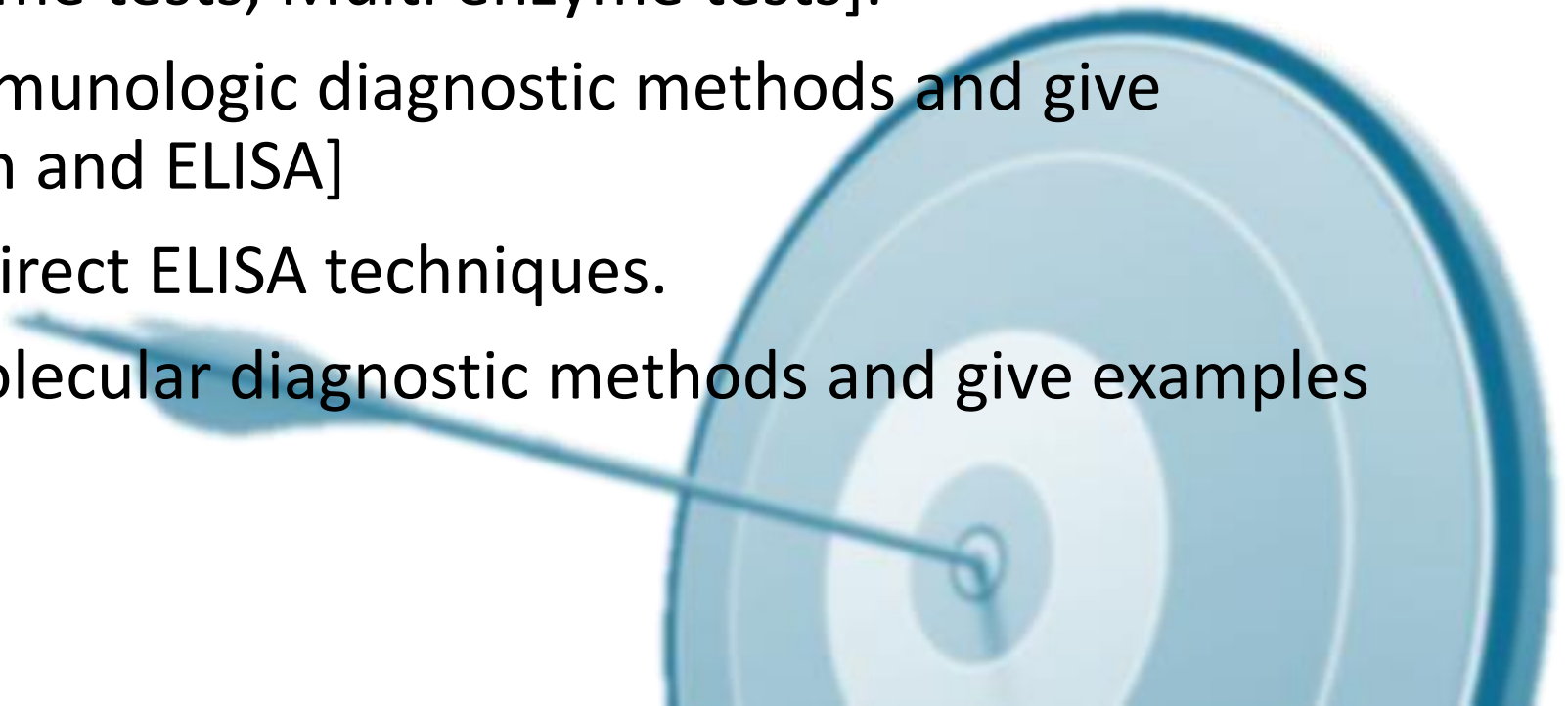
Lecture 5

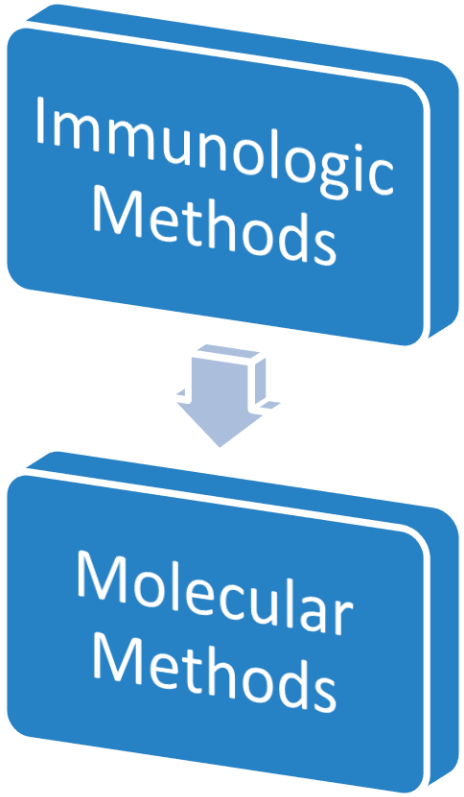
Identification of bacteria

Objectives

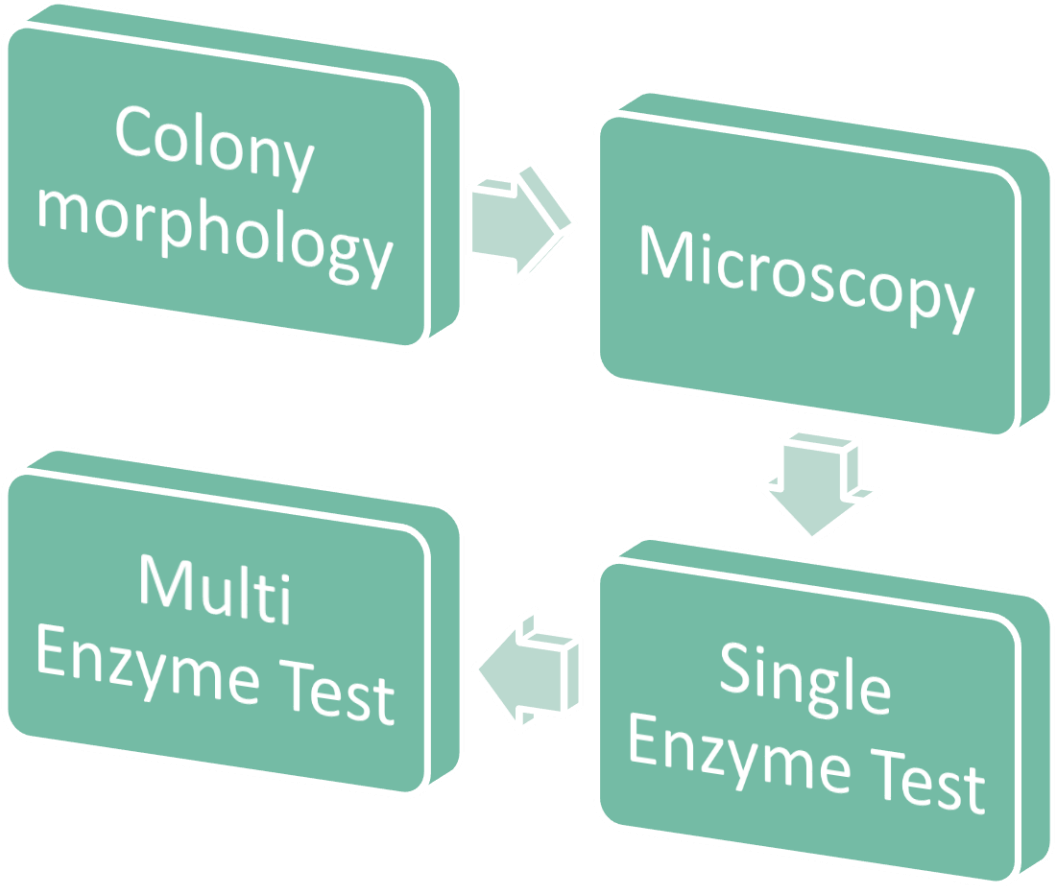
- Define bacterial colony and describe how to obtain pure culture
- Explain the principle and describe an example of each category of traditional culture-based identification methods [colony morphology, microscopy, single enzyme tests, Multi enzyme tests].
- Explain principles of Immunologic diagnostic methods and give examples [Agglutination and ELISA]
- Compare direct and indirect ELISA techniques.
- Explain principles of Molecular diagnostic methods and give examples [DNA probes and PCR].



