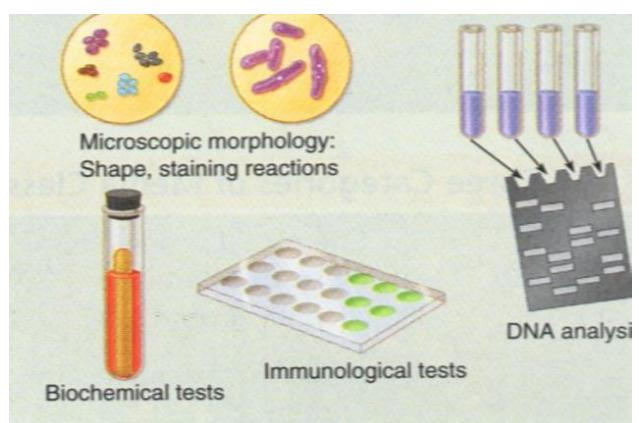
Inoculation



Inspection



Incubation



Identification

Microscopic examination

Is a morphological test

Provide foundational information

But many unrelated bacteria appear similar

❖ size and shape and arrangement

enough information for diagnosis of certain infections

❖ Stain reactions

- Gram stain distinguishes between Gram + and Gram – bacteria
- Ziehl-Neelsen stain divides them into acid fast and non acid fast

Special stains are necessary to bring out characteristics like flagella, capsules, spores and metachromatic granules

Microscopy Contrast

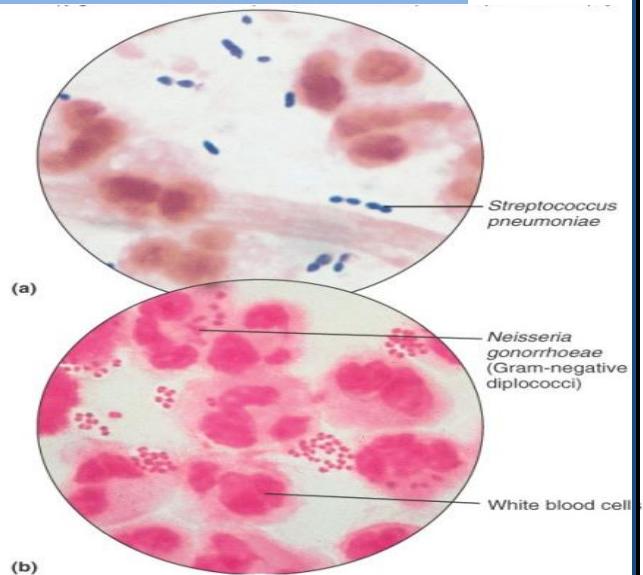
—use stains to enhance visualization; allow organism to stand out from background

1. Acid fast staining

—for staining of organisms with high degree of fatty (mycolic) acids—waxy

—render the cells resistant to decolorization: “acid-fast”

—*Mycobacterium* sp., *Nocardia* sp., *Cryptosporidium* sp. are acid-fast



Mycobacterium

- ❑ A 3rd type of cell envelope (high lipids content of cell wall)
- ❑ Not readily stainable with ordinary stains
- ❑ A strong stain e.g., concentrated carbol fuchsin + heat.
- ❑ Resist decolorization by strong mineral acids or acid-alcohol →

Acid-fast.

-Procedure

- Ziehl-Neelsen: heat drives in primary stain (carbolfuchsin)
- Kinyoun: higher conc. of phenol does not require heat
- Decolorize with acid-alcohol
- Counterstain with methylene blue or malachite green

Reagent	Acid Fast	Non acid fast
Carbol Fuschin with heat	Red (hot pink)	Red (hot pink)
Acid alcohol	Red (hot pink)	Colorless
Methylene blue	Red (hot pink)	Blue



Macroscopic examination

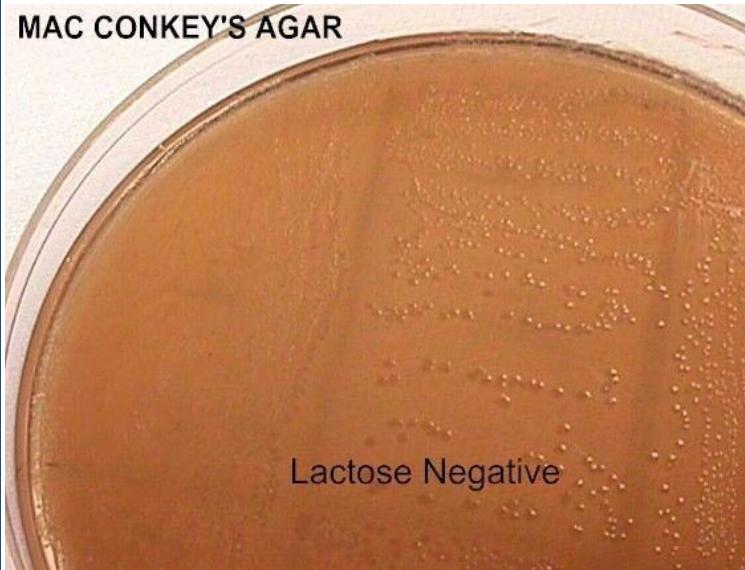
- ❖ Provides additional information for the identification of the bacterium. The characters revealed in different types of media are noted.
- ❖ While studying colonies on solid media following characteristics are observed :

Size, Shape, Margins, Surface, Their elevations, Edge, colour, structure, consistency, pigmentation, haemolysis.

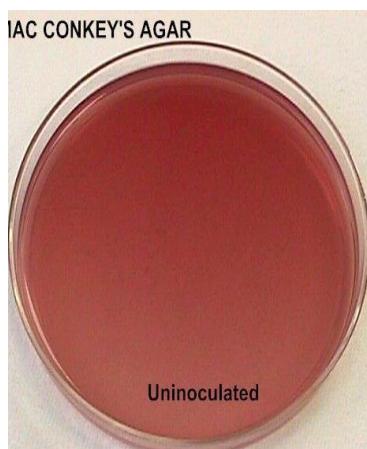
Colony form	Examples
Punctiform (pinpoint)	
Circular	
Filamentous	
Irregular	
Colony elevation	
Flat	
Raised	
Convex	
Colony margin	
Entire (smooth)	
Irregular	

Mac Conkey Agar a Minimal differentiating Medium

yellow colonies



pink colonies



Differential Medium - contains dyes, indicators or other constituents that give colonies of particular organisms distinctive and easily recognizable characteristics (e.g. McConkey Agar)

Differences may show up as colony size, media color, gas bubble formation and precipitate formation

Hektoen enteric agar: artificial medium



Salmonella growing on HE agar produces colonies with black centers (produces hydrogen sulfide).

6



Enterobacter produces acid on HE agar and turns the medium orange.

- contains bile salts and dyes (bromothymol blue and acid fuchsin) to inhibit non-pathogenic GNRs; non pathogens ferment lactose changing BTB to orange; pathogens *Salmonella* and *Shigella* are clear; ferric ammonium citrate detects H₂S production of *Salmonella* (black colonies)

❖ In fluid medium following characteristics are observed :

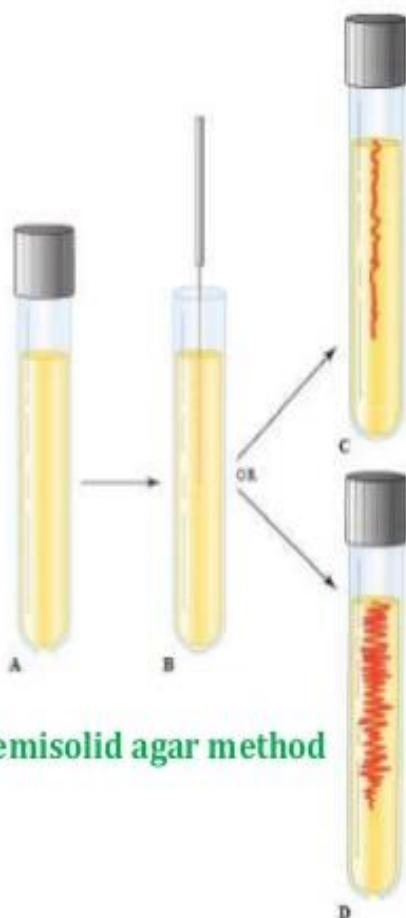
Degree of growth – Absence, scanty, moderate, abundant etc.

presence of turbidity and its nature

presence of deposit and its character

Nature of surface growth and odor

Motility test



Semisolid agar method

- **Motility of bacteria**

- Bacterium is **able to "swim"**
- Associated with presence of flagella
- Determined with *semisolid agar method*

- **Pattern of growth of *nonmotile* organism**

- Turbidity only seen along stab line
- Most cocci are unmotile

- **Pattern of growth of *motile* organism**

- Turbidity throughout the medium
- Most spiral-shaped and half of bacilli

Burton's Microbiology: Chapter 4

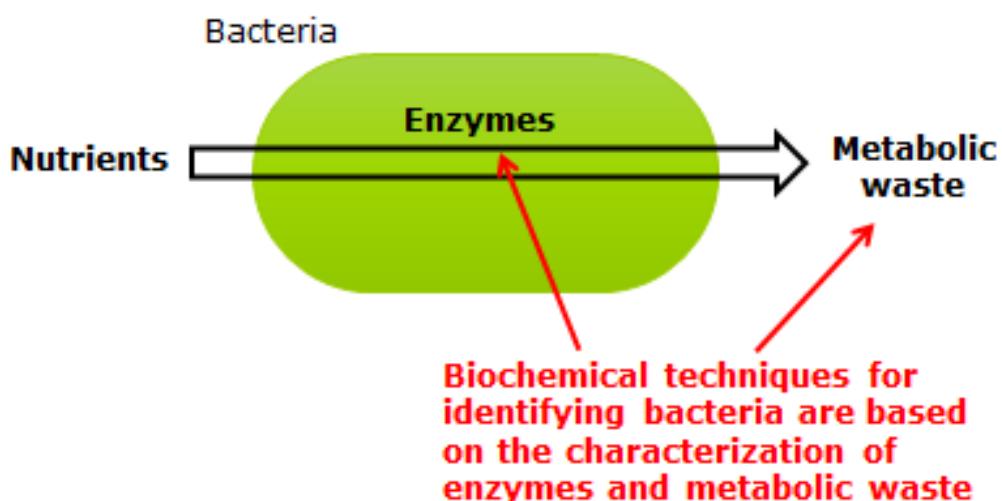
Biochemical testing

- Biochemical characteristic are traditional mainstay of bacterial identification.
- These include enzymes (catalase, oxidase, decarboxylase), fermentation of sugars, capacity to digest or metabolize complex polymers and sensitivity to drugs can be used in identification.

