6. Diagnosis of Microbial Growth

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Objectives

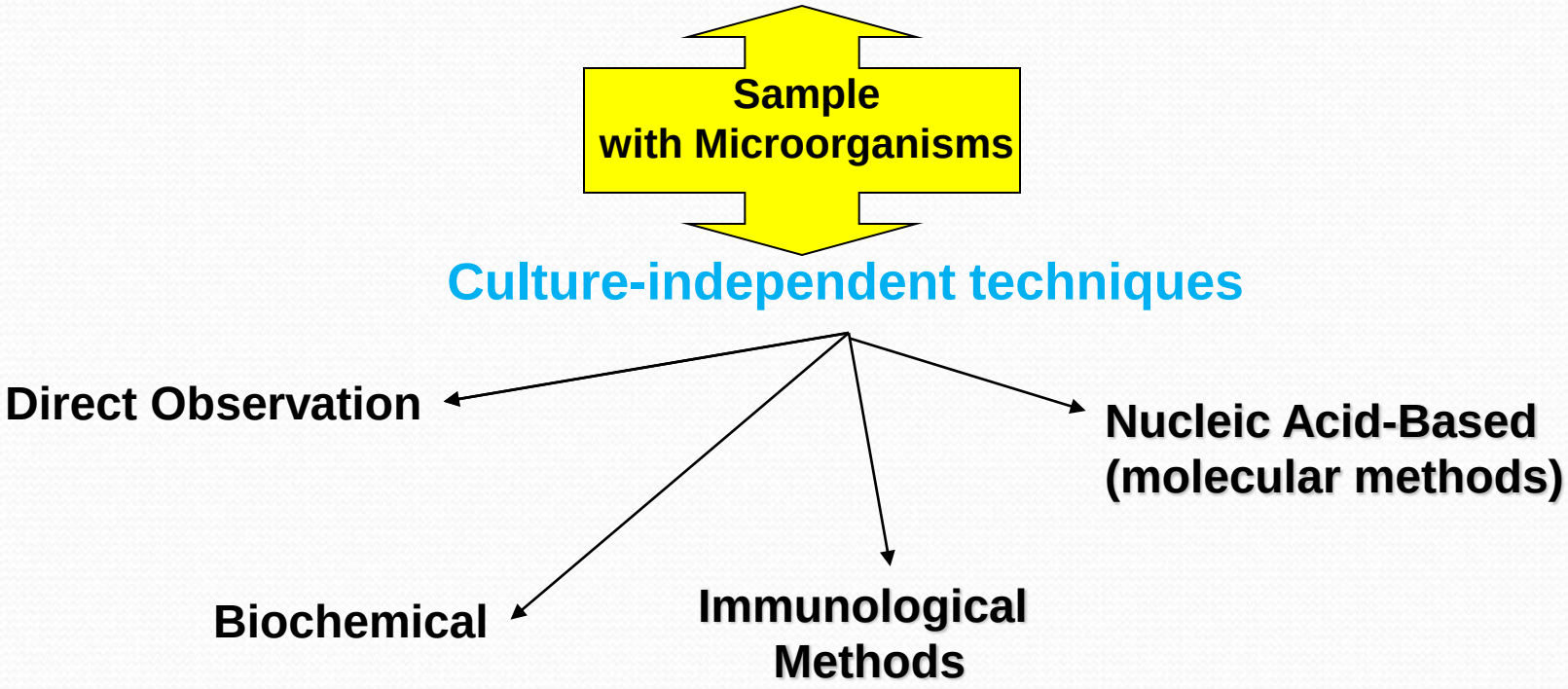
- Understand principals of culture and direct observation in microbiology
- Understand the main Immunological assay used in microbiology and describe antigen and antibody interaction
- Understand DNA structure and function, and describe the main genetic and molecular assay used in Microbiology
- Describe the main Biochemical assay used in Microbiology

Laboratory techniques in Microbiology

- Microscopy
- Culture – **Gold standard**
- Immunological assays
- Molecular assays
- Biochemical assays