Non culture based methods

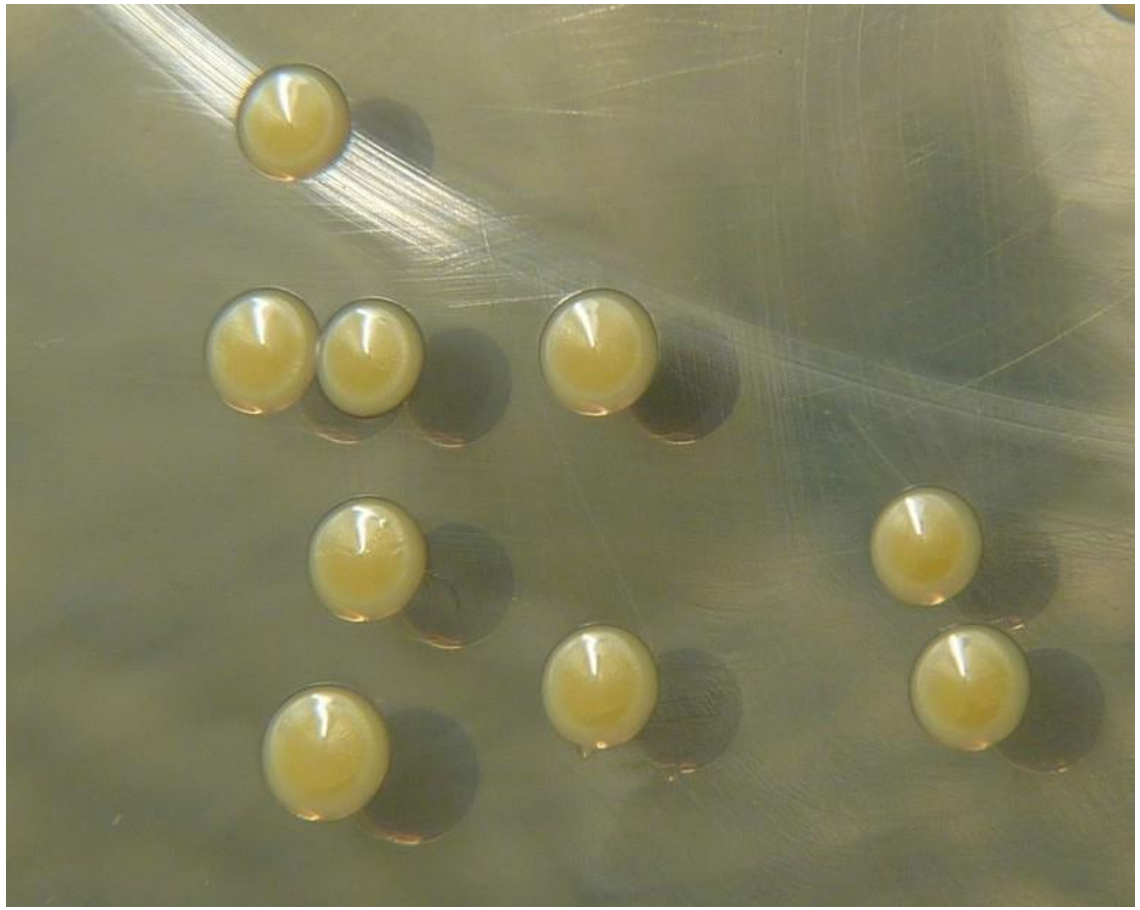


Traditional Culture based Methods



Colony

A **colony** is visible mass of large number of microorganisms, growing on or in solid medium.

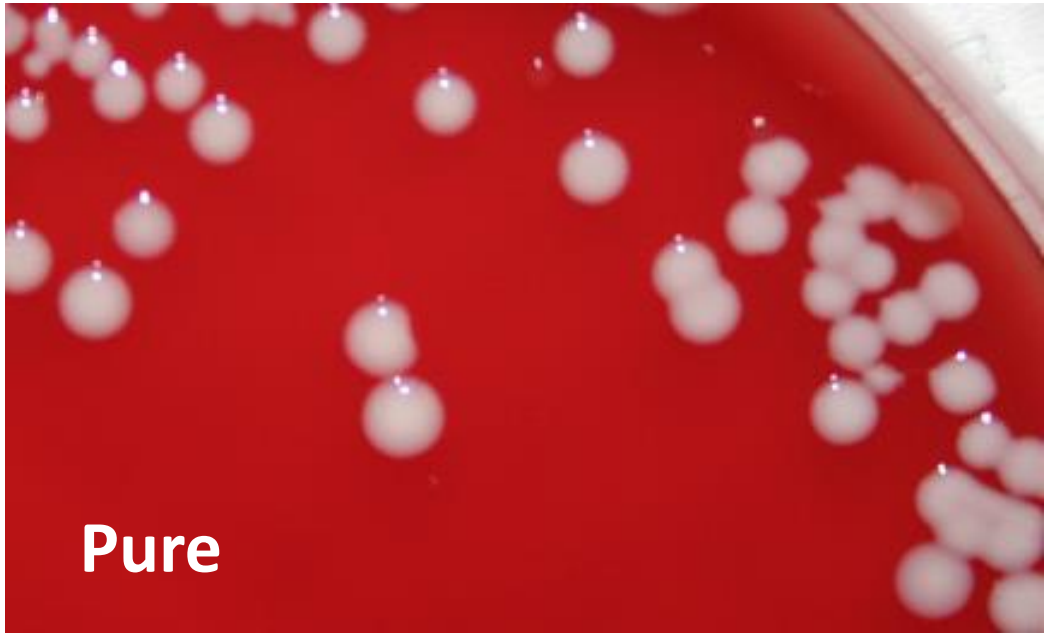


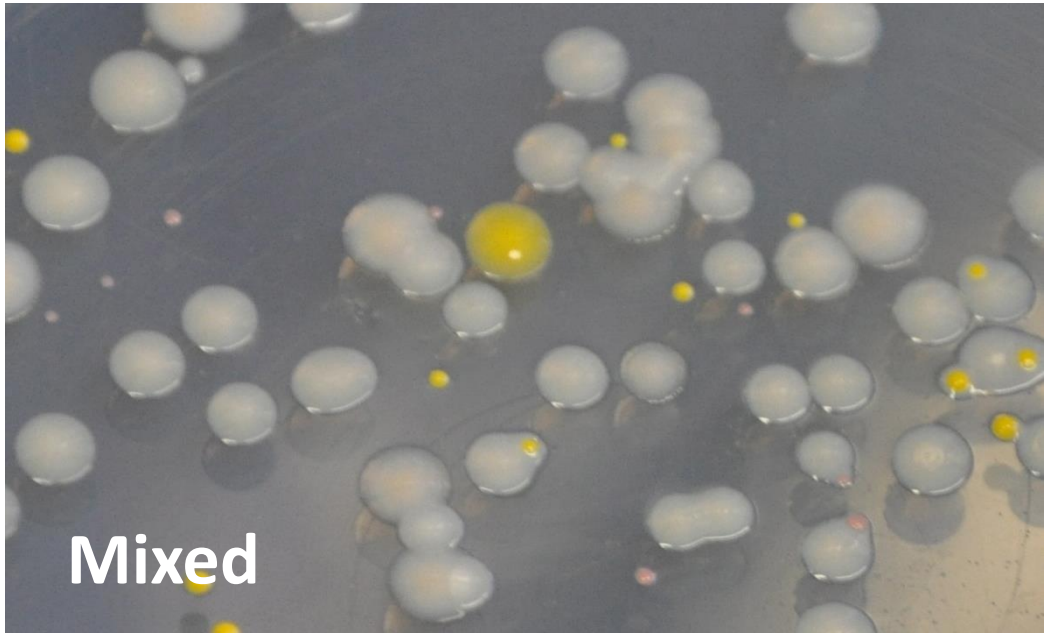
Pure Culture

- It is essential to start **identification** tests with **pure bacterial isolates** grown from a single colony.

- **Pure culture**, is a laboratory culture containing a **single** type of organism.

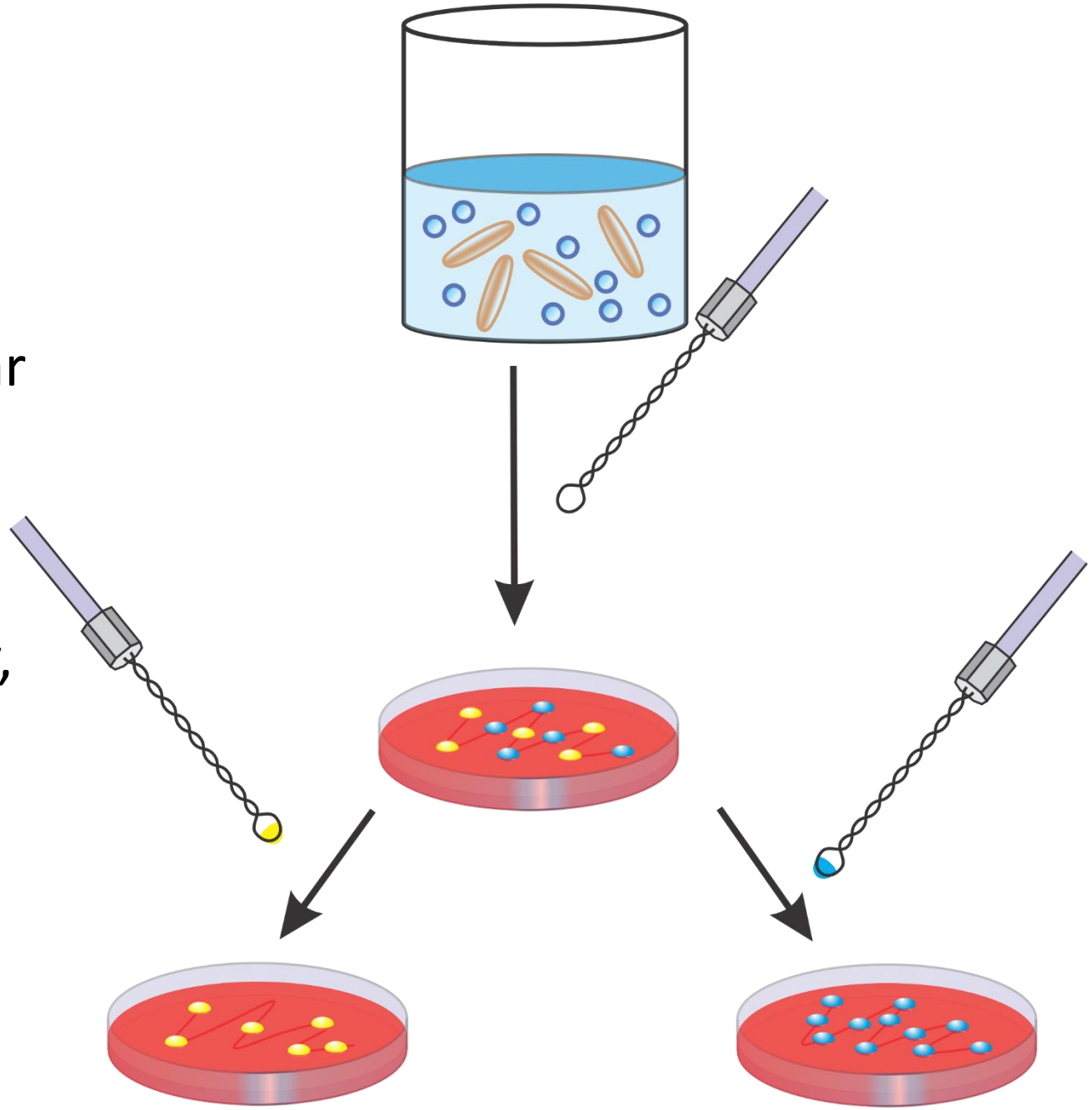
- A **pure culture** is usually derived from a **mixed culture** (one containing many species) by **streak plate** method.