Metabolism of the bacteria

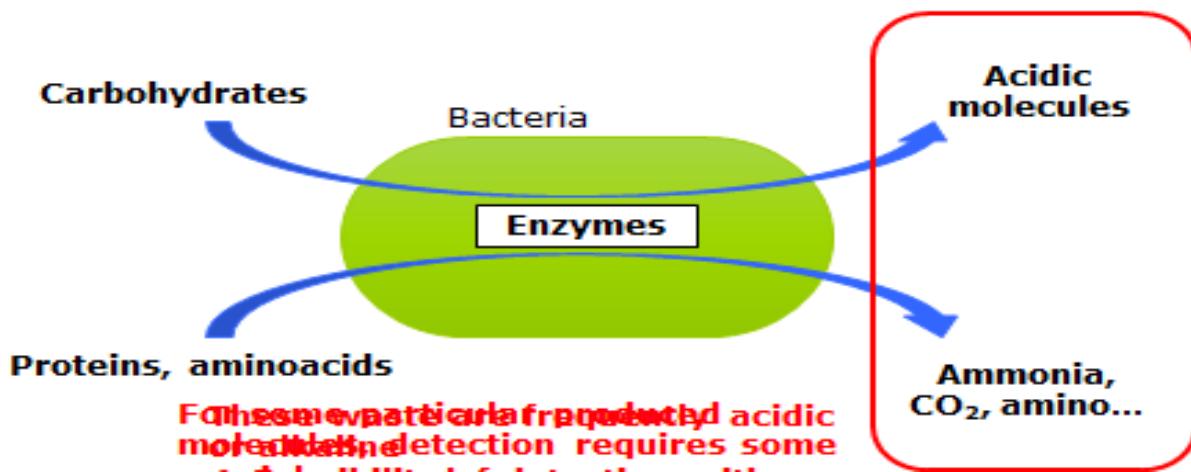
Bacteria are living cells who :

- consume nutrients (carbohydrates, proteins...)
- reject metabolic waste.



Metabolism of the bacteria

The main metabolic pathways :

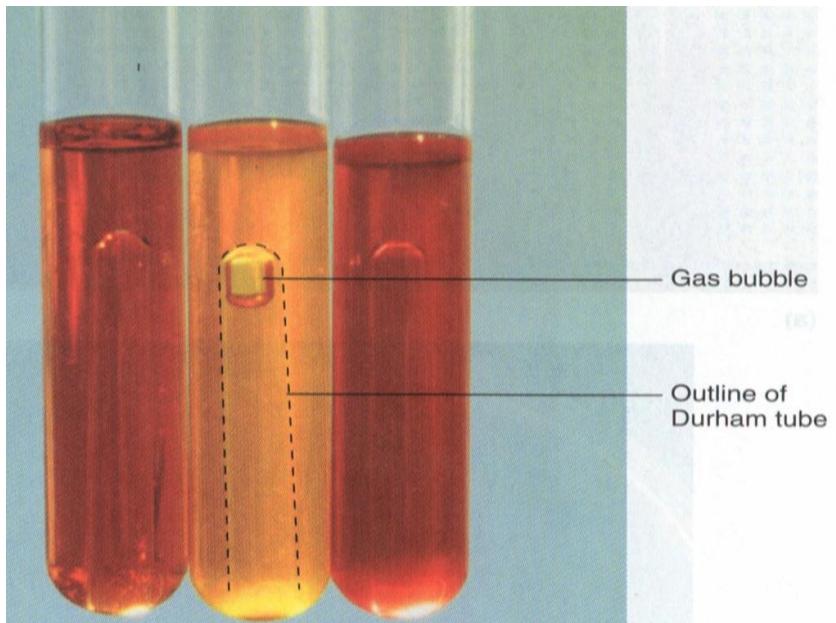


Carbohydrate fermentation

This medium show **fermentation (acid production)** and **gas formation**.

The small **Durham tube** for collecting gas bubbles.

Left-right:
Uninoculated negative control
Centre, positive for acid (yellow) and gas (open space).



Growth but no gas or acid

Application to the identification

Example : research the ability to use glucose



Yellow color :

- Acid pH
- Production of acidic waste by bacteria
- Proves the presence of enzymes which allow the use of glucose as nutrient by bacteria

Medium + **glucose** + pH indicator :
-green color for pH = 7
-yellow color for acid pH (pH <7)

→ **Bacteria « glucose + »**

Application to the identification

How to differentiate bacteria ???

Examples with two bacteria :

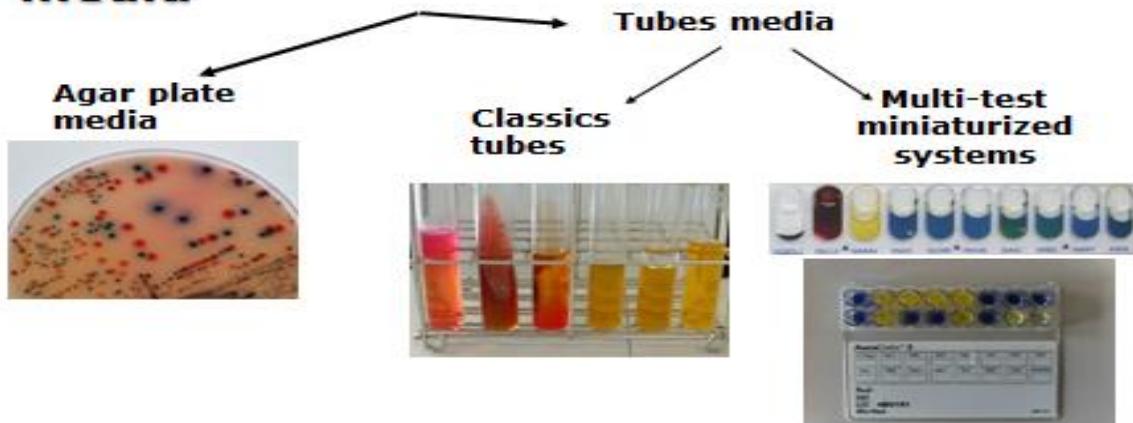
- *E.coli* can use as nutrient glucose, mannose, and arabinose but not amylose
→ Profile = GLU + MAN + ARA + AMY -

- *E.tarda* can use as nutrient glucose, but non mannose, arabinose and amylose
→ Profile = GLU + MAN - ARA - AMY -

Each bacteria has a specific biochemical profile

Identification media

Different packaging of identification media



A. Single enzyme tests

Catalase test

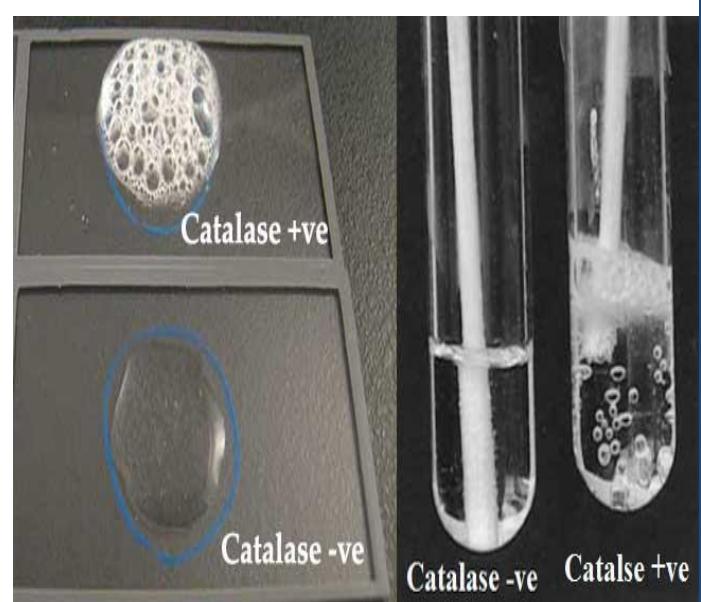
Some bacteria reduce O₂ and produce H₂O₂. H₂O₂ is toxic and bacteria must be able to protect itself from this product.

Catalase is a bacterial enzyme that decomposes hydrogen peroxide H₂O₂ into oxygen O₂ and water H₂O

Catalase test : is used to distinguish between staphylococci (positive) and streptococci (negative) and between aerobic and obligate anaerobic

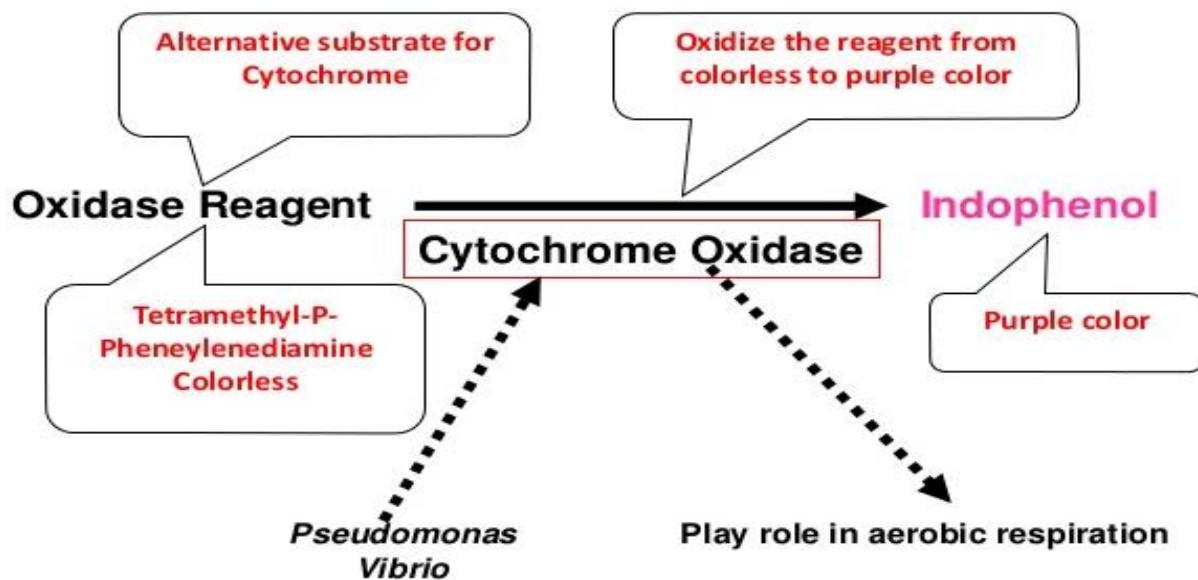
The presence of air bubbles indicates presence of catalase

- Place a small amount of a bacterial colony (18 to 24 hours old) on a clean glass slide.
- Add one to two drops of 3% hydrogen peroxide.
- **Positive:** Rapid bubble formation
- **Negative:** No bubble formation

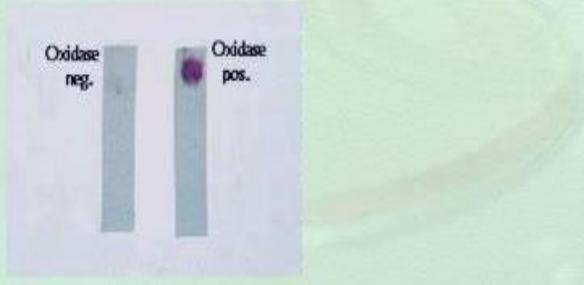


Oxidase test

It is used to determine if bacteria produces cytochrome oxidase which catalyzes the transfer of electrons in the chain respiration



- Oxidase reagent is added to filter paper with colony sample
 - Positive will change to purple color in 60 sec.
- Oxidase test can differentiate between gram negative bacteria within 2 groups
- **Pseudomonas**- oxidase positive
- **E. coli**- oxidase negative



Oxidase strips: The test used strips impregnated with a reagent TMPD, which is a redox indicator.