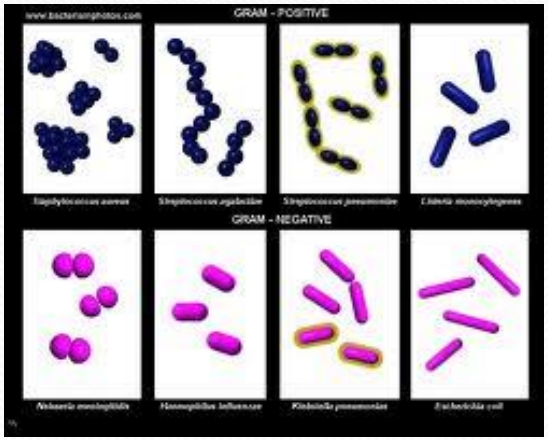
Testing Methods in Microbiology

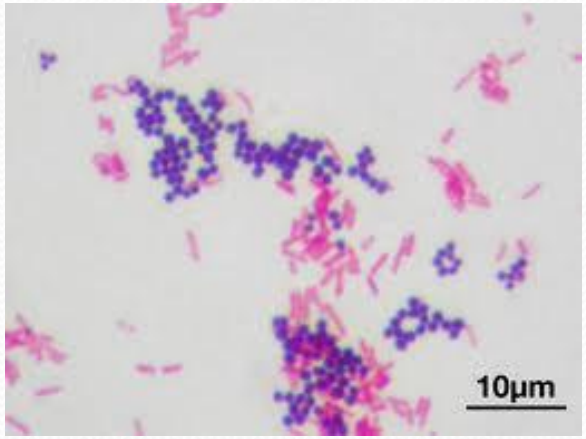
Culture-dependent techniques

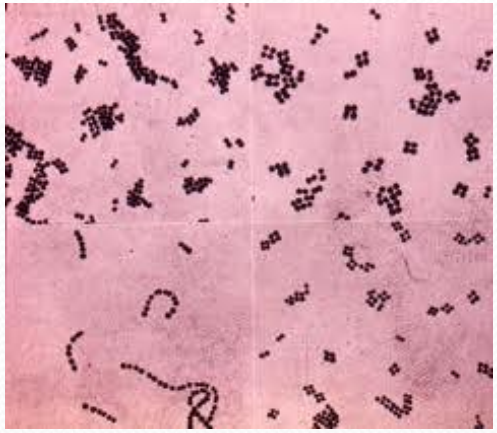


1. Direct Observation

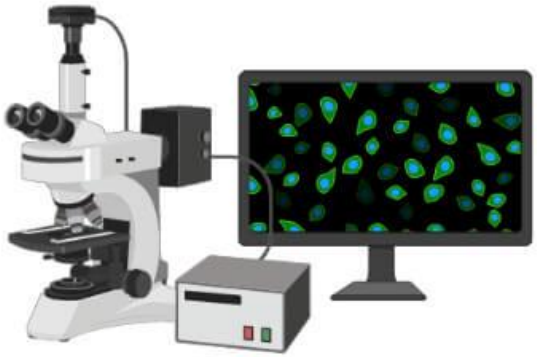
- Using light microscope to visualize bacterial shape and arrangement
- Using special stains to differentiates bacteria like gram stain and acid fast stain
- Quick and informative yet not definitive