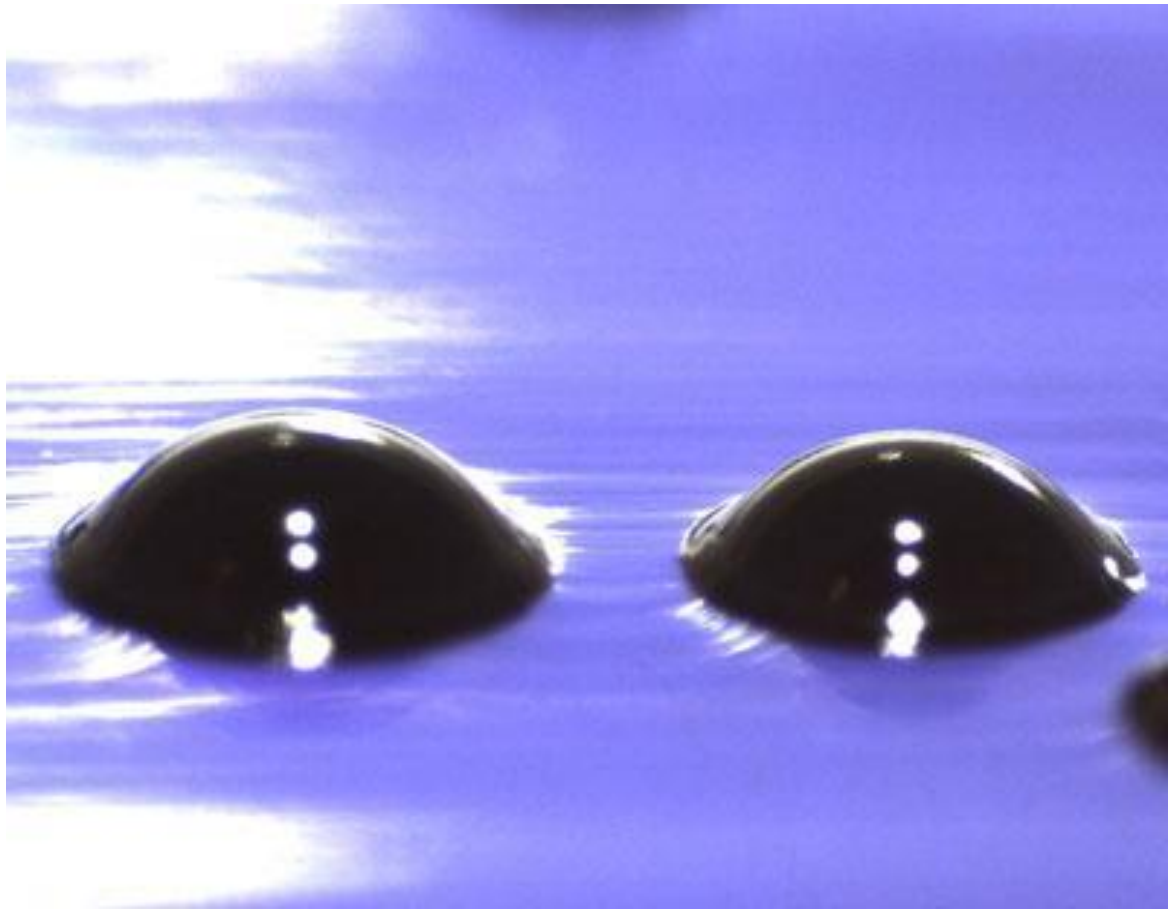
Streak Plate Method

- A small sample is **spread** by an **inoculating loop** over a solid agar plate to **disperse** the individual cells across the medium surface.
- This method separate **individual cells** so that, when they multiply, each will form a **discrete colony**.
- **Discrete colonies** may then be used to inoculate new plates.



A. Colony morphology

- A **colony** is a pile or mass of a sufficiently large number of cells, growing on or in solid medium, that they are visible to the naked eye.
- Different species of bacteria can produce very different colonies.



A. Colony morphology

Example of variation in colony morphology include:

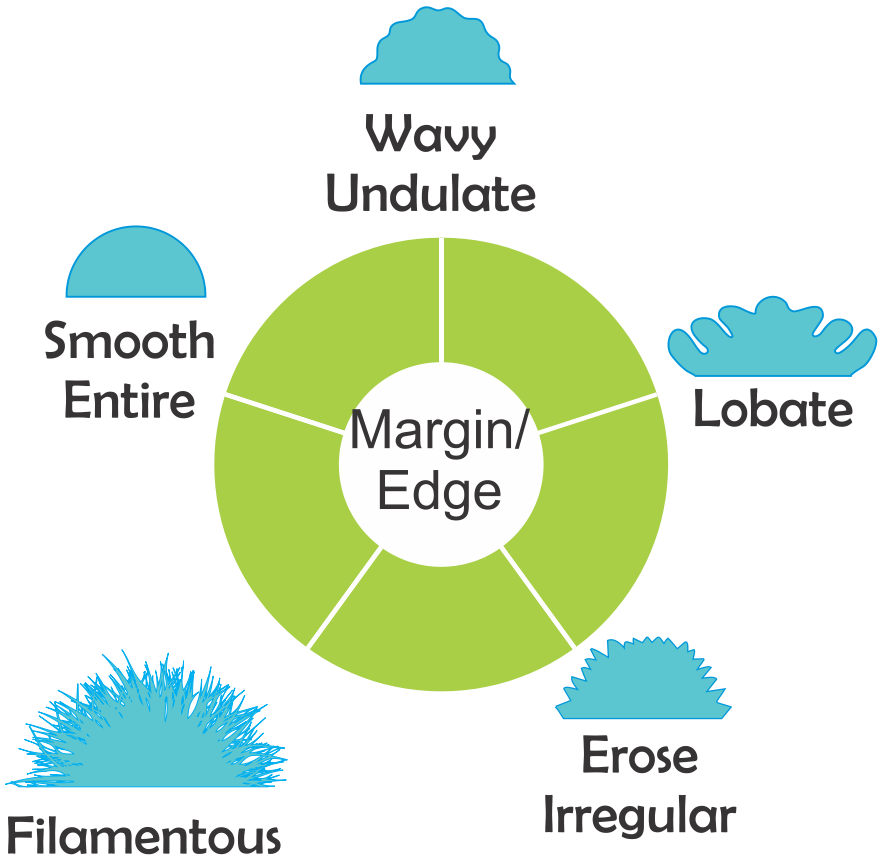
- Colony size,
- Colony Edge/margin
- Colony color
- Colony shape,
- Colony Elevation
- Presence of hemolysis on blood agar

etc…..

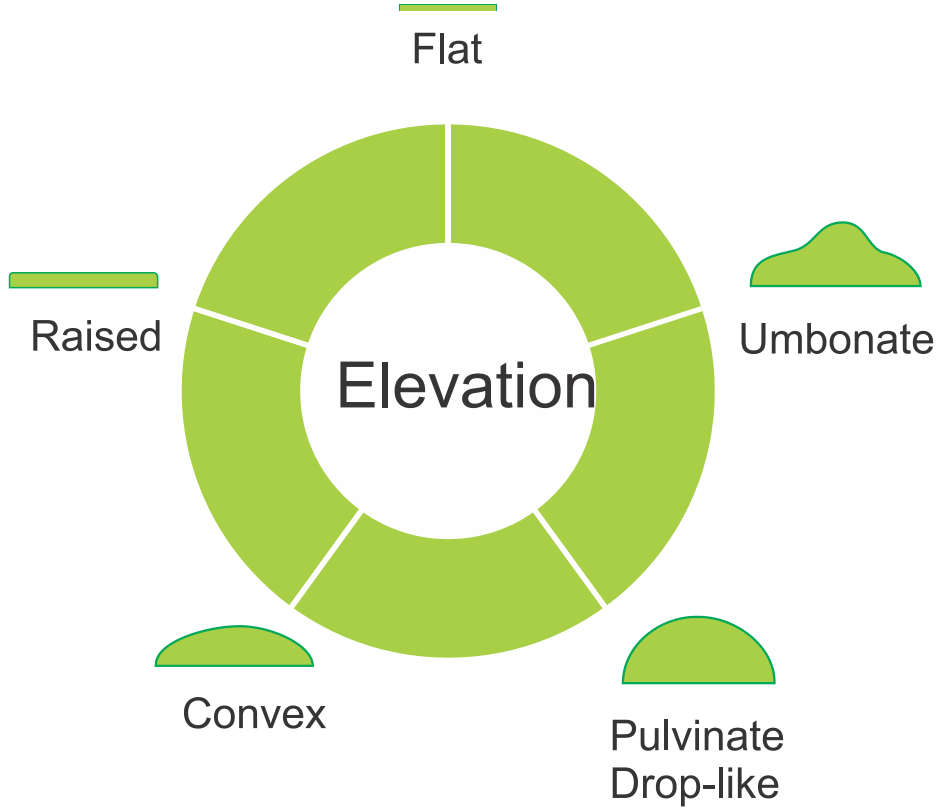
A. Colony morphology

Colony Shape

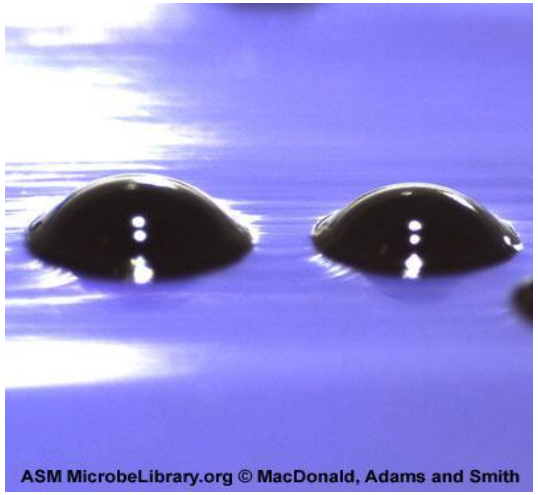
A. Colony morphology

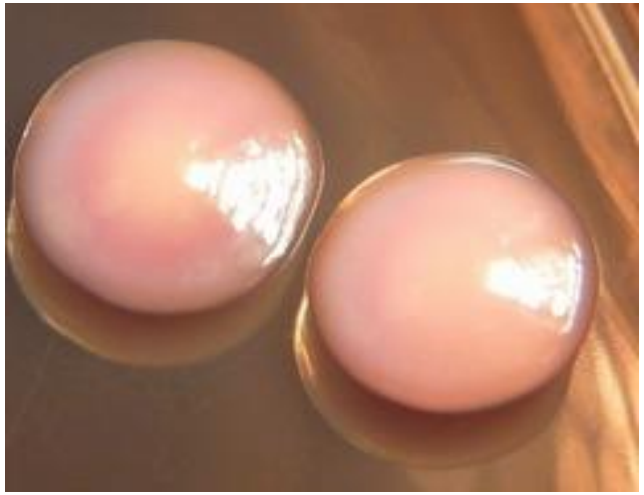


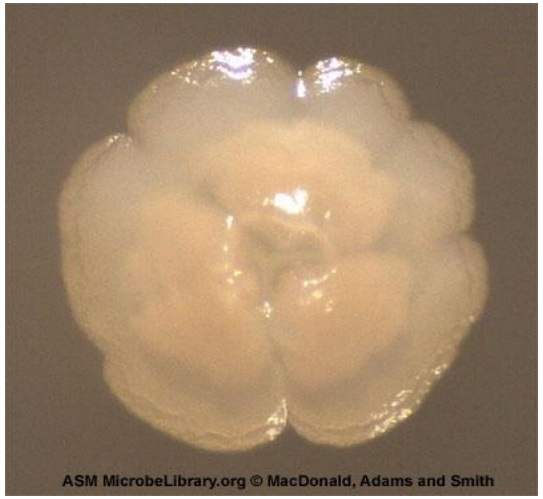
The shape of the edge or margin can be determined by looking down at the top of the colony.