Appearance of dark purple color indicates presence of oxidase



Indole test

Inoculate the test organism with tryptophane broth



Incubate 24h at 37C



After incubation , add 1 ml of Kova's reagent

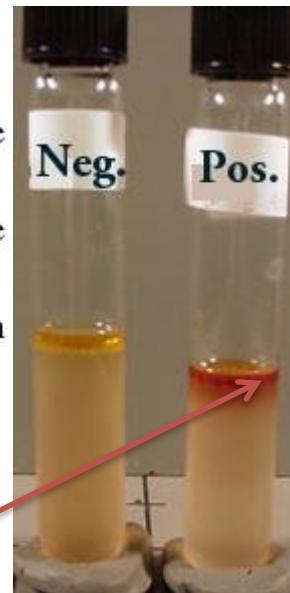
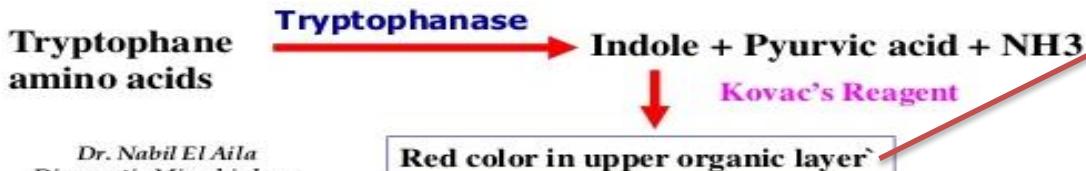


Shake the tube gently and read immediately

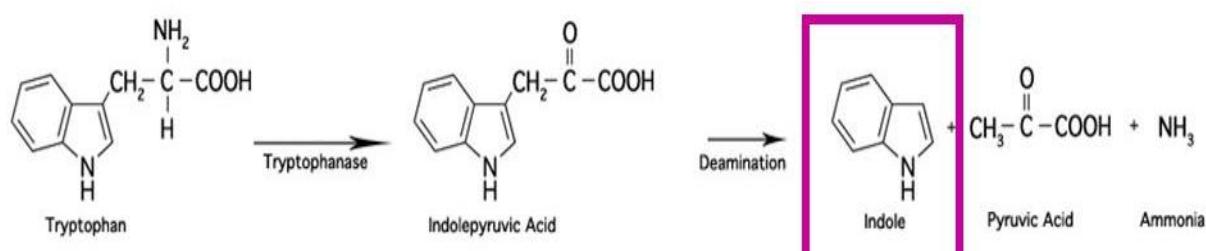
IMViC: Indole test

Principle

- Certain microorganisms can metabolize tryptophan by **tryptophanase**
- The enzymatic degradation leads to the formation of **pyruvic acid, indole and ammonia**
- The presence of indole is detected by addition of Kovac's reagent.



Indole is then tested for by a colorimetric reaction with p-dimethyl-aminobenzaldehyde (active chemical in Kovac / Ehrlich reagent) resulting in formation of red complex.



Test for indole with Kovac's reagent

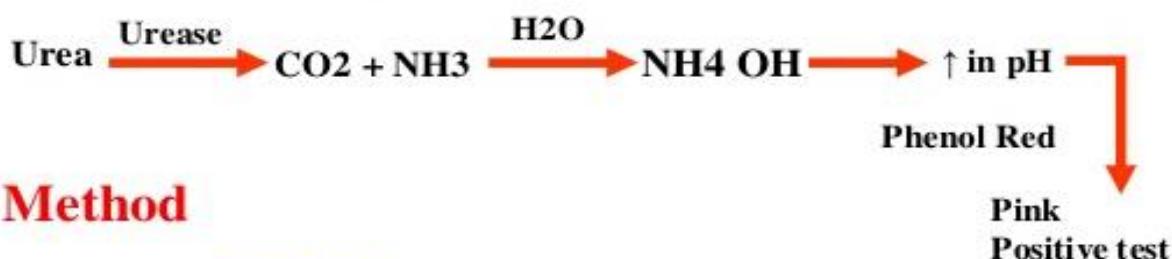
Urease test

- Use of urease strips method:
apply the organism and add drop of water-
- Use Urea agar medium:



Principle

- Urea agar contains urea and phenol red
- Urease is an enzyme that **catalyzes the conversion of urea to CO₂ and NH₃**
- Ammonia combines with water to **produce ammonium hydroxide**, a strong base which ↑ pH of the medium.
- ↑ in the pH causes phenol red to turn a deep pink. This is indicative of a positive reaction for urease

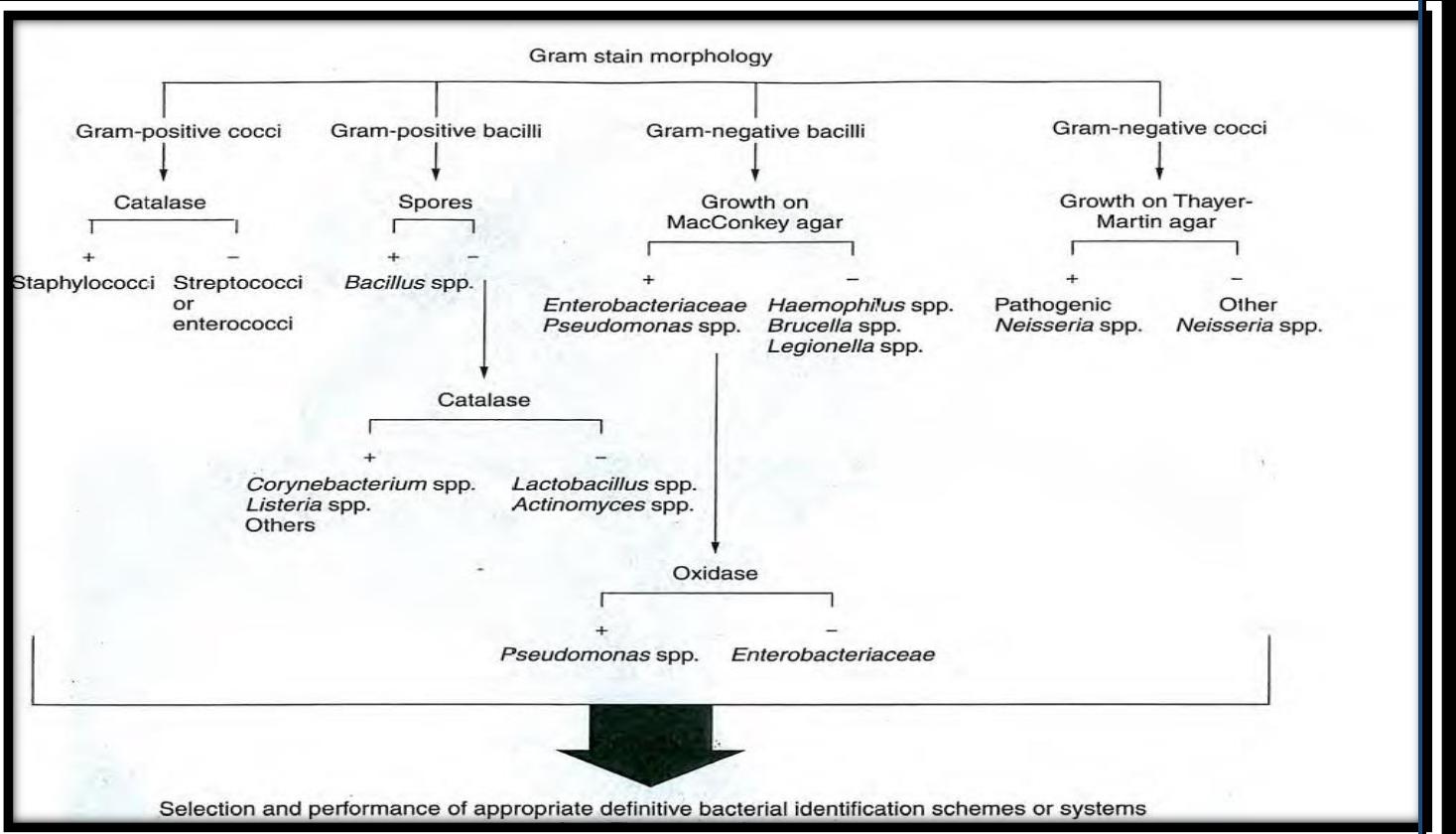


Method

- Streak a urea agar tube with the organism
- incubate at 37°C for 24 h



- Other biochemical tests of interest include
 - H₂S production
 - Amino acid degradation: detection of amino acid decarboxylase enzymes



B. Rapid test

Any of these test in rapid miniaturized system that can detect for 23 characteristics in small cups called **Rapid test: API strip**

- The info from the rapid test are input into a computer to help in identification of the organisms.

Rapid test: a biochemical system for the identification of **Enterobacteriaceae** and other Gram –ve bacteria.

- It consist of **plastic strips with 20 µl of dehydrated biochemical substrates** used to detect biochemical characteristics.

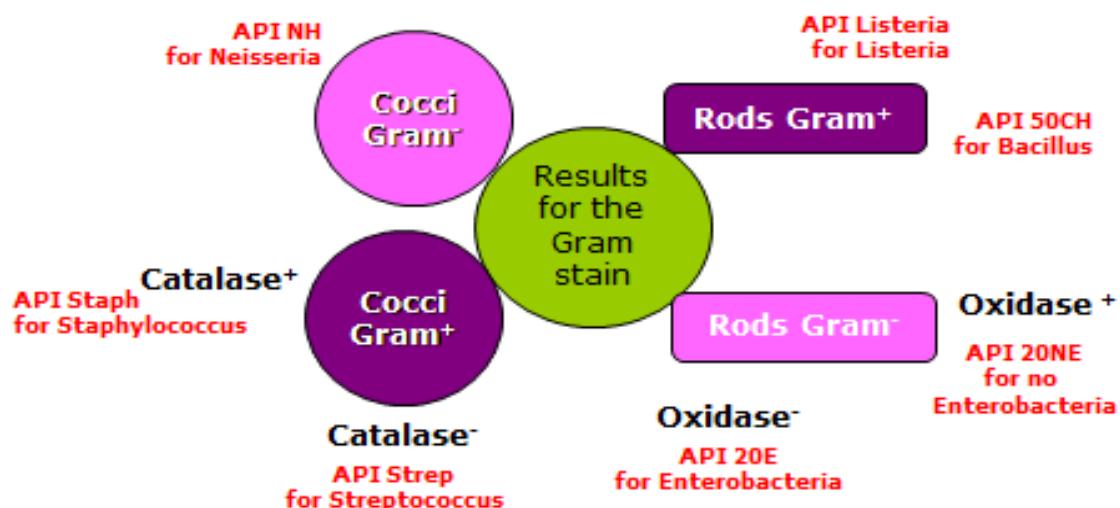
- The biochemical substrates are inoculated with pure cultures and suspended in physiological saline.
- After 5 hrs-overnight the 20 tests are converted to 7-9 digital profile.

With an unknown bacteria to identify, which API system use ?