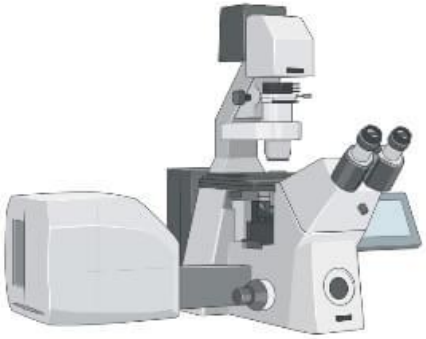
Light Microscope



Fluorescence Microscope

Electon Microscope

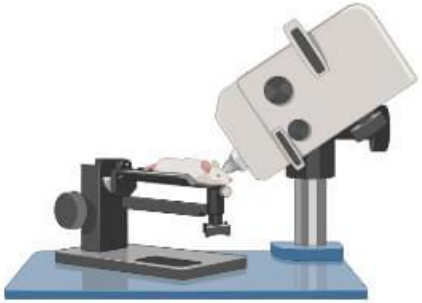
Stereo Microscope



Confocal Microscope

Atomic Force Microscope

Inverted Microscope



Retinal Imaging Microscope

2. Culture

- Culture: Microbes growing in/on culture medium
- Culture Medium: Nutrients prepared for microbial growth
- Agar: Complex polysaccharide used as solidifying agent for culture media in Petri plates, slants, and deeps
- Agar is not metabolized by microbes, liquefies at 100°C and solidifies ~40°C

Types of Media

- Media can be classified on three primary levels

1. Physical State
   - Liquid Media
   - Semisolid
   - Solid (Can be converted into a liquid)
   - Solid (Cannot be converted into a liquid)
2. Chemical Composition
   - Synthetic – exact formula
   - Non synthetic or complex - No exact formula
3. Functional Type
   - General
   - Selective

Bacterial Colonies on Solid Media

MSA

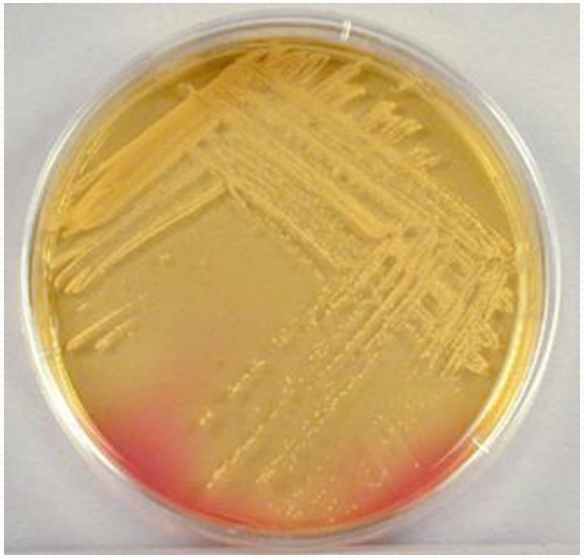
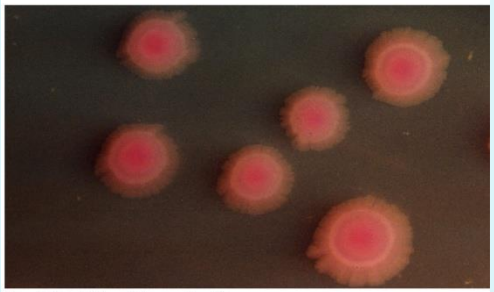


Figure 5-2c Brock Biology of Microorganisms 11/e



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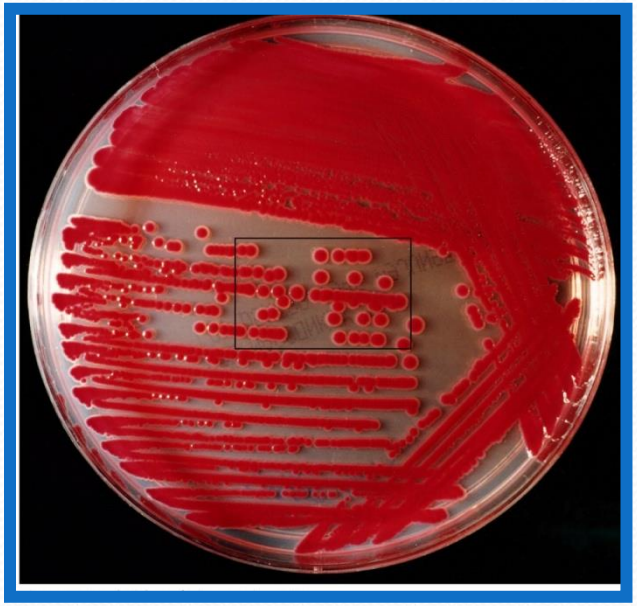
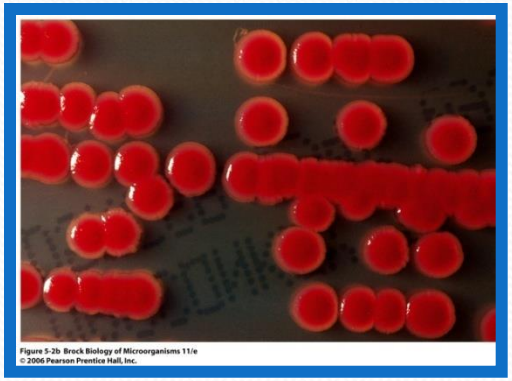
Blood agar