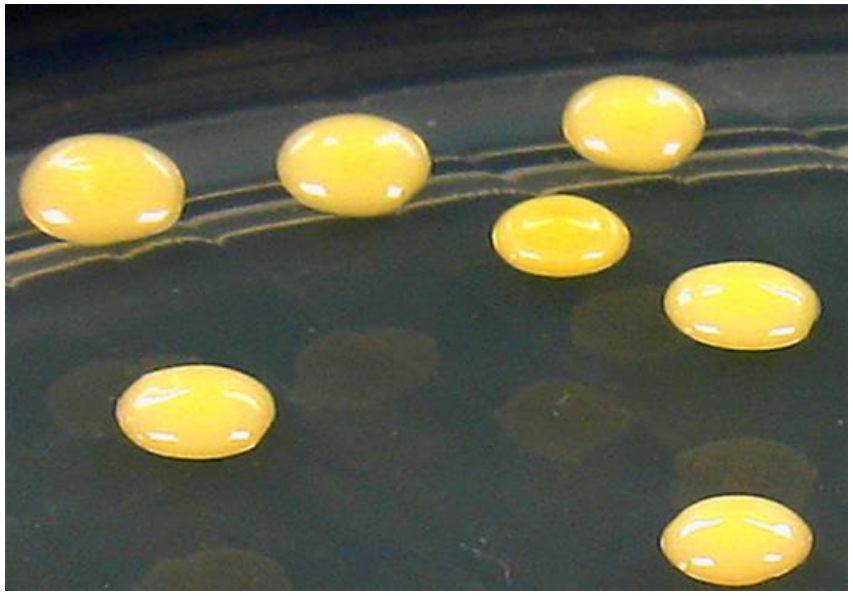


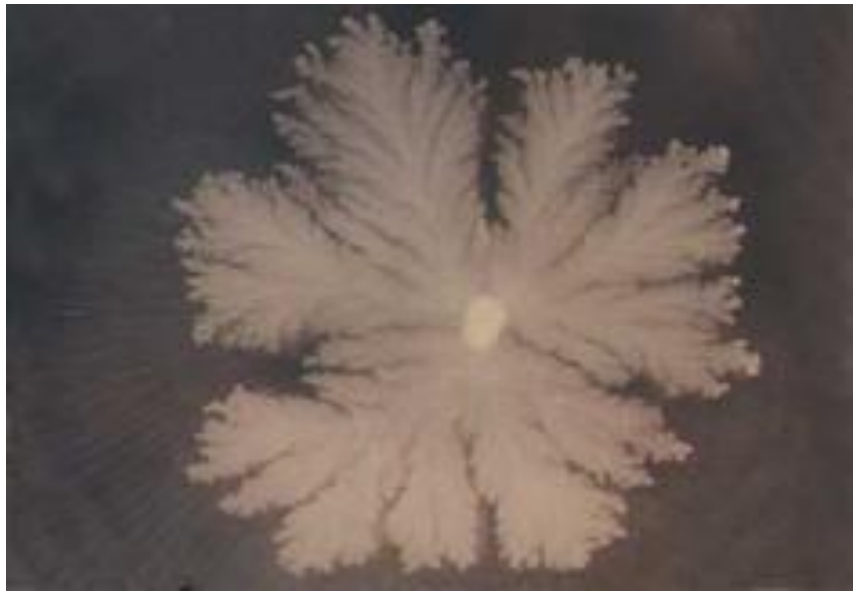
The nature of the colony elevation is apparent when viewed from the side as the plate is held at eye level.





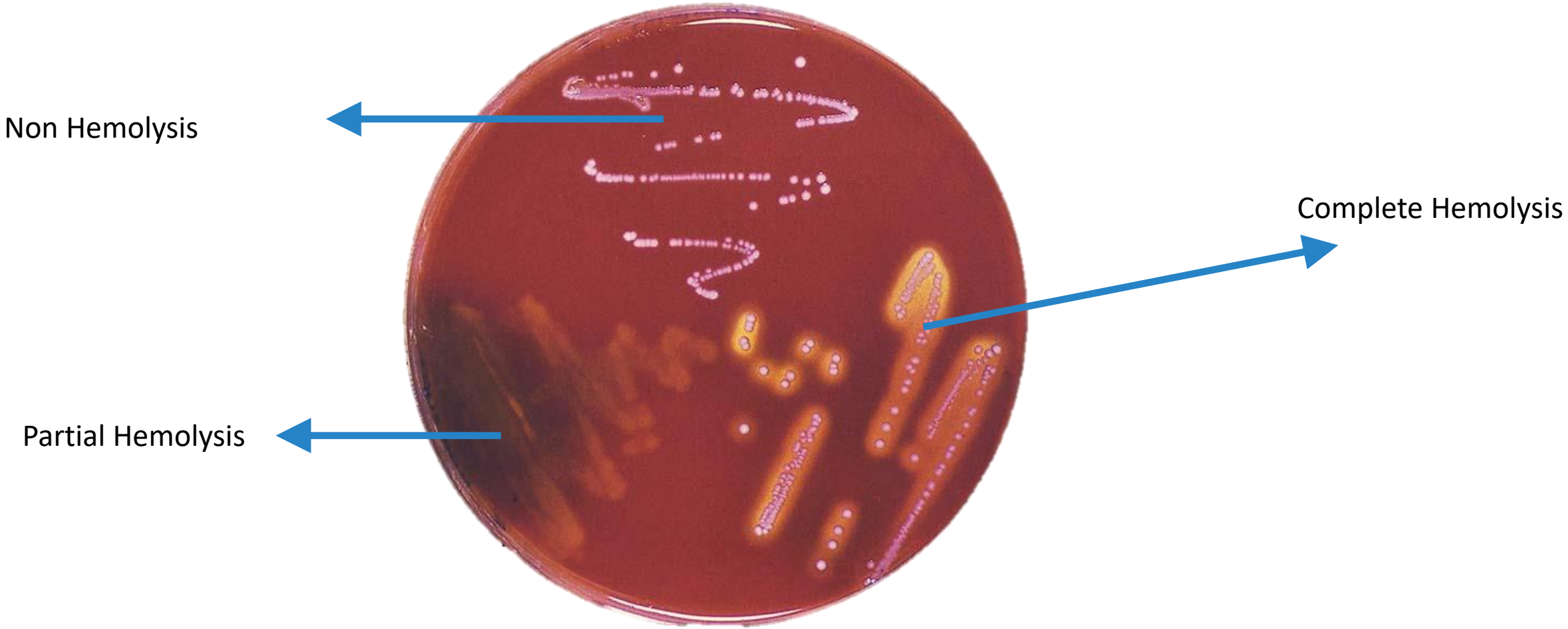






Examples of bacterial colonies showing diversity of morphologies.

Types of Hemolysis on Blood agar



B. Microscopy and Gram stain

To determine bacterial morphology and Gram reaction.

<u>Bacterial Morphology Include:</u>

- Size
- Spore
- Staining
- Shape
- Capsule
- Motility
- Arrangement

C. Single-enzyme tests

- Different bacteria produce varying spectra of enzymes.
- Tests that measure single bacterial enzymes are **simple**, **rapid**, and generally easy to interpret.

- ***Example → Catalase test***

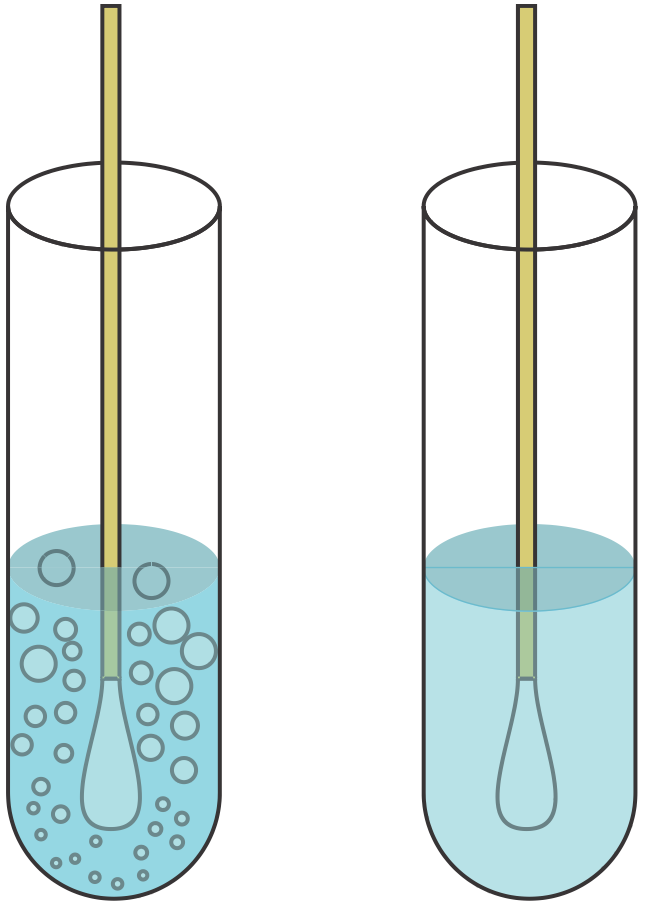
Catalase test

- The enzyme catalase catalyzes the degradation of hydrogen peroxide to water and molecular oxygen.

$$H_2O_2 \xrightarrow{catalase} H_2O + O_2.$$

Catalase test

Catalase-positive organisms rapidly produce bubbles when exposed to a solution containing hydrogen peroxide.