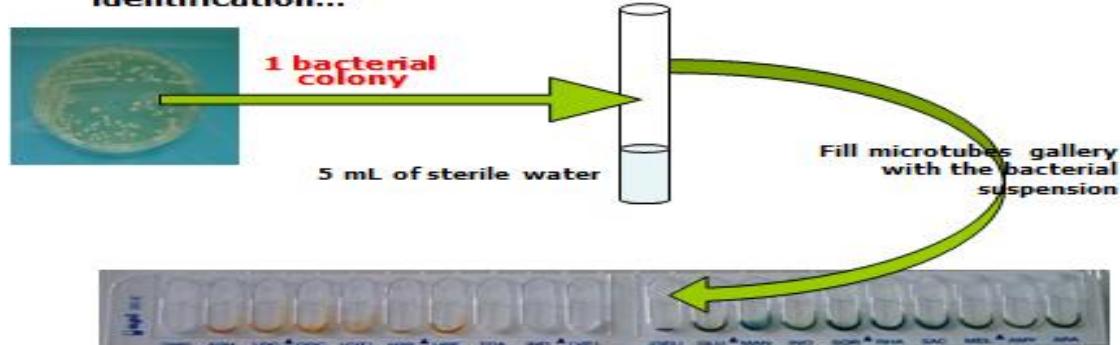
... before use API system, it's necessary to perform preliminary tests on bacteria to identify

- Preliminary test 1 : Gram stain + microscopic observation**
 - Distinguishes bacteria according to their form (cocci, rods) and their response to color (purple = Gram + bacteria, pink = Gram - bacteria)
- Preliminary test 2 : Bacteria respiratory enzymes test**
 - Distinguishes bacteria into groups according to the existence of two enzymes, « oxidase » enzyme, or « catalase » enzyme

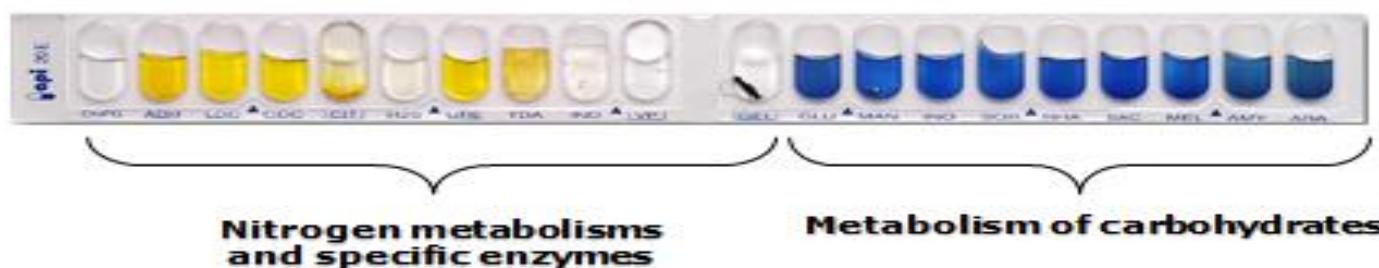
With an unknown bacteria to identify, which API system use ?



Introducing the API 20E system for Enterobacteria identification...



View of API 20E just after seeding...



24h / 37° C....

ONPG (β galactosidase); ADH (arginine dihydrolase); LDC (lysine decarboxylase); ODC (ornithine decarboxylase); CIT (citrate utilization); H₂S (hydrogen disulphide production); URE (urease); TDA (tryptophan deaminase); IND (indole production); VP (Voges Proskauer test for acetoin); GEL (gelatin liquefaction); the fermentation of glucose (GLU), mannitol (MAN), inositol (INO), sorbitol (SOR), rhamnose (RHA), sucrose (SAC); Melibiose (MEL), amygdalin (AMY), and arabinose (ARA); and OXI (oxidase).

API 20 E after incubation...Positive results for all tests :



API 20 E after incubation...Negative results for all tests :

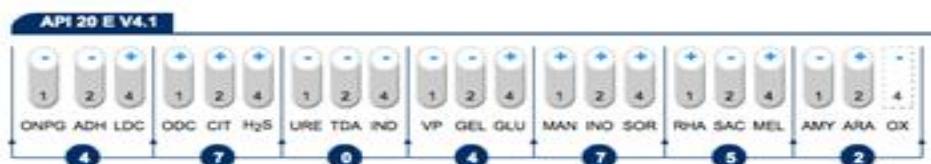


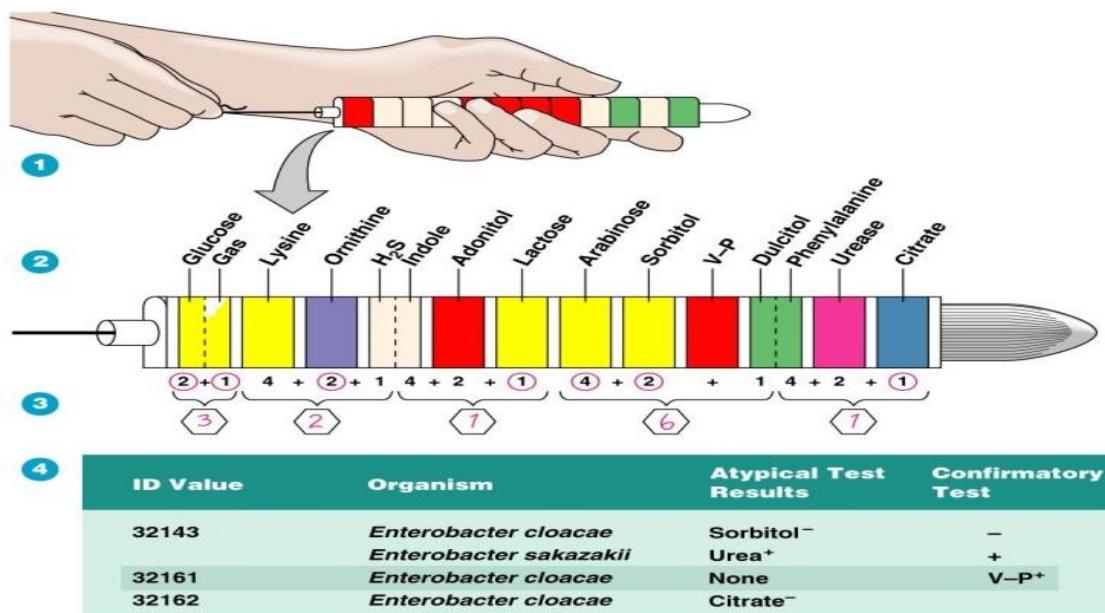
Example of results for bacteria to test :

1-Reading results :



2-Entering results in the database software:





Commercial ID systems

Rapid and innovative automated systems
Examples

- ARIS (Trek)
- MicroScan (Dade-Behring/Seimens)
- Phoenix (Becton-Dickinson)
- **Vitek (bioMérieux)**



- Andromas = automated system of bacterial identification using **mass spectrophotometry**
 - developed by a French team of researchers from Necker hospital in Paris
- It is a Physical detection** of molecules (usually **proteome**) contained in bacteria cytoplasm by **mass spectrometry**

Limitation of phenotypic methods

- ❖ Inability to cultivate on artificial medium.

Ex-Treponema pallidum

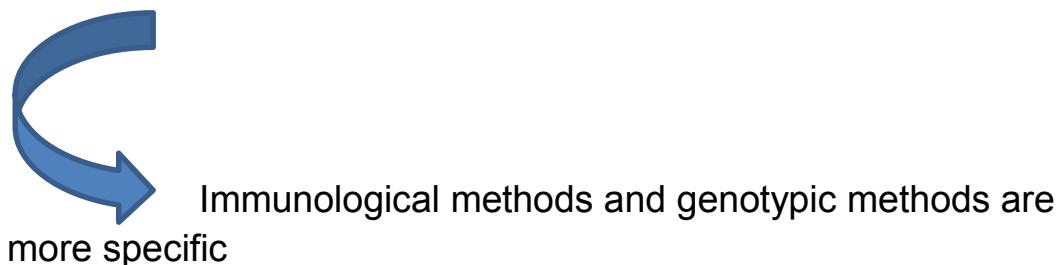
Environmental researchers estimate that **< 1%** of microorganisms **are culturable** and therefore it is not possible to use phenotypic methods of identification.

These microorganisms are called viable nonculturable (VNC).

- ❖ Fragility of organisms and failure to survive when transported.
- ❖ Fastidious nature of some microorganisms.

Ex-Bartonella, Leptospira

- ❖ Administration of antibiotic before specimen is obtained.



Immunological methods

Immunological Methods for Identification and Detection of Microorganisms' Antigens in the Pure Culture or in the Specimen

- Bacteria possess many **highly specific structures that may be antigenic** (capsular polysaccharides, flagellar proteins, exotoxins, and several cell wall components. These **antigens**, which present on whole bacteria or are at free state as bacterial exotoxins may be demonstrated with specific **antigen-antibody reactions** *in vitro*.
- Using the **antibodies** of known specificity bacterial identification can be made by formation of **antigen-antibody complex**.

Immunological methods involve the interaction of a microbial **antigen with an antibody** (produced by the host immune system).

• **Lab kits** based on this technique is available for the identification of many microorganisms.

Serology

- A science that attempts to detect signs of infection in a patient's serum such as Ab for a specific microbe
- Serological tests based on Abs specifically binding to Ag.
 - Ag of known identity will react with Ab in an unknown serum sample.
 - Known Ab can be used to detect Ag in serum
- Ag-Ab reactions are visible by clumps, precipitates, color changes or release of radioactivity.
- The most effective tests have high specificity and sensitivity.

- Serological methods are useful in determining the identity of strains (serotypes) of microorganisms that differ in antigenic

composition of a structure or product on the bacteria cell surface

- Serological methods determine the identity of species as well as relationship between organisms.
- Serological methods studies blood serum for evidence of infection and other parameters by evaluating antigen-antibody reactions in vitro

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:

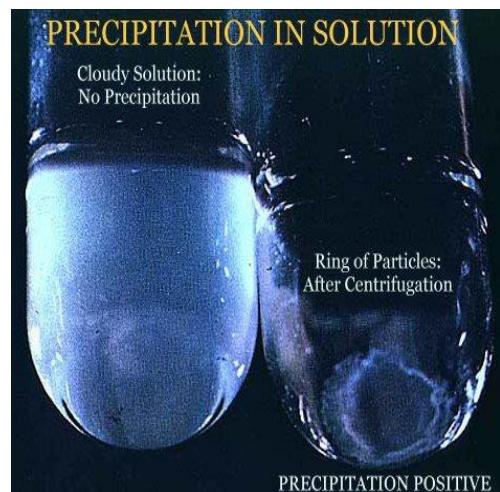
- Detection of specific and non specific antibodies (IgM & IgG) by using antigens or organisms

Numerous types of serologic test -differ in their speed and sensitivity.