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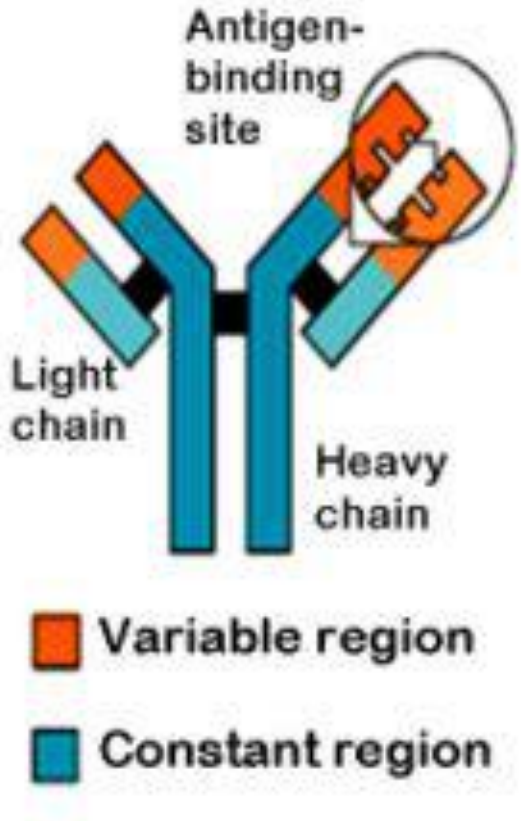
3. Immunologic Methods

- Any assay that relies on the characterization of antigen and/or antibody reaction
- Antibodies can reveal the history of a patient's contact with microorganisms or other antigens
- Serology: the branch of immunology that traditionally deals with *in vitro* diagnostic testing of the serum

- Antigen: any “thing”, foreign to the immune system. e.g. bacteria, viruses, (or their parts), pollen, etc

- Antibody: proteins produced
  by the immune system
  which help defend against antigens

- Antigen/antibody interaction



Visualizing Antigen-Antibody Interactions

Antigen- antibody interaction occurs at molecular level and can not be seen directly

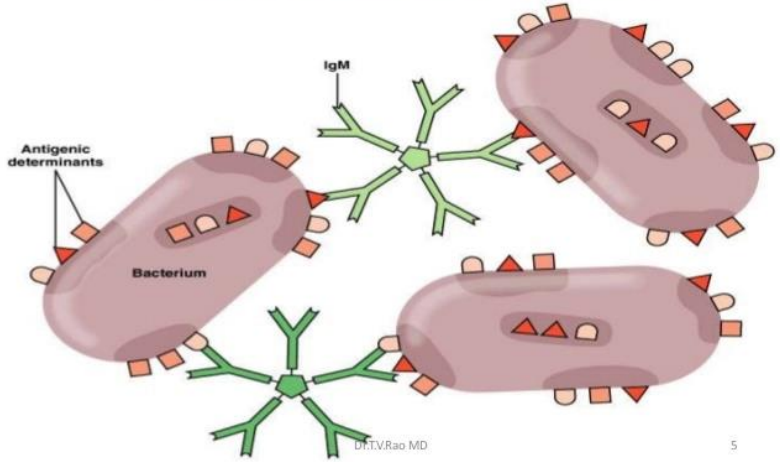
To visualize antigen-antibody interaction mutiple strategies can be applied:

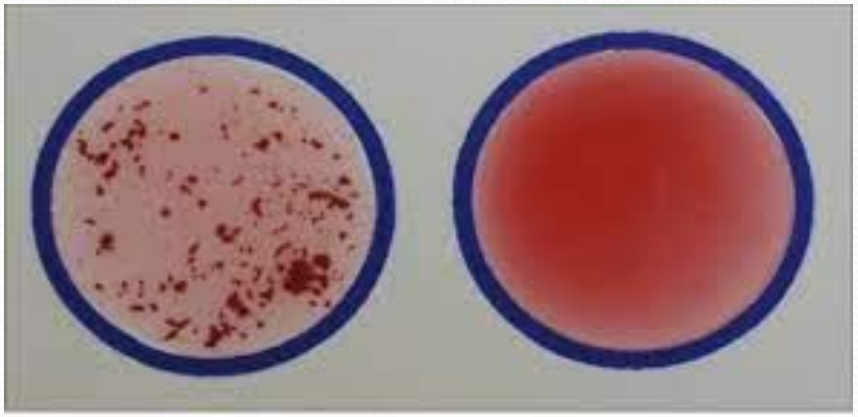
1. **Agglutination**
2. Precipitation
3. Immunodiffusion
4. Complement fixation
5. Fluorescent antibody tests
6. Other Immunoassay tests

Agglutination Test

- Agglutination: antigens are whole cells such as red blood cells or bacteria with determinant groups on the surface
- Antibodies cross-link the antigens to form visible clumps
- Performed routinely to determine ABO and Rh blood types
- Widal test: tube agglutination test for diagnosing salmonella and undulant fever
- Latex agglutination tests: tiny latex beads with antigens affixed

Agglutination Test





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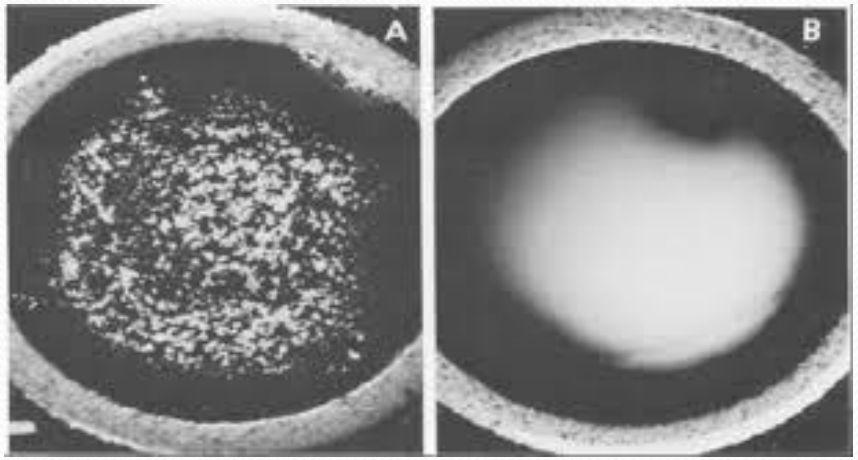
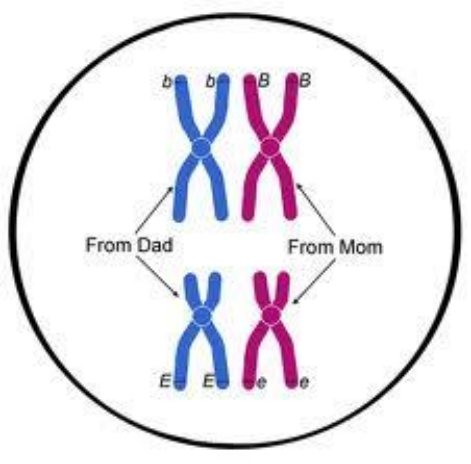
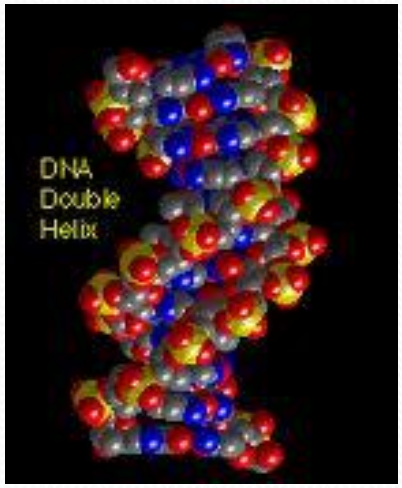