Catalase positive test

Catalase negative test

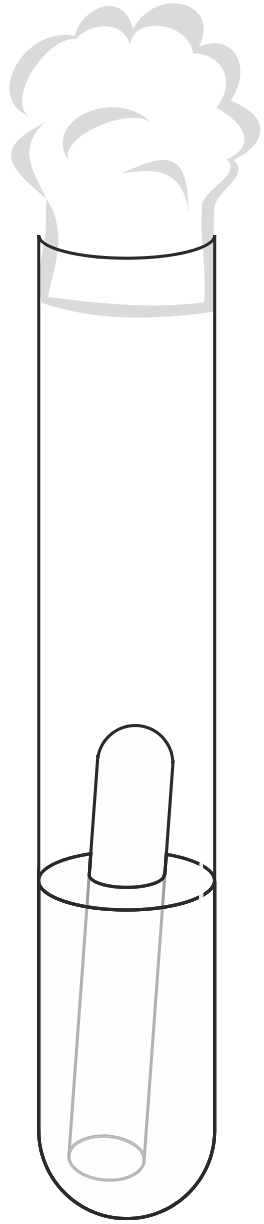
D. Tests based on the presence of metabolic pathways

- These tests measure the presence of a metabolic pathway in a bacterial isolate, rather than a single enzyme.
- Example → **Sugar fermentation tests.**

Sugar fermentation tests:

To determine the ability of the bacteria to ferment a particular sugar the bacteria is inoculated in a test tube containing:

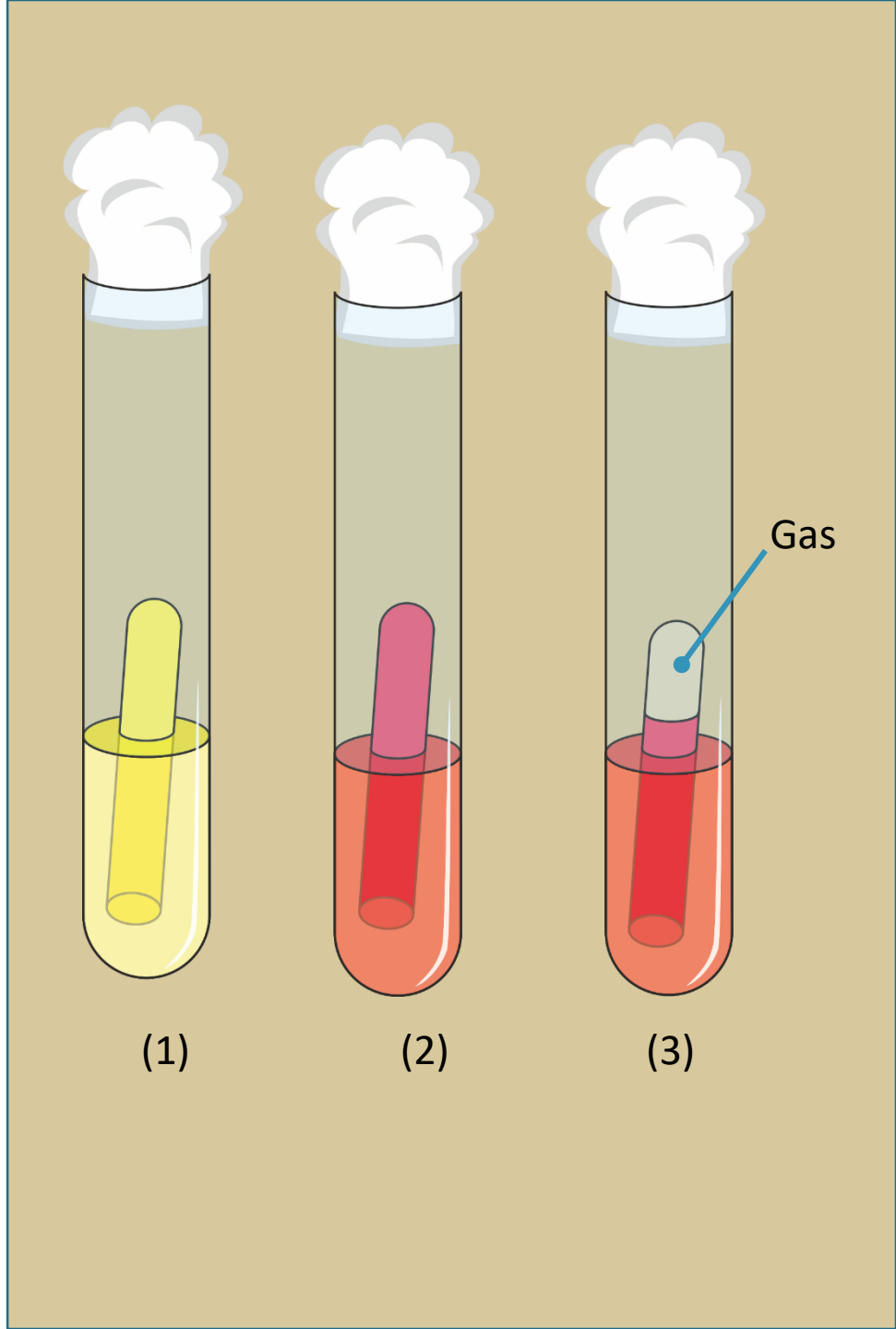
- The test **sugar solution**.
- A pH **indicator** which change its color if acid is produced.
- An **inverted tube** which is used to detect gas production.



Sugar Fermentation tests

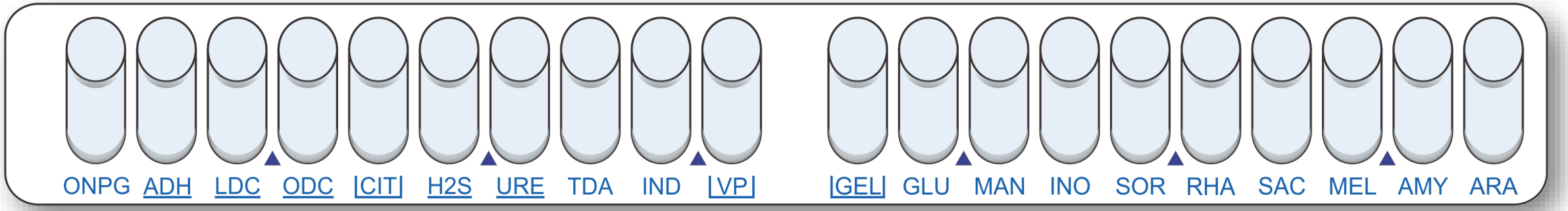
There are three possible results:

- Bacteria can’t ferment the test sugar (1).
- Bacteria can ferment the test sugar with production of gas (2).
- Bacteria can ferment the test sugar but without gas production (3).



Commercial biochemical tests

- API 20E is a common commercial test for bacterial identification.
- It consists of 20 microtubes containing **dehydrated substrates** to detect various bacterial **metabolic pathways**.
- The test is inoculated with a bacterial suspension.
- After incubation, color changes in the microtubes indicate the presence or absence of the bacteria's ability to metabolize a particular substrate.



Commercial biochemical tests

All Negative API Strip

All Positive API Strip

Uninoculated API strip

E. Automated systems

Microbiology laboratories are increasingly using automated methods to identify bacterial pathogens.

F. Immunologic Detection of Microorganisms

Immunologic techniques can be used for identification of microorganisms by:

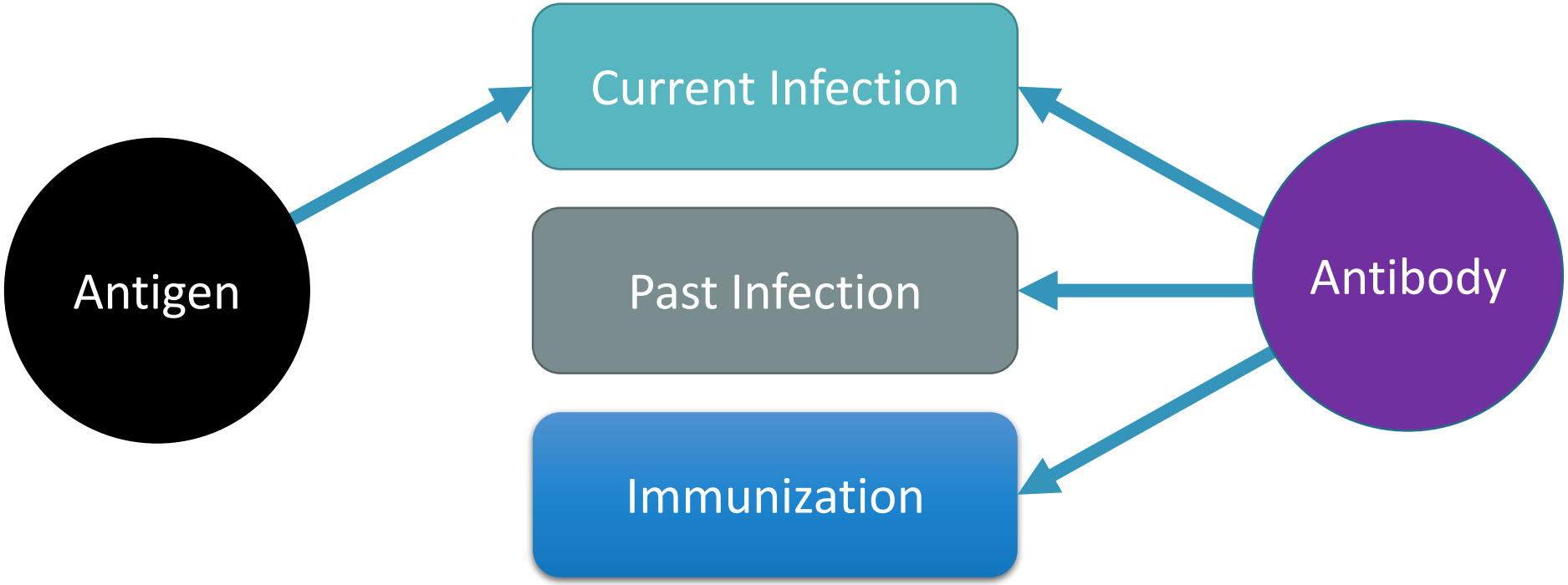
1. Detection of bacterial **antigens** in specimens or cultures: using known specific antibody.