1. Precipitation tests
 - (a) Immuno diffusion
 - (b) Immunoelectrophoresis
2. Agglutination tests
3. Neutralization
4. Complement fixation
5. Immuno fluorescence
6. Radioimmunoassay (RIA)
7. Enzyme-Linked Immuno Sorbent Assay (ELISA)
8. Western Blotting

A. Precipitation reactions

- Precipitation is the **interaction of a soluble Ag with an soluble Ab** to form an **insoluble complex**.
- The complex formed is an aggregate of Ag and Ab.
 - Precipitation reactions occur maximally only when the **optimal proportions** of Ag and Ab are present



B. Agglutination tests

- More sensitive
- Agglutination occurs due to the cross-linking of particulate antigens by antibody molecules.
- **Agglutination is the visible clumping of insoluble particles (bacteria cells), whereas precipitation involves the aggregation of soluble molecules**

Types of Agglutination Reactions

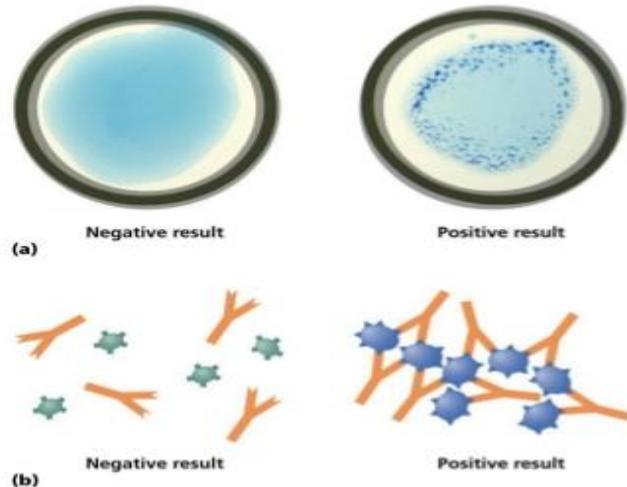
(a) Direct agglutination reactions

These reactions can be performed on slides (rapid tests) or on microliter plates or tubes for Antibody titration

- In a thick suspension of the bacteria, the binding of specific antibodies to surface antigens of the bacteria causes the bacteria to clump together in visible aggregates.
- This type of agglutination is called bacterial agglutination. Because tube testing allows more time for antigen-antibody reaction, it is considered to be more sensitive than slide testing.

1. Slide agglutination (Qualitative Test)

- Unknown Ag
- Add known antiserum (Ab)



Used for serotyping and sero grouping exp salmonella, vibrio cholera and E.coli

Exp: Salmonella .

Antigenic structure of Salmonella

- Two sets of antigens
- Detection by serotyping
- **1 Somatic or O Antigens** contain long chain polysaccharides (LPS) comprises of heat stable polysaccharide commonly.
- **2 Flagellar or H Antigens** are strongly immunogenic and induces antibody formation rapidly and in high titers following infection or immunization. The flagellar antigen is of a dual nature, occurring in one of the two phases.

- Serotype is determined by O antigens present **and** phase 1/phase 2 H antigens
- Over 2400 different serotypes
 - Over 1400 common to humans
 - No commercial kit available

- Commercially available polyvalent antisera designated **A, B, C1, C2, D, E**, and **Vi** are commonly used to preliminarily group *Salmonella* spp.
- The antisera A through E contain antibodies against somatic ("O") antigens
- the Vi antiserum is prepared against the capsular ("K") antigen of *S. typhi*
- Typing is performed using a slide agglutination test.
- S. typhi* is positive with Vi and group D.

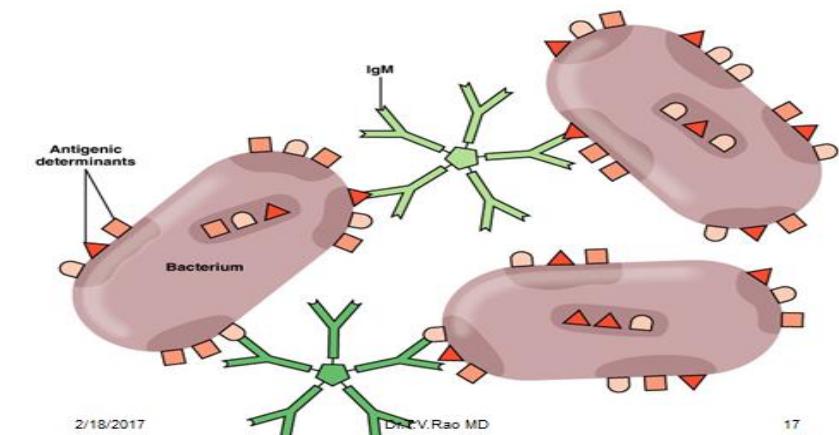
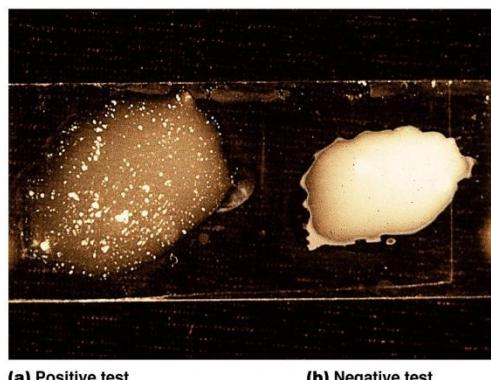
Antigen: isolated *Salmonella* in suspension

Antibody: specific antisera against *Salmonella*

Place test *Salmonella* in a drop of saline on a slide

Add a drop of antiserum, mix and rock slide for approx. 1 minute

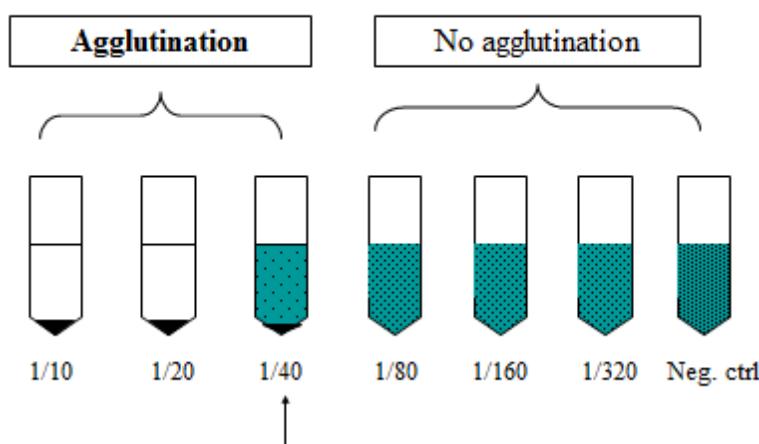
Examine for agglutination



2.

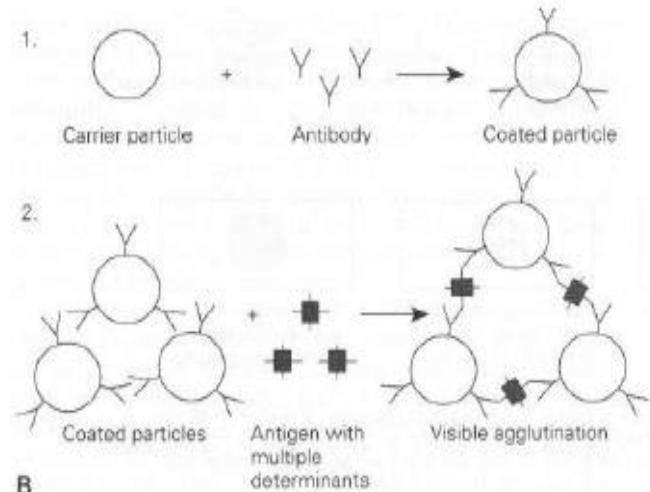
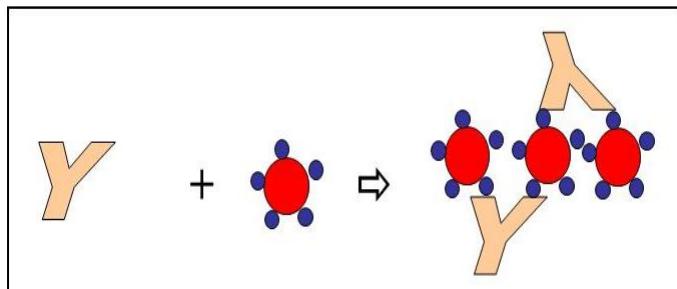
Tube Agglutination Test

Identify and titrate antibodies in the patient's serum



b. Passive agglutination test (PAT)

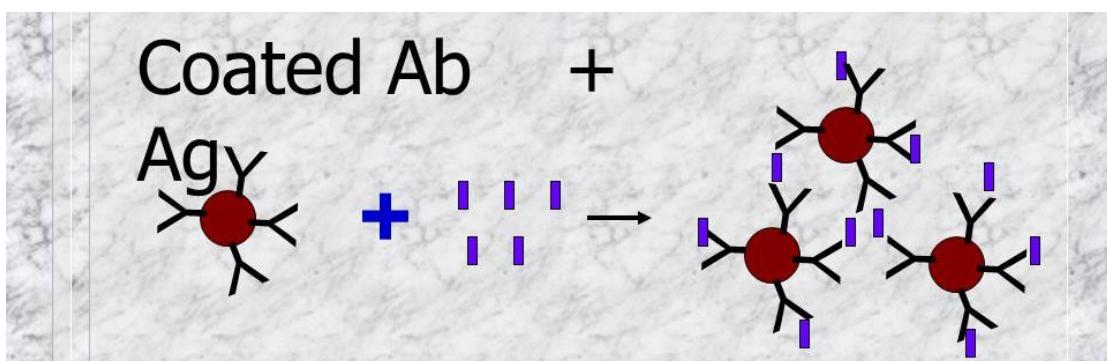
- ❖ Chemically link soluble antigen to inert particles such as LATEX or RBC
- ❖ Addition of specific antibody will cause the particles to agglutinate



Applications

- Measurement of antibodies to soluble antigens

- ❖ Reverse PAT: antibody linked to LATEX



e.g. Lancefield grouping in Streptococci.

Streptococcus Group A Colour enhanced latex test for the detection of extracellular antigens of Streptococcus.



Streptococcus

II. Classification based on serologic reactivity of cell wall

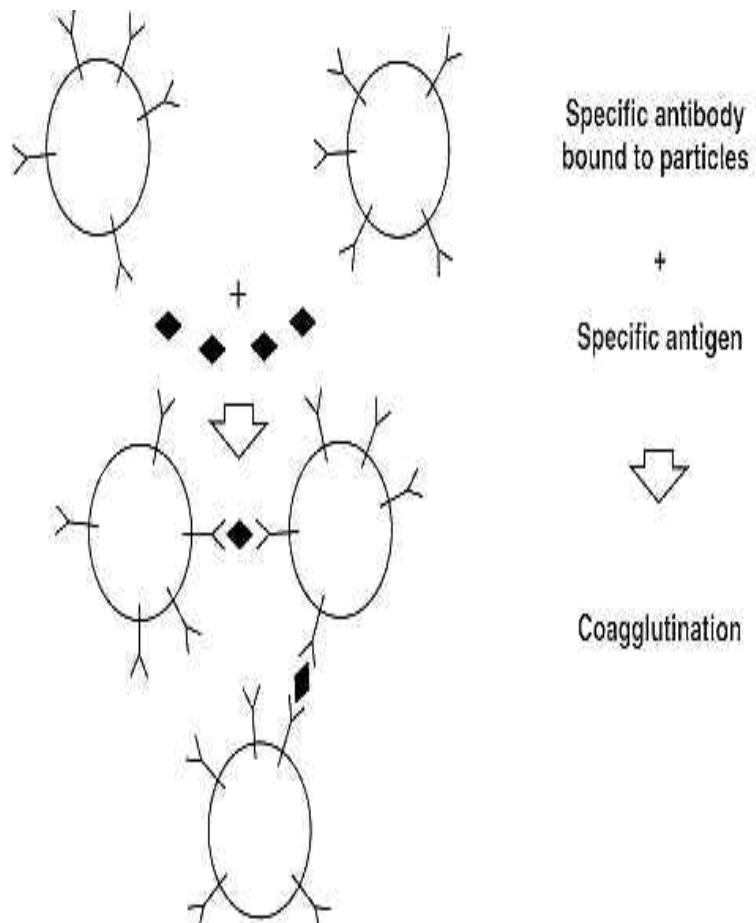
polysaccharide Ags as originally described by R. Lancefield