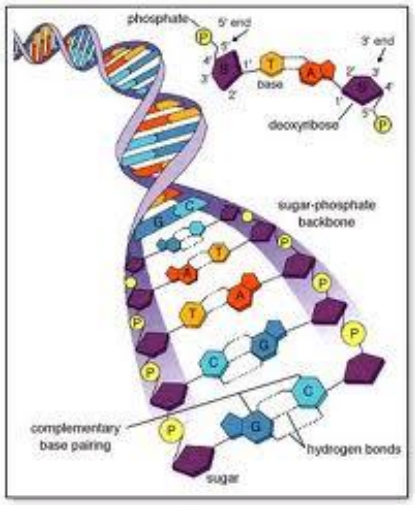
4. Molecular Methods

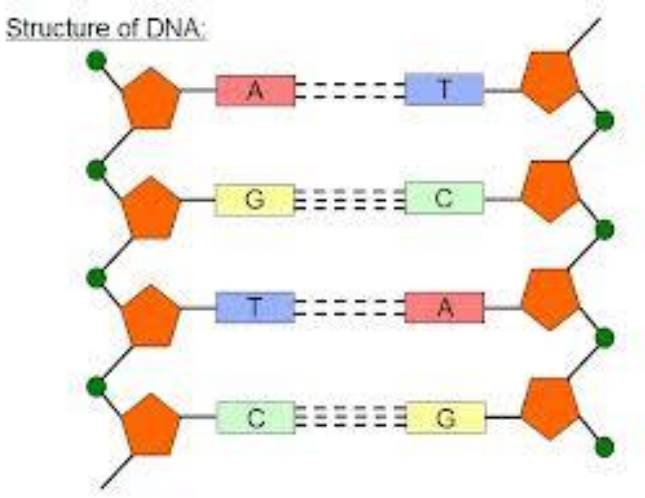
- Every organism contain unique species specific DNA sequence (NUCLEIC ACID) that differentiate it from other organisms
- DNA carry all the inherited characteristics of each organism
- Molecular methods make the species specific DNA visible