2. Detection in a patient's serum of **antibodies** that are directed against microbial antigens (Serology).

Immunologic Detection of Microorganisms

The presence of **antibodies** or **antigens** in patients' sera or other body fluids is determined by performing an **immunoassay procedure**, such as:

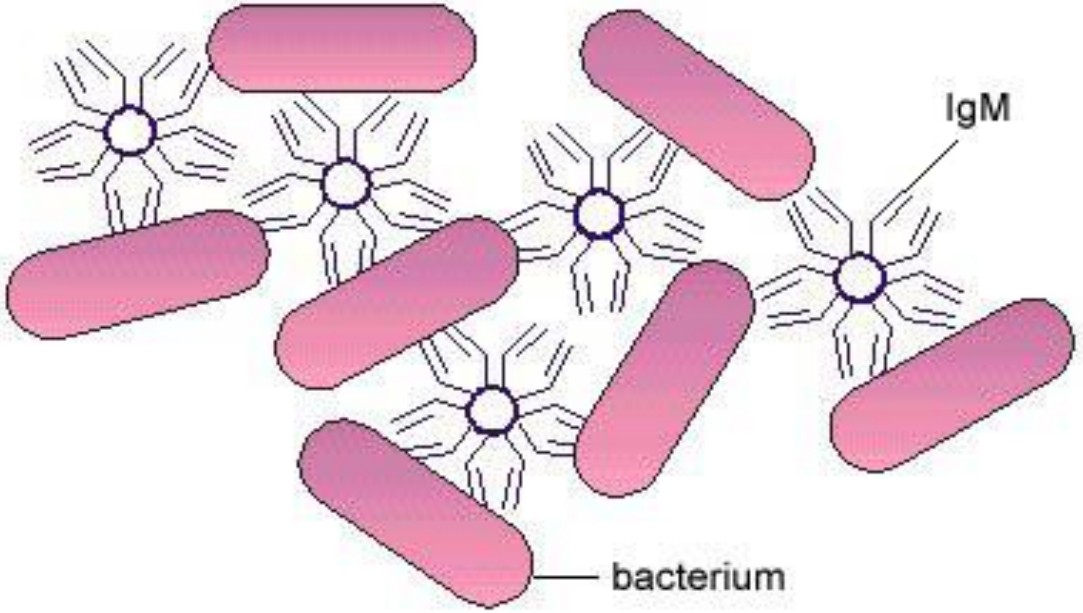
- Slide Agglutination.
- **Enzyme-linked immunosorbent assay (ELISA).**



- Detection of **bacterial antigens** provides a direct evidence that the patient is currently infected.
- Detecting **antibodies** to a particular pathogen may represent:
  - ❶ present infection,
  - ❷ past infection, or
  - ❸ prior vaccination against that pathogen.

Agglutination test

Agglutination is the visible **clumping** of **particulate** (insoluble) antigen e.g. bacteria or red blood cells with its specific antibody.



Agglutination occur due to the **cross-linking** of the particulate antigens by antibody molecules

Agglutination Test

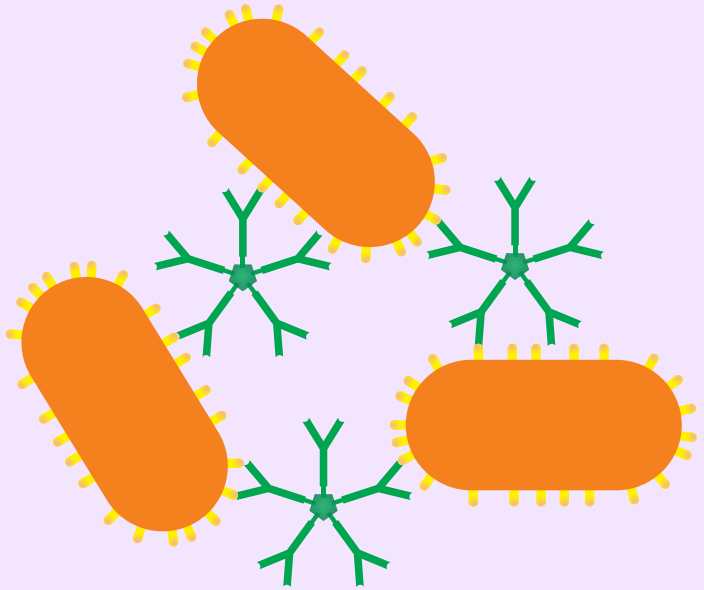
Agglutination reactions may be:

- **Direct:**

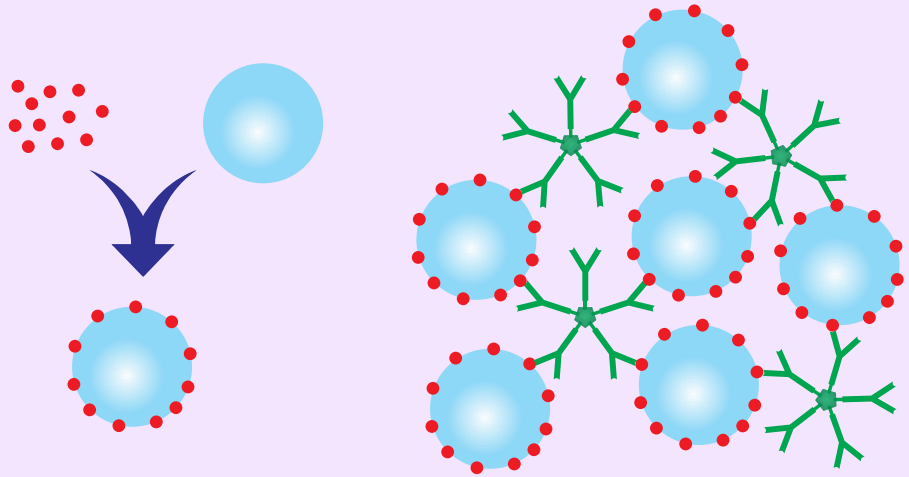
  -Antigens are found naturally on cell surface.

- **Indirect:**

  -Known antigen is attached to a “carrier” particle.



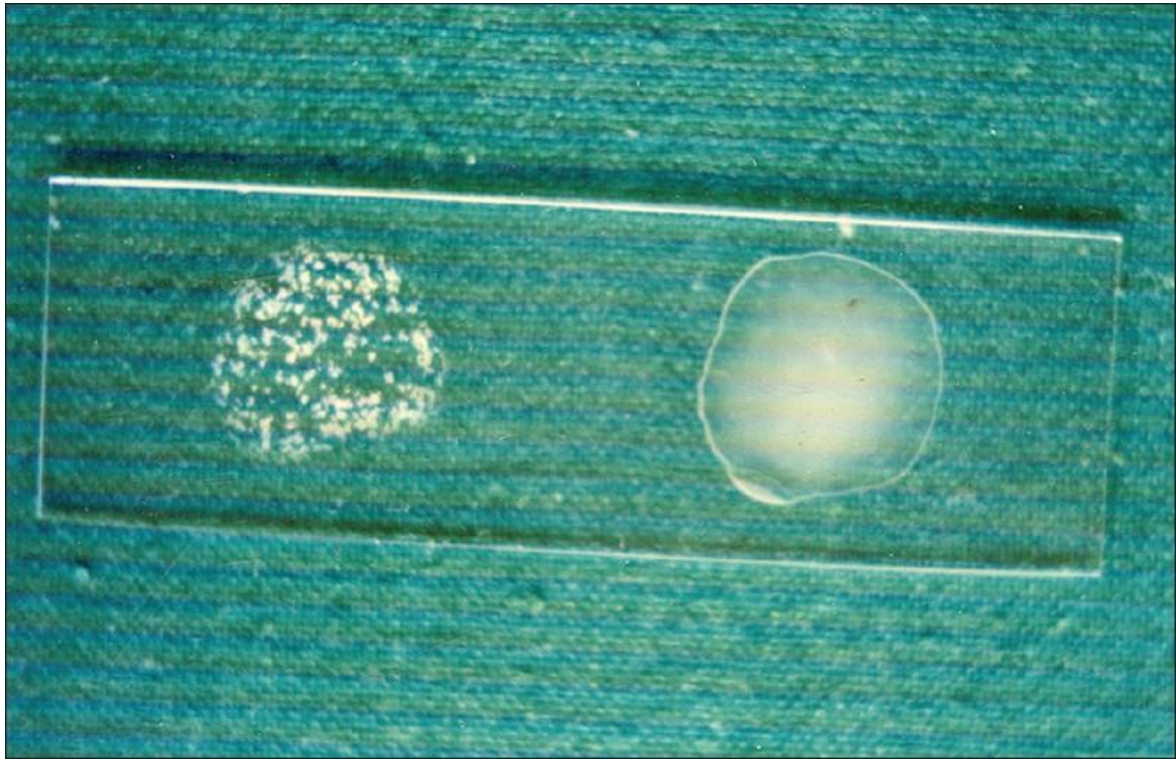
Direct agglutination



Indirect agglutination

Slide agglutination test

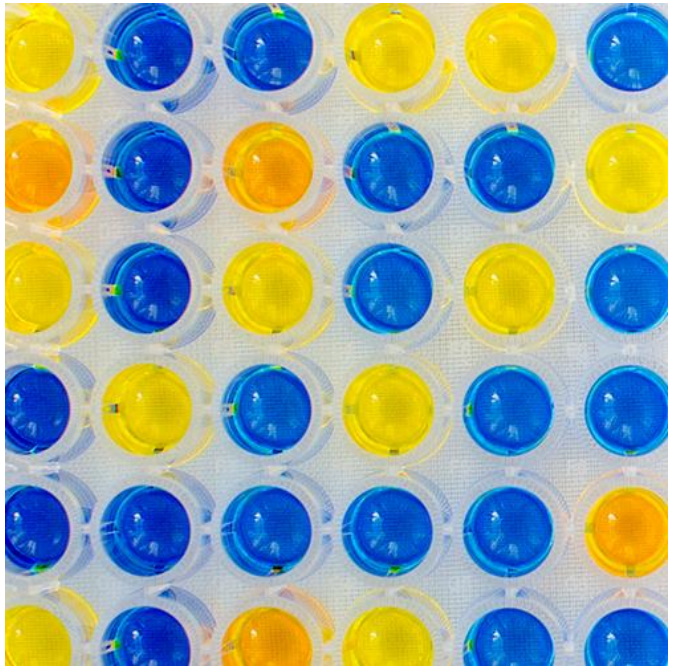
- Slide agglutination test can be used for rapid identification of bacteria.
- A drop of bacterial suspension is mixed with specific antibacterial antibody on a microscopic slide.
- This test is repeated with different antibodies to detect which antibody causes agglutination.