- >18 group-specific Ags (Lancefield groups A-U) were established according to the *carbohydrate (C) Ag* present in the cell wall.
- **Group A:** *S. Pyogenes* - causes pharyngitis, skin infections
- **Group B:** *S agalactiae* - causes neonatal septicemia, meningitis
- **Group C:** *S. equisimilis* – endocarditis, bacteremia, meningitis
- **Group D:** Enterococci – UTI, endocarditis
- **Group H:** *S. sanguis* – endocarditis, dental caries
- **Group K:** *S. salivarius* - endocarditis, caries

c. Coagglutination test

- Agglutination test in which inert particles (latex beads or heat-killed *S aureus* Cowan 1 strain with protein A) are coated with antibody to any of a variety of antigens and then used to detect the antigen in specimens or in isolated bacteria

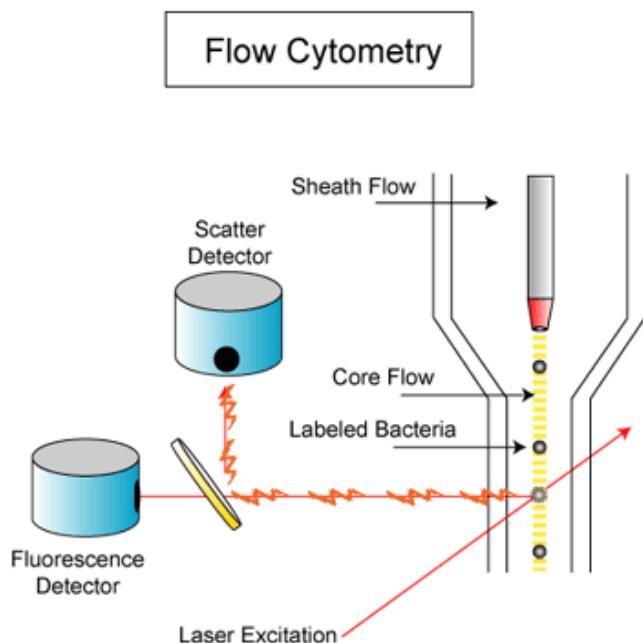
- *S.aureus*: most frequently used because it has protein A in its outer surface that naturally adsorbs the Fc portion of the antibody



- Highly specific but not as sensitive as latex agglutination.
- Used for identification of streptococci, *Neisseria meningitidis*, *Neisseria gonorrhoeae*, *Vibrio cholera* O139 and *Haemophilus influenzae*.

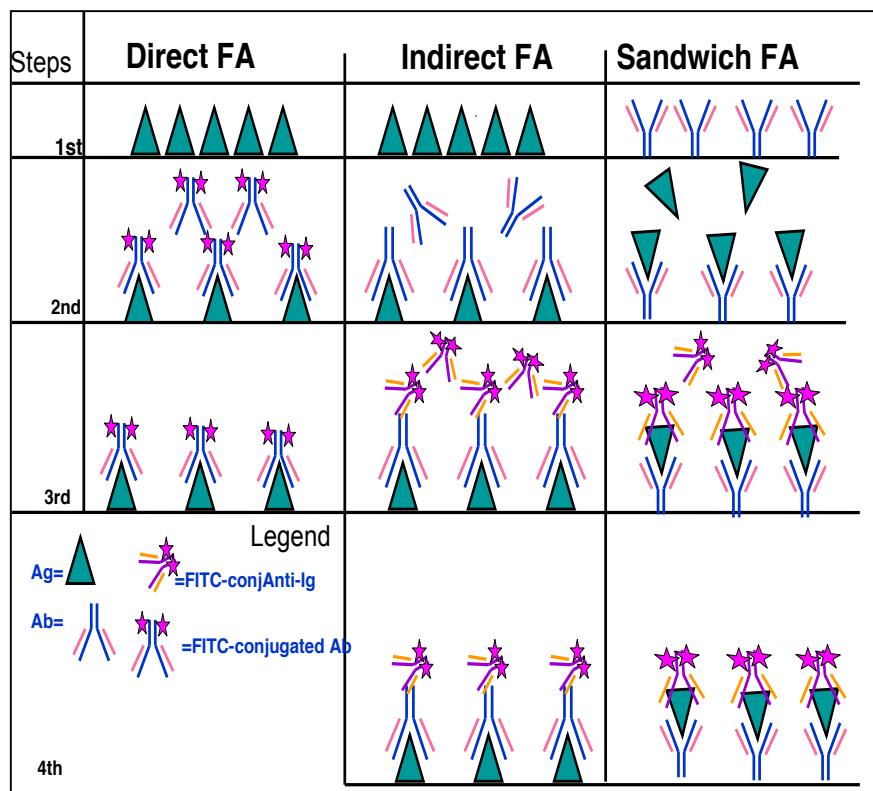
C. Immuno fluorescence

- ❖ Classical techniques are not successful in identification of microorganisms that cannot be cultured (exp. Bacteria parasite)
- ❖ What do is the name of microorganisms that cannot be cultured?
- ❖ **Flow cytometry** allows single or multiple microorganisms detection an easy, reliable and fast way.
- ❖ In flow cytometry microorganisms are identified on the basis of the **cytometry parameters** or by means of certain dyes called **fluorochromes** that can be used independently or bound to specific antibodies



- Immuno-fluorescence

- Use fluorescein isothiocyanate labeled-immunoglobulin to detect antigens or antibodies according to test systems
- Requires a fluorescent microscope
- Exp detection of vibrio cholera



- **Advantages:** Sensitive and specific and Can be used for discrepant analysis
- **Limitations:** Expensive (Reagents and equipment), Subjective, Cross reactivity, Non-specific immuno-fluorescence
- **Time taken:** few minutes to few hours.

Problems with Serology: Other Health conditions interfere

Immunocompromised patients often give a reduced or absent humoral immune response.

Patients with infectious mononucleosis and those with connective tissue diseases

Patients receiving blood or blood products may give a false positive result due to the transfer of antibody

Genotypic methods

- The initiation of new molecular technologies in genomics is shifting traditional techniques for bacterial classification, identification, and characterization in the 21st century toward methods that examine the genetic material of the organisms.
- Genotypic testing is particularly useful in the case of organisms that are difficult to identify.
- Increasingly genotypic techniques are becoming the **sole means of identifying** many microorganisms because of its **speed and accuracy**.
- Genotypic methods of microbe identification include the use of :
 - Nucleic acid probes: nucleic acid hybridization
 - PCR
 - Nucleic acid sequence analysis
 - 16s rRNA analysis
 - particular use for identifying prokaryotes impossible to grow in a culture
 - The 16S rRNA gene A gene found only in prokaryotes
 - It can be species specific - differentiating one bacterial strain from another
 - It codes for the small subunit of ribosome
 - once the 16S molecule is sequenced, it can then be compared to the sequences of known organisms
 - RFLP
 - Plasmid fingerprinting.

Advantage of genotypic methods over phenotypic methods

SPEED,ACCURACY, COST

ability to detect nonviable organisms that are not retrievable by cultivation based method.

identification of bacteria grown in culture

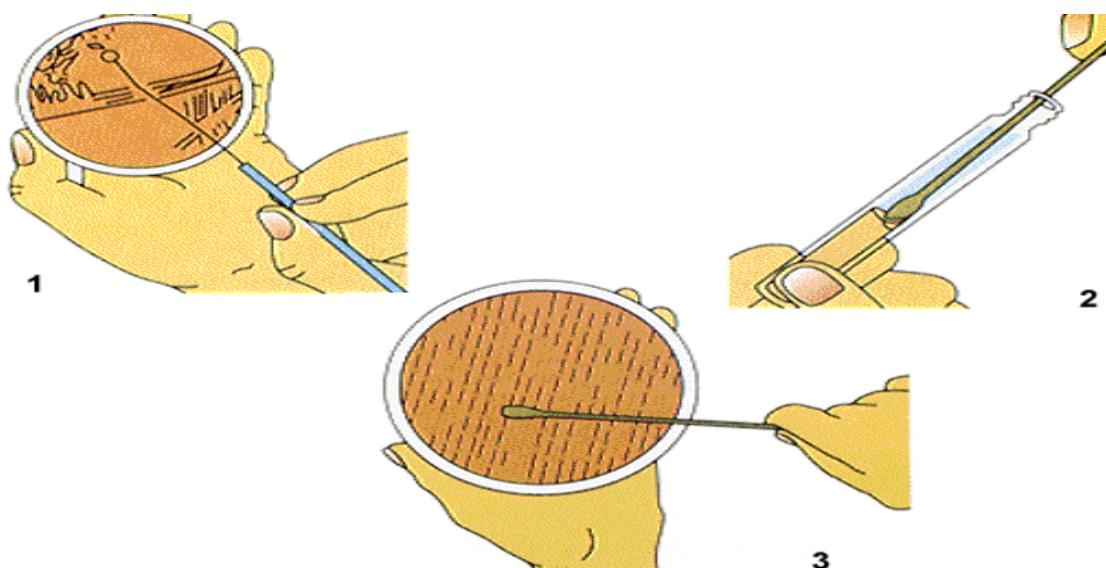
- 1)Slow growing bacteria
- 2)Common pathogen exhibit unusual phenotypic traits.

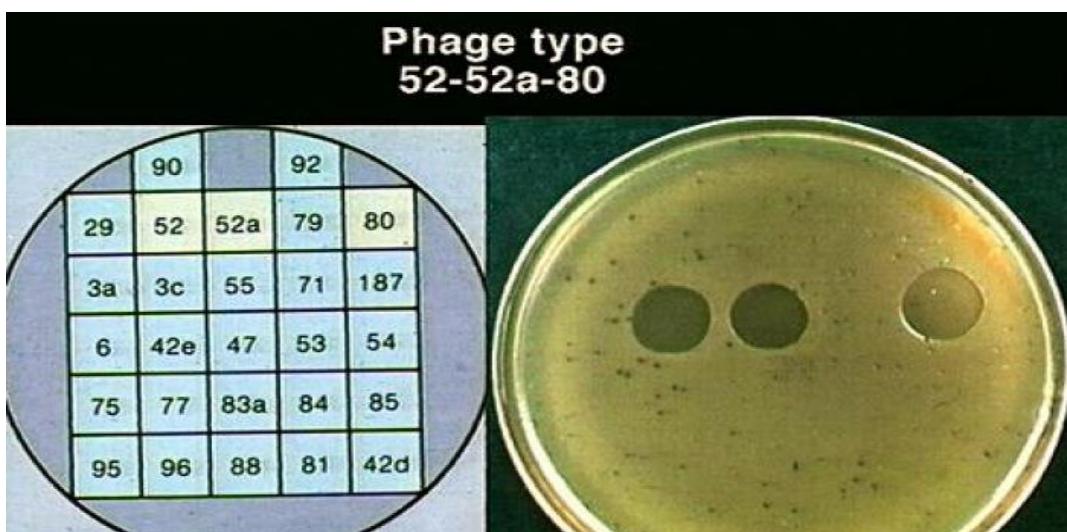
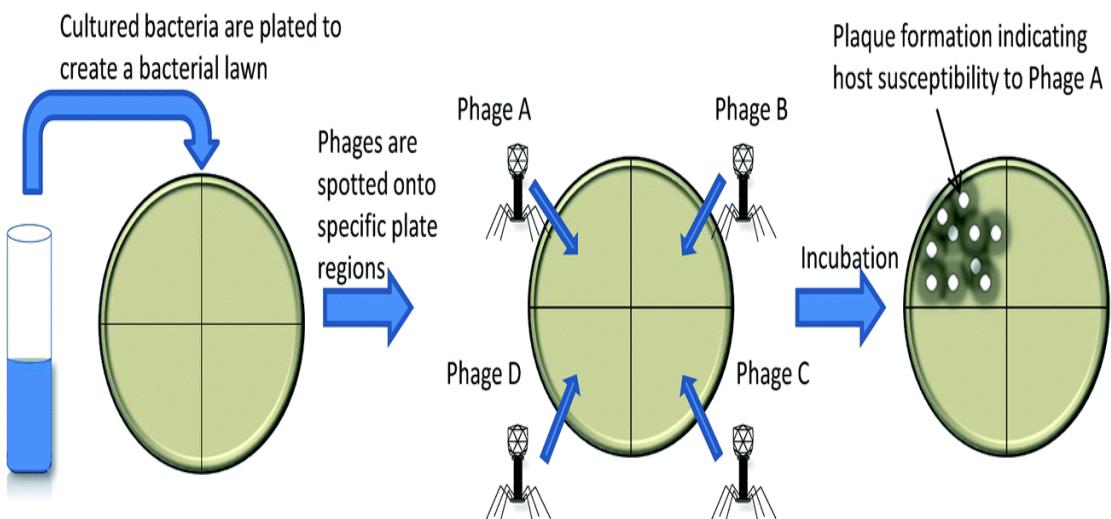
Bacteriophage typing