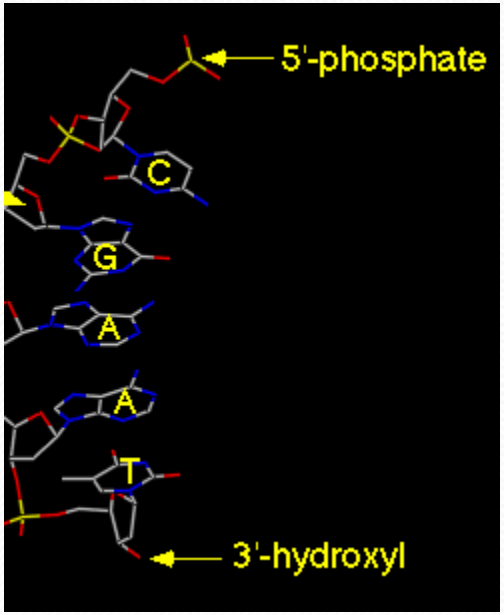
DNA Structure











DNA is formed by 4 nucleotides

A is for adenine

G is for guanine

C is for cytosine

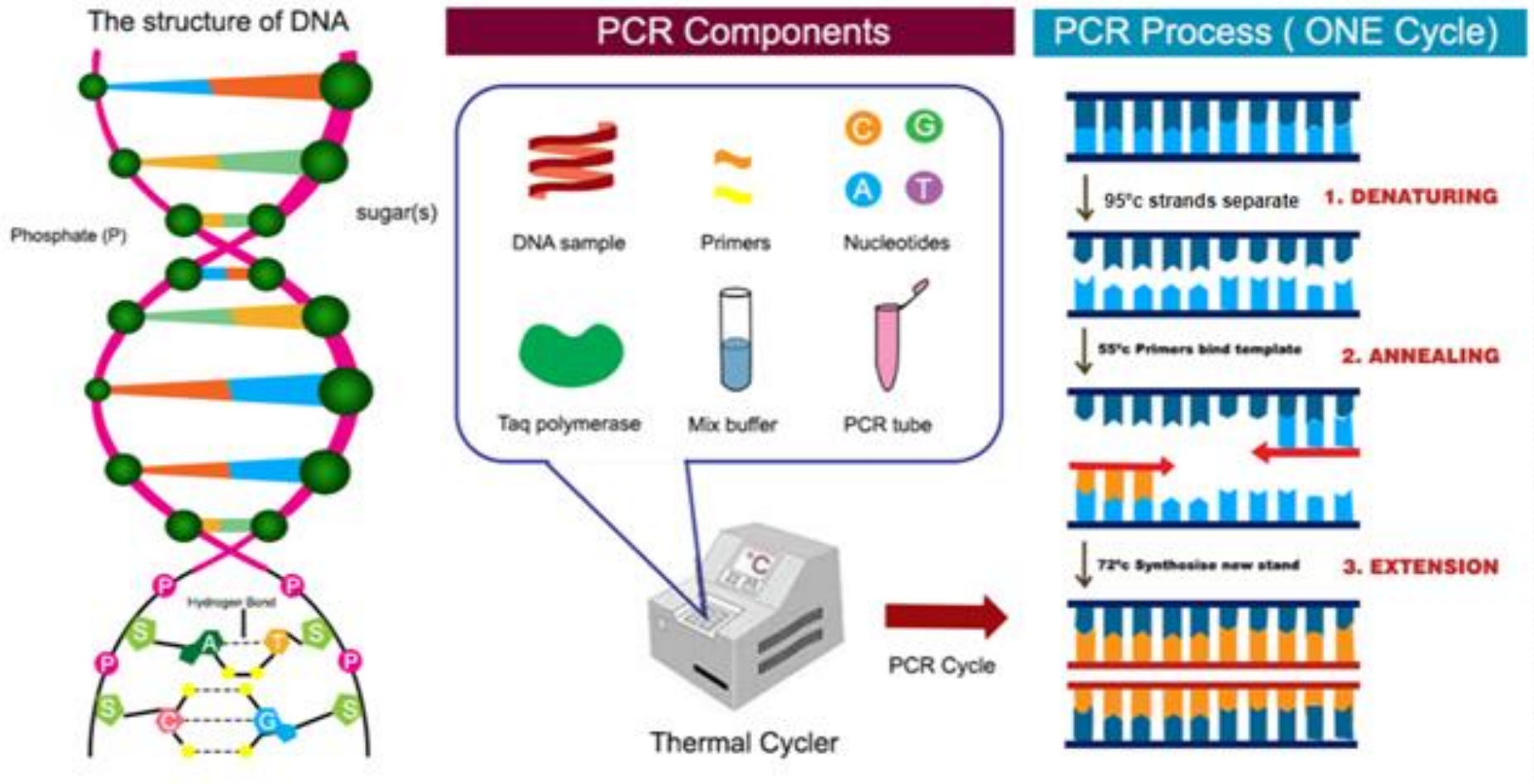
T is for thymine

Molecular Methods

- **Polymerase chain reaction (PCR)**
- DNA hybridization
- Nucleic acid sequence analysis
- Plasmid fingerprinting.