Enzyme-linked immunosorbent assay (ELISA)

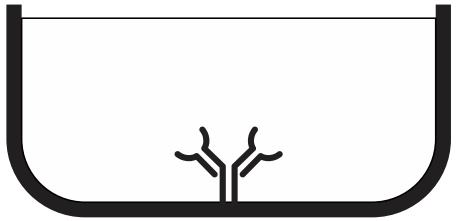
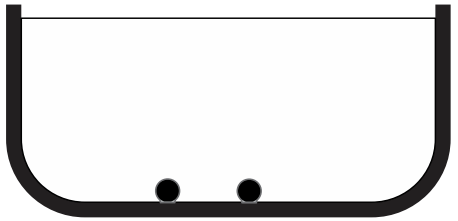
- Enzyme-linked immunosorbent assay (ELISA) is a **sensitive** assay useful in detecting either antigens or antibodies in **low concentrations** in a patient's body fluids.

- There are two types of ELSIA technique:
  - **Direct ELISA** technique is used to detect **antigen** in specimens.
  - **Indirect ELISA** to detect and measure **antibodies** in serum.



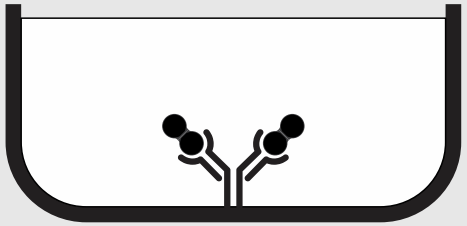
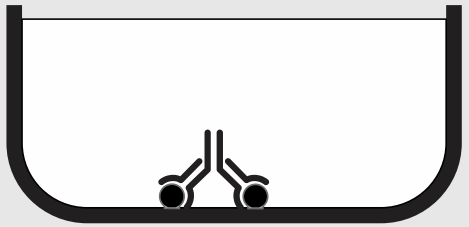
Microtitration plate:
Positive Test is indicated by color change.

Enzyme-linked immunosorbent assay (ELISA)

Direct ELISA (Sandwich method)	Indirect ELISA
For ANTIGEN detection	For ANTIBODY detection
1  Well of microtitration plate is coated with specific known antibody	 Well of microtitration plate is coated with known antigen.

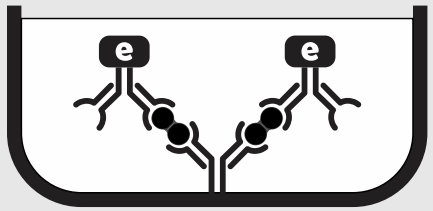
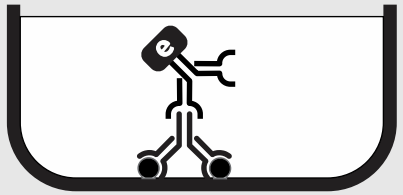
Enzyme-linked immunosorbent assay (ELISA)

2

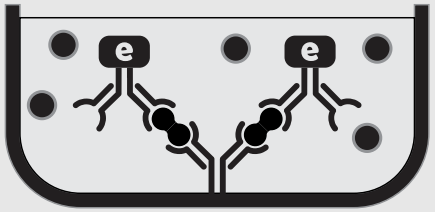
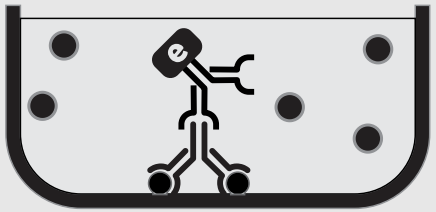
Direct ELISA		Indirect ELISA	
	Specimen containing antigen is added. The antigen (if present) combine with the antibody		Patient serum is added. The patient's antibody (if present) combines with the antigen

Enzyme-linked immunosorbent assay (ELISA)

3

Direct ELISA		Indirect ELISA	
	After washing, enzyme labelled antibody is added which attaches to the antigen.		After washing, enzyme labelled anti-human globulin is added which attaches to the antibody.

Enzyme-linked immunosorbent assay (ELISA)

Direct ELISA		Indirect ELISA	
	After washing a substrate is added which is hydrolyzed by the enzyme to give color change.		After washing, a substrate is added which is hydrolyzed by the enzyme to give color change.

Microtitration plate
Positive Test is indicated by color change

G. Molecular Methods

- The main objective of the molecular methods of bacterial identification is direct recognition of **pathogen-specific nucleic acid sequences** in the test material.
- These methods are used in particular in the search for bacteria that are very **difficult to culture**, or proliferate very slowly.

Molecular Methods

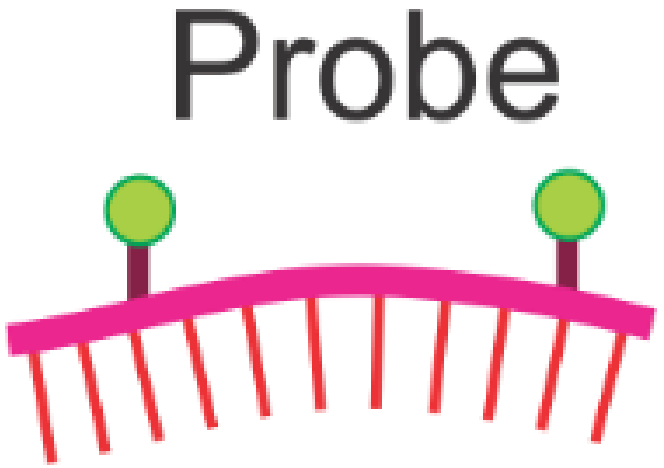
Molecular methods include:

- DNA Probes.
- Nucleic Acid Amplification.

DNA probes.

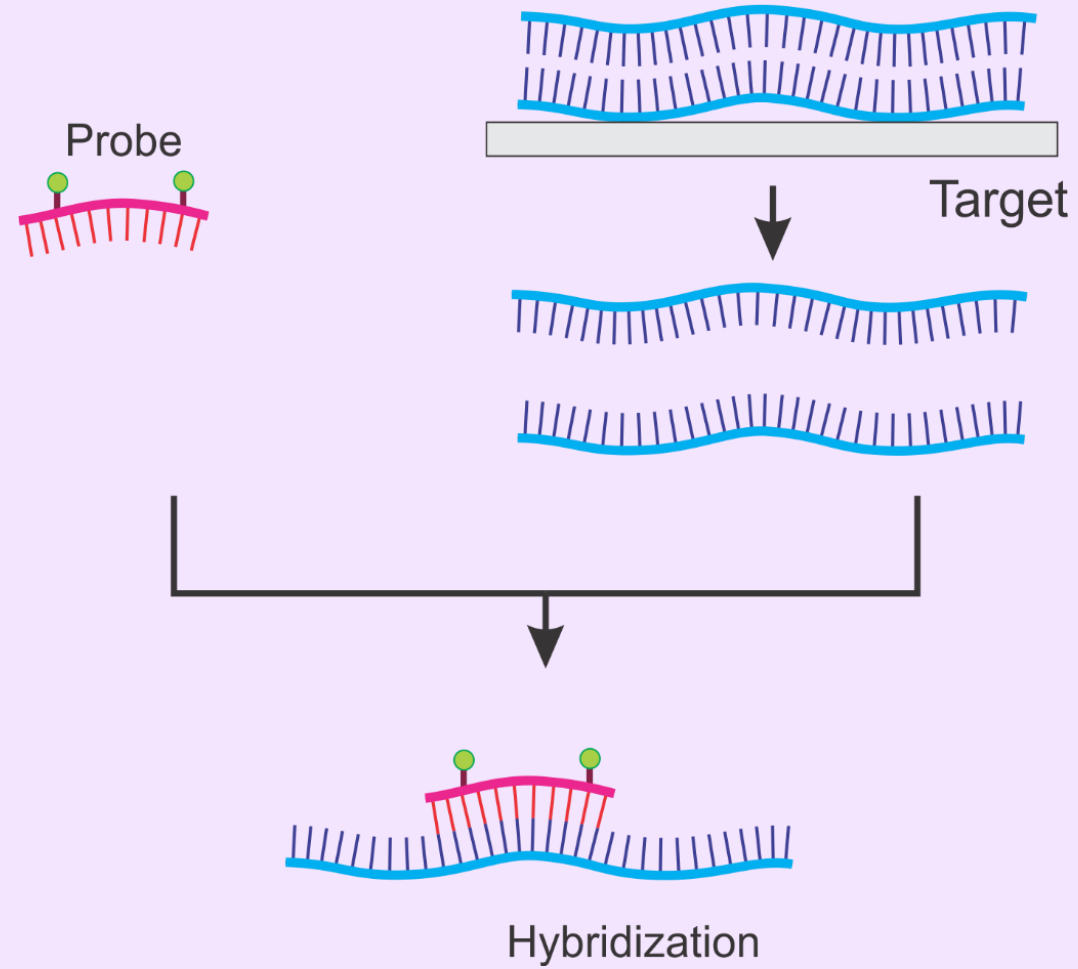
DNA probes are:

- Small segments of complementary DNA.
- **Labeled** with a **marker** molecule (such as enzymes or radioactive label).



