
بسم الله الرحمن الرحيم

Lab diagnosis of Bacterial infection

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LECTURE OBJECTIVES

1. Describe the bacteriological approach for diagnosis of bacterial infections of different body specimen.
2. Describe general principals of immunologic and nucleic acid based methods for identification of an organism

Bacterial infection

- Bacterial infection in the body can lead to inflammation. Signs and symptoms vary according to the site and severity of infection. It can be
  - Sepsis, endocarditis, arthritis, catheter associated infections
  - Pneumonia, pleuritis, lung abscess, tuberculosis
  - Bacterial meningitis,
  - urinary / genital tract infections and bacterial vaginosis
  - bacterial cellulitis, peritonitis, food poisoning,
  - Wound infections

Diagnosis requires history, physical exam, radiographic finding and lab data.

Diagnosis of infectious disease

Laboratory investigations are important in the diagnosis, treatment and surveillance of infectious disease. These investigations may be general investigations, bacteriological cultures and its identification.

- Specimen are examined to detect, isolate and identify pathogens (**bacteriologic** approach),
- Serologic tests to detect antibody or antigen against the organism in the patient's serum (**immunologic (serologic)** approach).

Diagnosis of an infections

1. Bacteriologic approach: The laboratory diagnosis of an infectious disease begins with the collection of an appropriate clinical specimen for examination or processing in the laboratory. It includes

   a) ***Right*** specimen,

   b) at the ***right*** time (collection), collected

   c) With ***right*** aseptic procedure

   d) In the ***right*** container (sterile)

   e) on the ***right*** way (transportation)

   f) to the ***right*** laboratory.

   g) Provide essential information

Specimen collection is *the back bone for isolation and identification of causative agents*

Specimen selection

Specimen must be representative of the disease, i.e.

1. Throat & Nasopharyngeal swab
2. Broncheal & broncho alveolar wash
3. Rectal swab & stool
4. Urine
5. Skin & mucous membrane scraping
6. Sterile body fluid: CSF, pleural, joint fluid
7. Blood, bone marrow & tissue specimens
9. Serum for Ab or Ag (proteins) testing

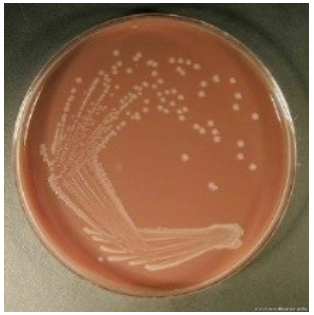
Specimen Collection

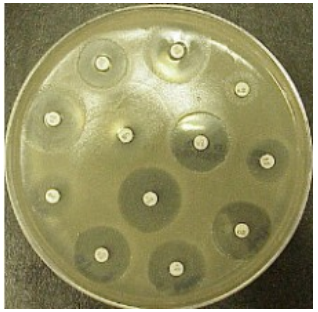
1. Appropriate specimen,
   a) Tissue are excellent and should be kept moist in few drops of sterile normal saline,
   b) Aspirate, Blood, CSF, urine, bone marrow etc in sufficient quantity.
   c) swab are least desirable
2. Time of collection
   a) In acute phase: agents are more likely to be isolated
   b) Before Antibiotic therapy
   c) First morning specimen is best for some specimens,
3. Specimen containers: should be **sterile**, leak proof, disposable; and should not be reused.
4. Selection of appropriate anatomic site

Lab Identification of Microorganisms

The laboratory approach to identify the microorganism are

1. Staining and observing the microorganism in the microscope.
2. Obtaining a pure culture of the organism by inoculating it onto a bacteriologic media.
3. Identifying the organism by using biochemical reactions, growth on selective media, DNA probes, or specific antibody reactions. (and antibiotic sensitivity).