Polymerase Chain Reaction (PCR)

- PCR is widely used for the identification of microorganisms.
- Sequence specific primers are used with PCR in the amplification of DNA or RNA of specific pathogens.
- PCR allows for the detection even if only a few cells are present and can also be used on viable nonculturables
- The presence of the appropriate amplified PCR product confirms the presence of the organisms

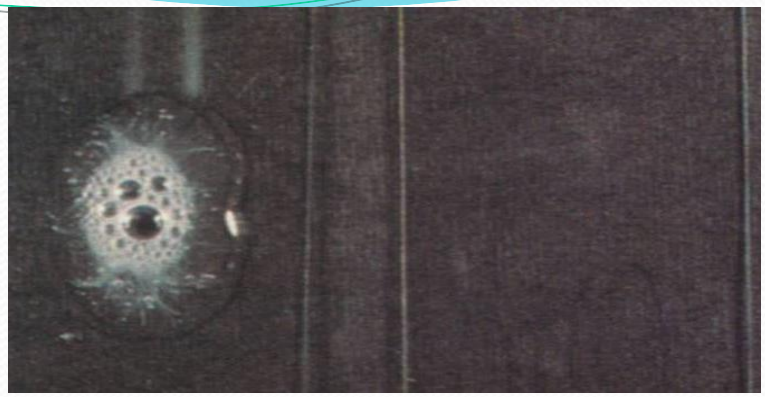

The structure of DNA
Phosphate (P)
sugar(s)
Hydrogen Bond
PCR Components
DNA sample
Primers
Nucleotides
Taq polymerase
Mix buffer
PCR tube
Thermal Cycler
PCR Cycle
PCR Process (ONE Cycle)
95°c strands separate
1. DENATURING
55°c Primers bind template
2. ANNEALING
72°c Synthesise new stand
3. EXTENSION

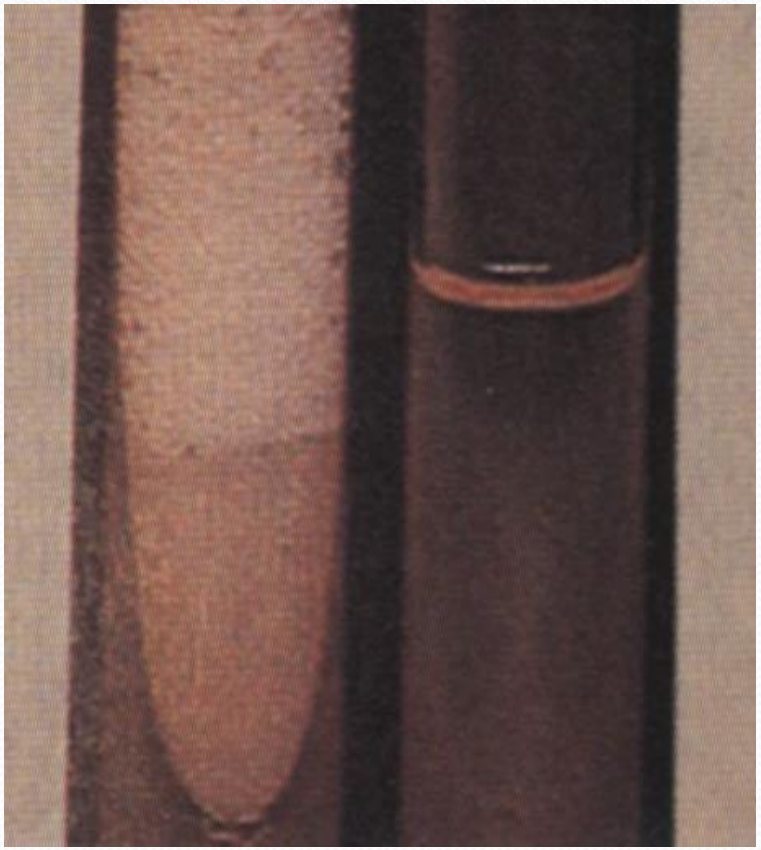
5. Biochemical Tests

- The microbe is cultured in a media with a special substrate and tested for an end product
- Prominent biochemical tests include carbohydrate fermentation, acid or gas production and the hydrolysis of gelatin or starch
- Many of these test used in rapid system for quik detection of certain infection called Rapid test

Catalase Test

- This test is used to identify organisms that produce the enzyme, catalase
- This enzyme detoxifies hydrogen peroxide by breaking it down into water and oxygen gas
- Place a drop of H_2O_2 on the culture. A positive reaction show gas bubbles





Thank you....