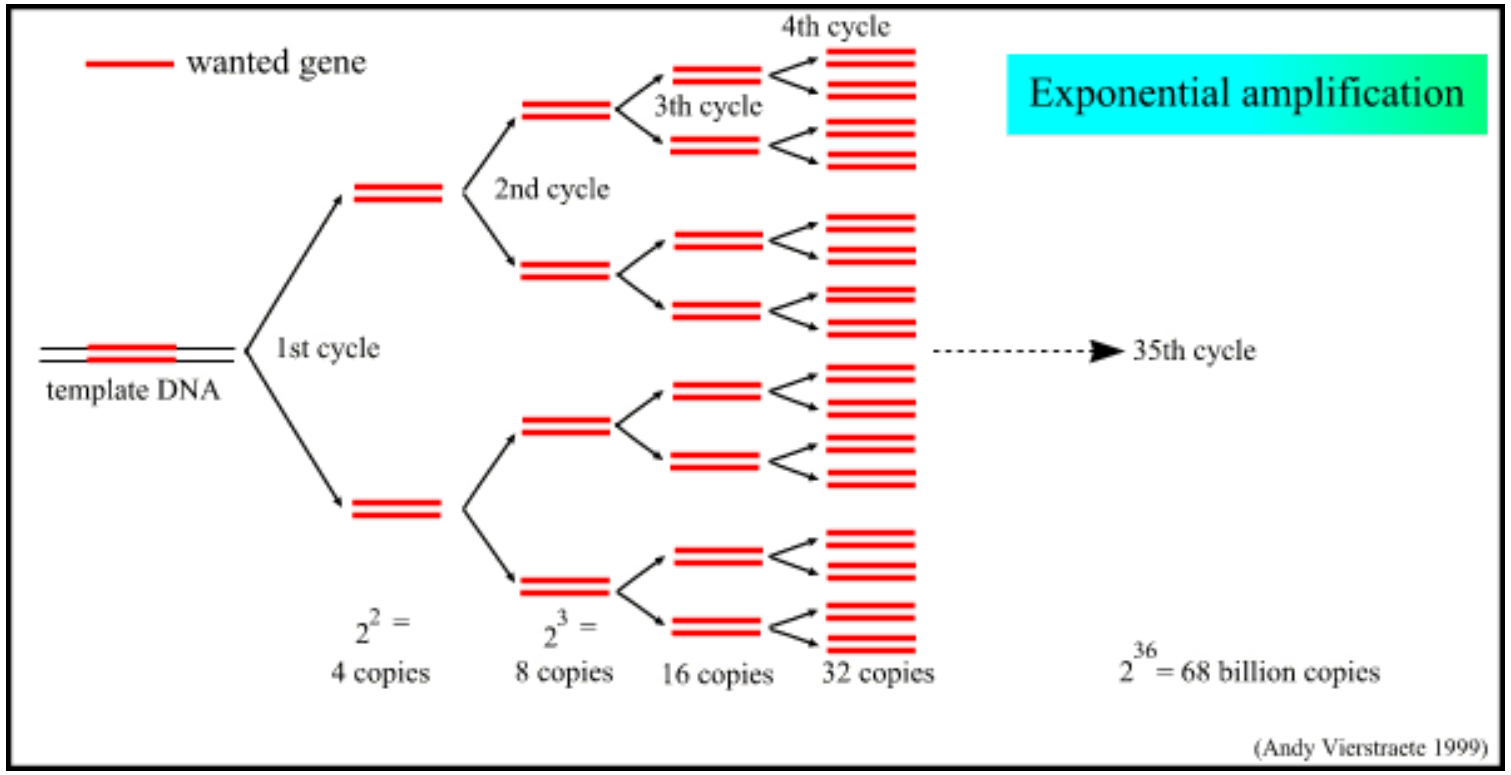
DNA probes.

- The DNA probes **hybridizes** (attaching to) its complementary nucleic acid sequences in the test sample.
- The presence and the quantity of hybrids is determined by the detection of the label.



Nucleic Acid Amplification:

The polymerase chain reaction (PCR) is a scientific technique to <u>amplify</u> a specific sequence in single or a few copies of DNA strands to generate thousands to millions of copies.



Quizzes

1. Nucleic acid amplification can be done by:

A. Polymerase chain reaction (PCR)
B. DNA probes
C. Enzyme-linked immunosorbent assay (ELISA)
D. API-20E

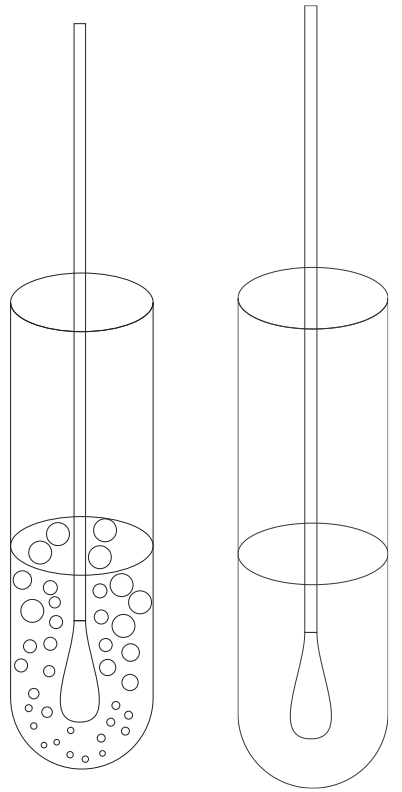
2. Detection of bacterial antigen in patient sample indicates _____?

A. Present infection
B. Past infection
C. Previous immunization
D. All of the above

3- Study the following diagram and then fill in the spaces:

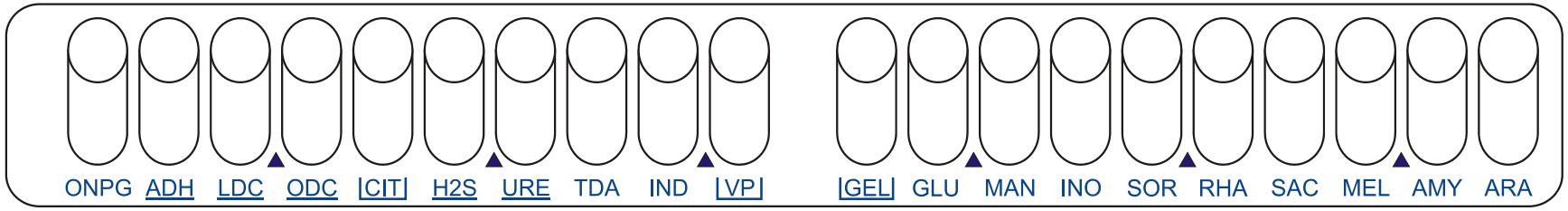
- This test is: ……
- The inverted tube is used to detect: ……
- The change of the color of the tube indicate ……

4- Study the following diagram and then fill in the spaces:



A. The name of the test is: ……
B. The tube contain a solution of: ……
C. Positive test is indicated by: ……
D. Tube b is …… (positive/negative)

Study the diagram and answer:



5. **The name of the above test is:**

A. Polymerase chain reaction (PCR)
B. DNA probes
C. Enzyme-linked immunosorbent assay (ELISA)
D. API20E

6. **This test is used to detect :**

A. Bacterial Nucleic acid
B. Bacterial Antigens
C. Bacterial Morphology
D. Bacterial Metabolic pathways

7. A visible mass of large number of microorganisms, growing on or in solid medium is called _____

A. Colony
B. Mold
C. Biofilm
D. Probe

8. A small segments of complementary DNA labeled with a marker molecule is called:

A. Plasmid
B. Antigen
C. Probe
D. mRNA
E. Antibody

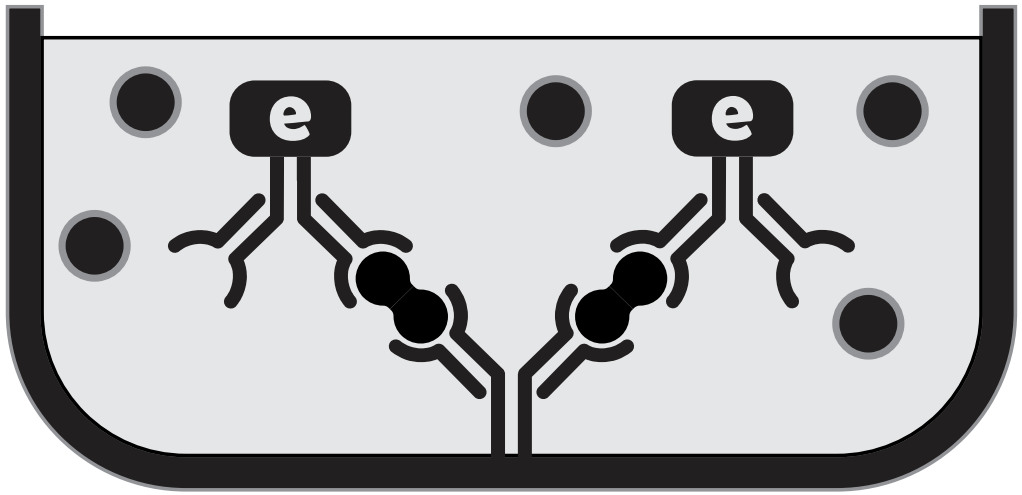
9. Complete what abbreviations stand for:

A. ELISA: ……..

B. PCR: ……..

10. Study the diagram and fill in the spaces:

A. The Name of the above test is:
   ……
B. The test is used for detection of:
   ……
C. The wells are coated with:
   ……
D. Is the above test direct or indirect:
   ……



11. Study the diagram and fill in the spaces:

A. The Name of the above test is: _____
(ELISA/ PCR/ API20E)

B. The wells are coated with: _____
(Antigen/Antibody)

C. The test is used for detection of: _____
(Antigen/ Antibody)

D. Is the above test _____
(direct / indirect)

E. In positive tests there will be _____
(color change/ gas bubbles/ precipitation / agglutination).