LAB

- **Observation**
  - Macroscopy
  - Microscopy
  - Staining
    - Gram, AFB, Giemsa
    - Morphological characteristics
- **Pure culture (inoculation)**
  - Antibiotic susceptibility
- **Identification**
  - Bioch reactions
  - Growth on selective cultures
  - DNA probes
  - Antibody detection

- **Immunological**
  - Antigen in pt's serum
  - Antibodies in patient serum
  - PCR & DNA probes

Isolation, Identification & diagnosis of bacteria infections

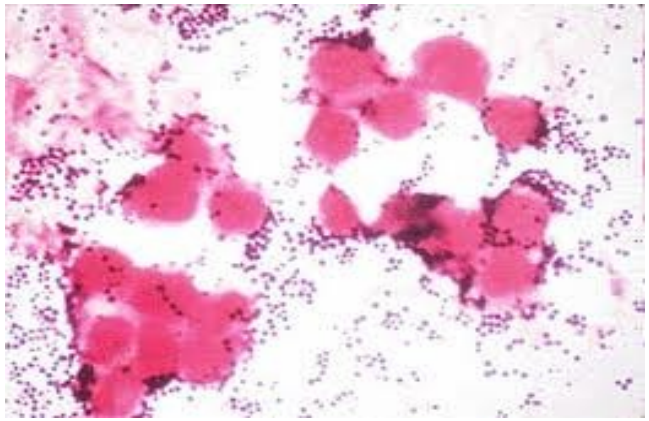
Identification criteria

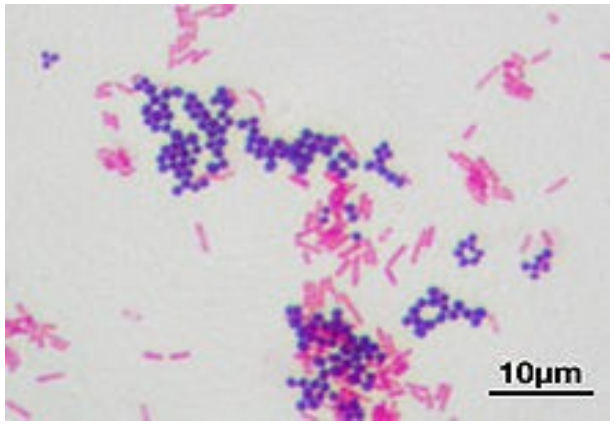
Microscopic morphology

a. Gram stain reaction: positive or negative

b. Shape: cocci, rods, coccobacilli, vibrio

c. Arrangement: single, pairs, chains





1. Biochemical reactions
2. Susceptibility or resistance to antibiotics
3. Serotyping

Diagnosis

laboratory methods

Serological procedures for the detection & rise of antibody levels

1. IgM in single serum
2. Rise in paired serum

Blood TLC & DLC

Culture & Bacterial isolation

Detection of antigens (proteins, enzymes)

Soluble Ag ELISA, SPRIA, RPHA, CIEP

Detection of nucleic acid in the specimen (very costly)

1. Molecular methods (PCR)

CIEP (Counterimmunoelectrophoresis),
SPRIA (solid phase radioimmunoassay),
RPHA (reverse passive hemagglutination),

IMMUNOLOGIC METHODS

1. These are used to identify pathogen,
2. Evaluate course of an infection, acute or chronic infection, or
3. to determine nature of an infection i.e. primary or secondary infection.

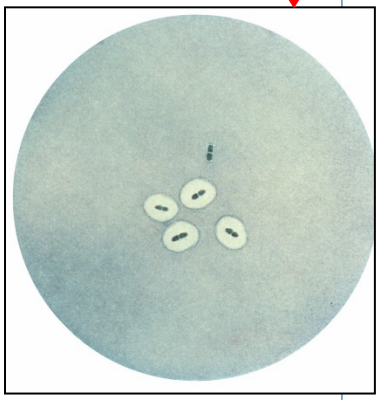
IMMUNOLOGIC METHODS

There are essentially two basic approaches:

1. using known antibody to identify the microorganism, and
2. using known antigens to detect antibodies in the patient's serum.