- ❖ Bacteriophage is a virus that infect bacteria
- ❖ Some of bacteriophage can infect only a single strain of bacteria
- ❖ Phage typing is a method used for detecting of different bacterial strains within a single species.
- ❖ **Classifies** bacterial organisms according to susceptibility of the bacteria to **lysis** by the panel of bacteriophage.
- ❖ Phage typing has played useful in epidemiologic roles for *S.aureus* & *S.enterica* serotype Typhi.
- ❖ Bacteriophage typing is based on the **specificity of phage surface receptor** for the bacteria surface receptor.
 - Only those phages that can **attach to the surface receptors** can cause lysis.

The procedure

- A plate is **heavily inoculated** so that there is no uninoculated areas.
 - The plate is **marked off** in squares (15-20 mm) and each square **inoculated** with a drop of suspension for **different phages**.
- The plate is incubated for 24 hrs then observed for plaques.
- The phage type is reported as a specific **genus and species** followed by **the types** that can infect the bacterium.
 - E.g. 10/16/24 means that the bacteria is sensitive to phages 10, 16 and 24.
 - Phage typing remain a **tool for research and reference labs**.





Exp typing *staphylococques aureus*

- **Based on coagulase**
 - Coagulase" +": e.g., *S. aureus*
 - Coagulase" -": e.g., *S. epidermidis* & *S. saprophyticus*
- **Phage typing**

S. aureus: 3 **phage groups**, 26 phage types.

 - group 1: TSST-1-producing strains
 - group 2: exfoliative toxin-producing strains
 - group 3: enterotoxin-producing strains

----is of epidemiological value

Bacteriocin typing

- Bacteriocins are bactericidal **antibiotic like** substances.
- Apparently **protein** in nature which are produced by many bacteria.
- Have a killing action on strains of the **same or closely related species**.
- The **narrow specificity** of their action and their protein nature distinguish them from other(classical) antibiotics.
- The first discovery was reported by **Gratia in 1925** of a highly specific antibiotic produced by *E. coli* and active against other strains of the same species.

Bacteriocin typing

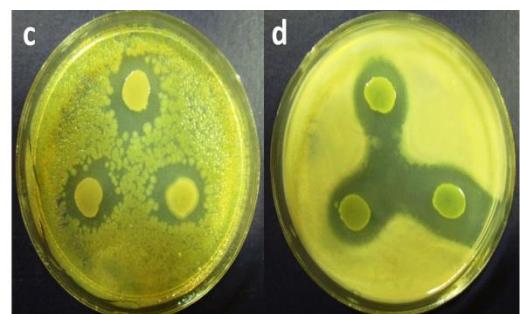
- Done for identification of isolated strains : as they are **same or different**.
- A strain may be typed by:
 - 1) Activity of their bacteriocin against a set of indicator strains of same or closely related species, or
 - 2) Pattern of their susceptibility to the bacteriocin of a set of indicator strains.
- If the isolates are **same strains**, their bacteriocin production and susceptibility patterns will be **identical**.

Screening for bacteriocin production

Agar well diffusion assay

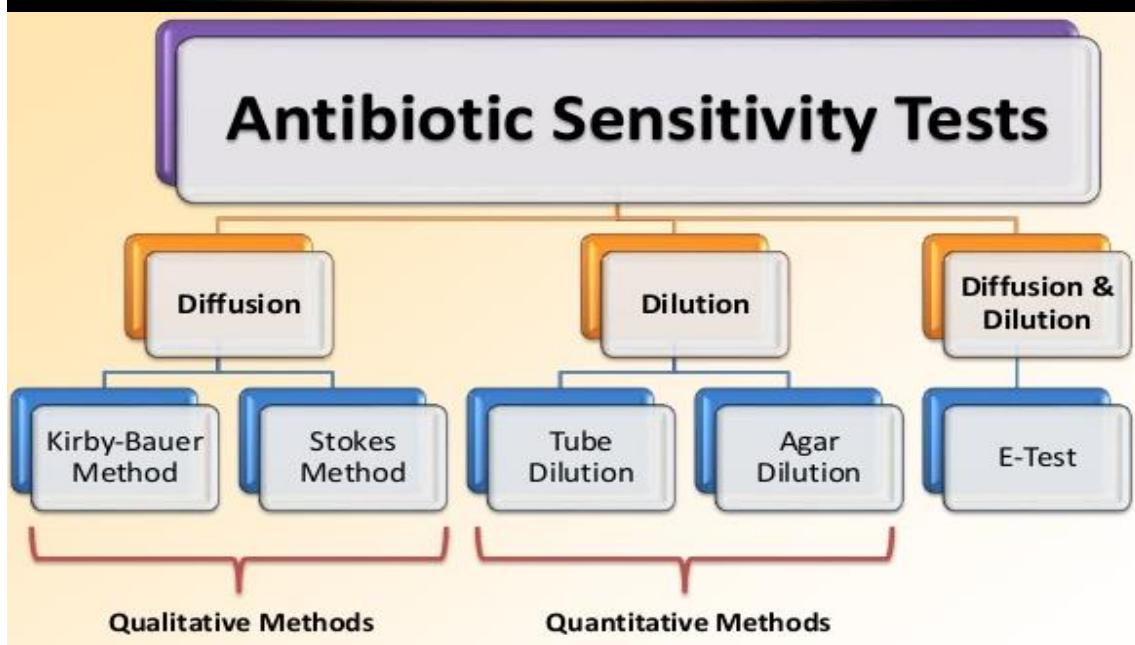
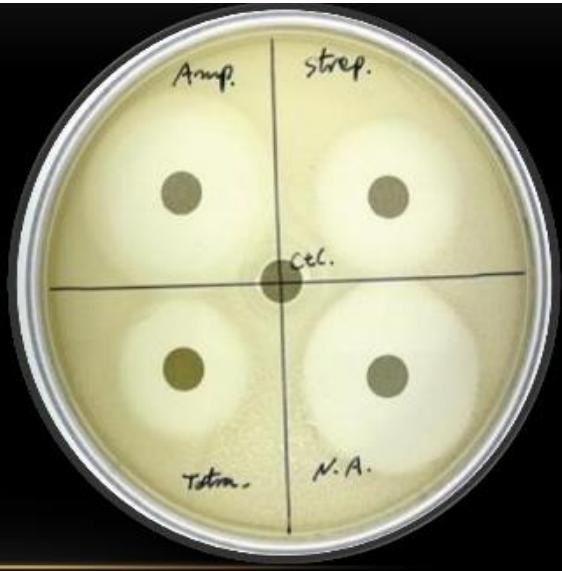


- The *Lactobacillus* strains were able to produce bacteriocin whose antibacterial activity was analyzed by agar well diffusion assay.
- The antibacterial substance produced by *Lactobacillus* inhibited pathogen such as *Staphylococcus aureus* and *E. coli*.
- Clear zone was observed around the wells.
- The inhibition zone obtained in *E.coli* plate :- 5-8mm
- The inhibition zone obtained in *Staphylococcus* plate :-4-9mm.



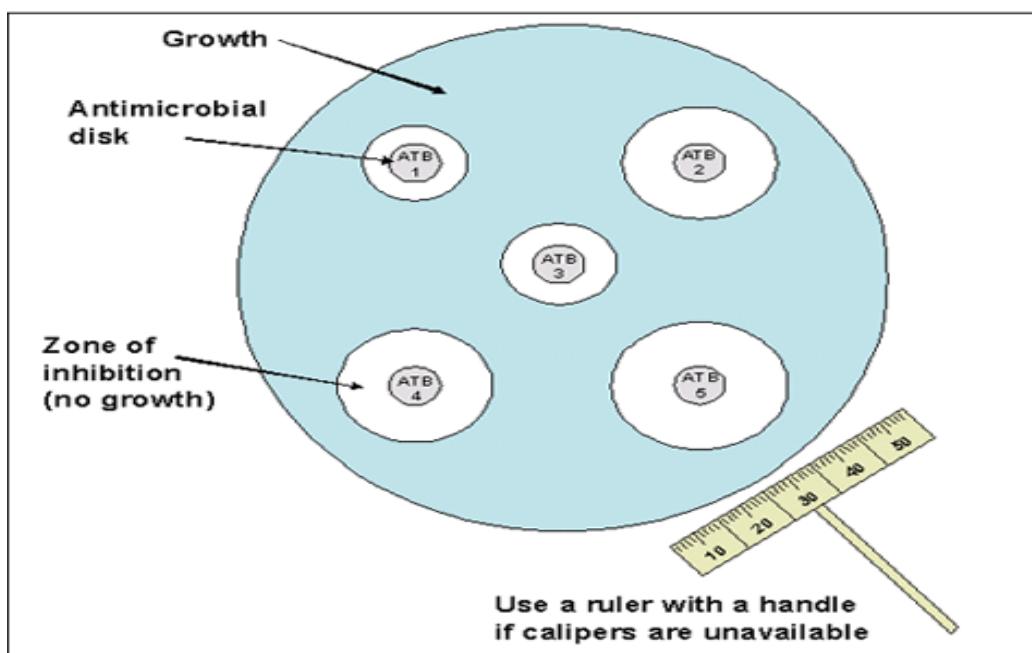
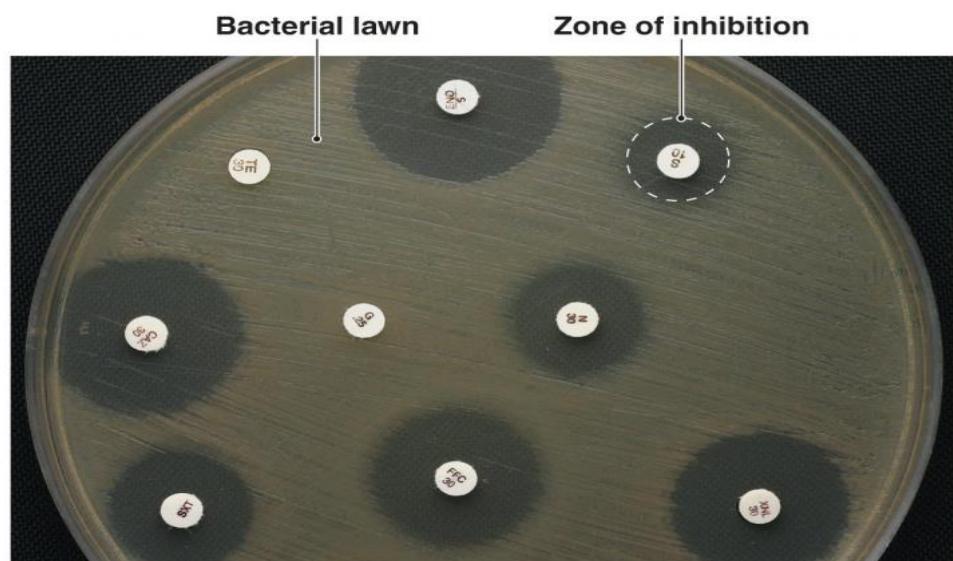
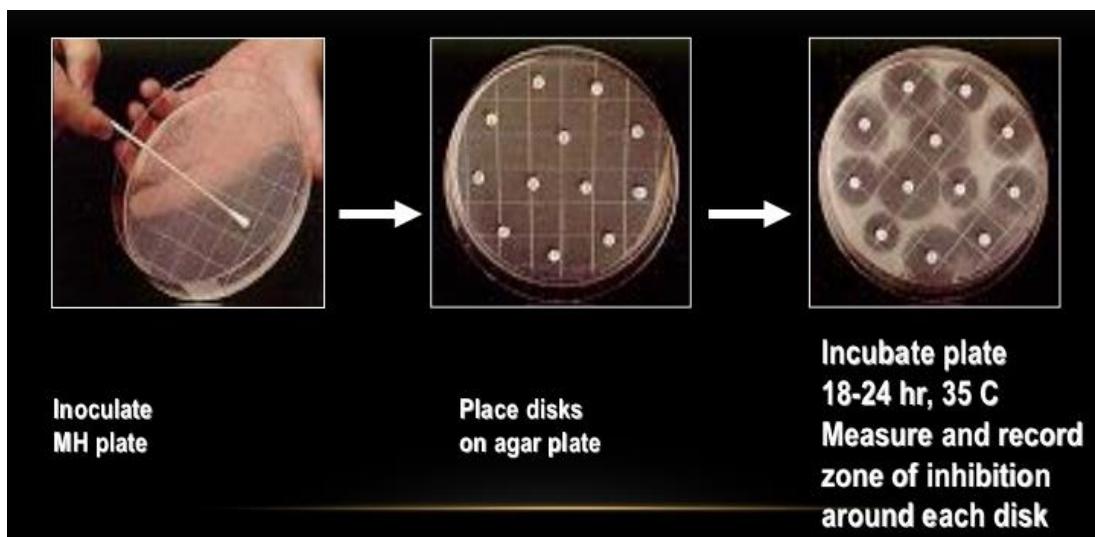
Antibacterial susceptibility testing

- Antimicrobial susceptibility tests measure the ability of an antibiotic or other antimicrobial agent to inhibit bacterial growth *in vitro*. This ability may be estimated by either the dilution method or the diffusion method.



Disk diffusion (Kirby Bauer):

- swab bacterial suspension on agar plate
- place small filter paper disks impregnated with a standard amount of antibiotic
- incubate overnight
- zone of inhibition of bacterial growth = measure of susceptibility (predefined cutoffs):
 - large zone = susceptible
 - small / no zone = resistant
 - zone between the above = intermediate



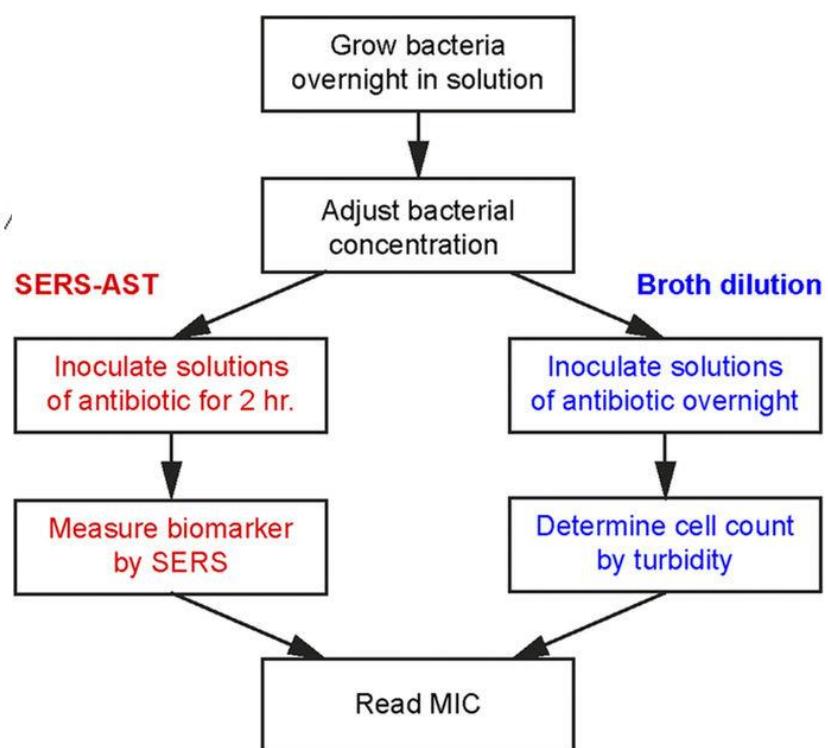
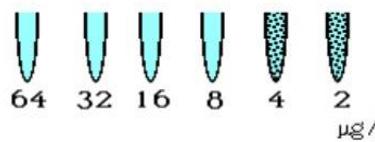
Dilution antibiotic sensitivity test

- ❖ a laboratory test used to determine the minimum concentration of antibiotic that required to control the infection (complete inhibition of the m.o)
- ❖ **Minimum Inhibition Concentration (MIC)**
 - ❖ The lowest concentration of antimicrobial agent that inhibits bacterial growth/ multiplication
- ❖ **Minimum Bactericidal Concentration (MBC) or Minimum Lethal Concentration (MLC)**
 - ❖ The lowest concentration of antimicrobial agent that allows less than 0.1% of the original inoculum to survive

MIC (continued): Measurement methods

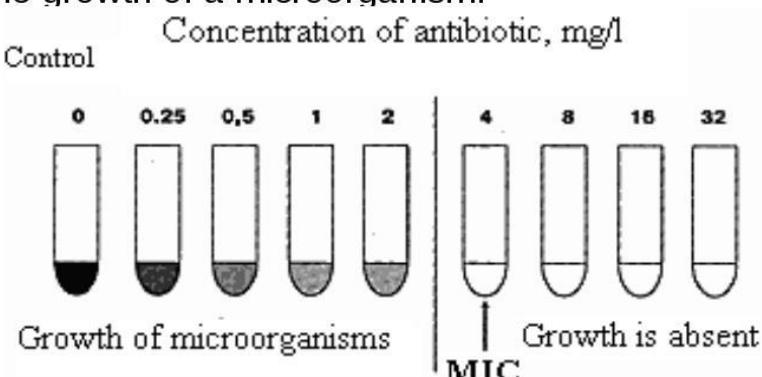
Broth dilution:

- Bacteria inoculated in culture broth + antibiotic (various concentrations) and incubated
- MIC = the lowest concentration of antibiotic which inhibited bacterial growth

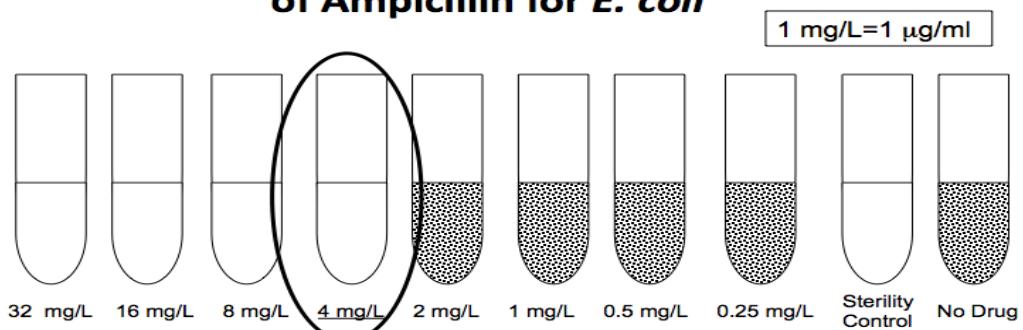


Definition of MIC by Tube Dilution Test

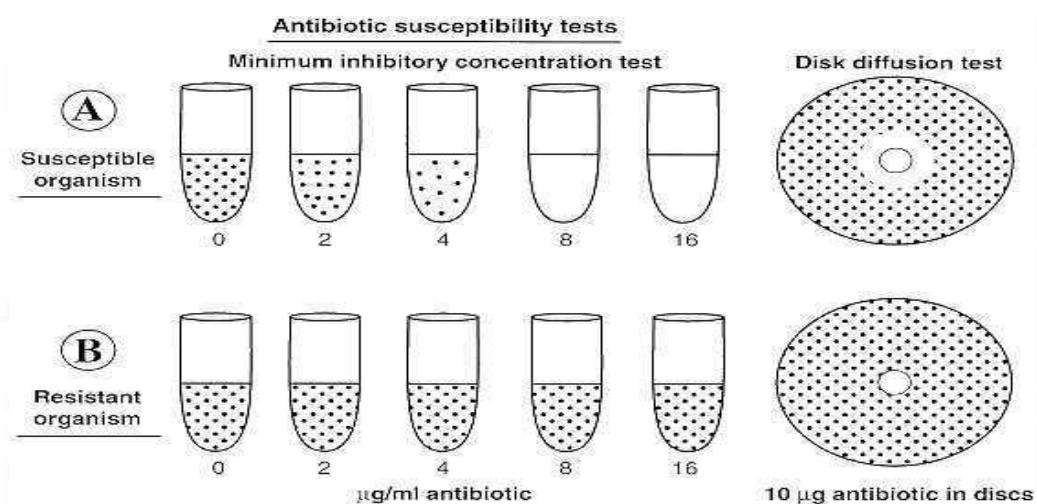
- Minimal inhibitory concentration (MIC) is the lowest concentration of an antibiotic that will inhibit the growth of a microorganism.



Minimal Inhibitory Concentration (MIC) of Ampicillin for *E. coli*



- Serial 2-fold dilutions of ampicillin in Mueller-Hinton broth
- Inoculum $5 \times 10^5 \text{ CFU/ml}$ (clear, non-turbid); Incubate 18 hrs. @ 35° C .
- Assess turbidity, visually
- Concentration of drug in first tube without visible turbidity is the **MIC** (4 mg/L)



Microbe identification