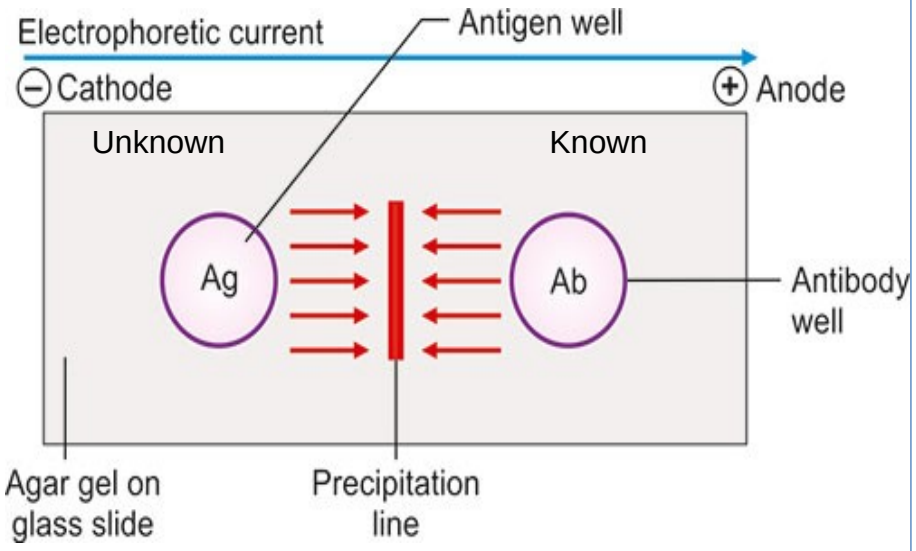
Identification of a microorganism with known antiserum

1. **Capsular Swelling (Quellung) Reaction** capsule swells in the presence of homologous antiserum. *(Str. pneumoniae, H. influenzae* type b, and *N. meningitidis* groups A and C are identified).
2. **Slide Agglutination Test** Antisera against *Salmonella* and *Shigella* can agglutinate them.
3. **Latex Agglutination Test** Latex beads coated with specific antibody are agglutinated in the presence of homologous bacteria or antigen.
4. **Counterimmunoelectrophoresis Test**
5. **Enzyme-Linked Immunosorbent Assay**
6. **Fluorescent Antibody Tests**



Counterimmunoelectrophoresis Test

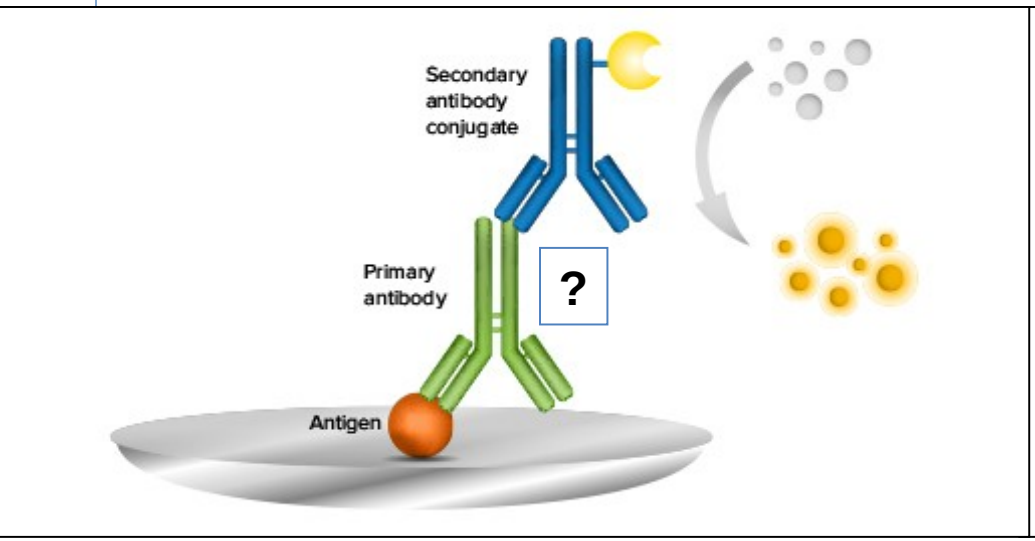
- In this test, the **unknown bacterial antigen** and a **known specific antibody** move toward each other in an electrical field. If they are homologous, a precipitate forms within the agar matrix. The test can be used to detect the presence in the spinal fluid of the capsular antigens of
  - *H. influenzae,*
  - *N. meningitidis,*
  - *Str. pneumoniae,*
  - and group B streptococci.

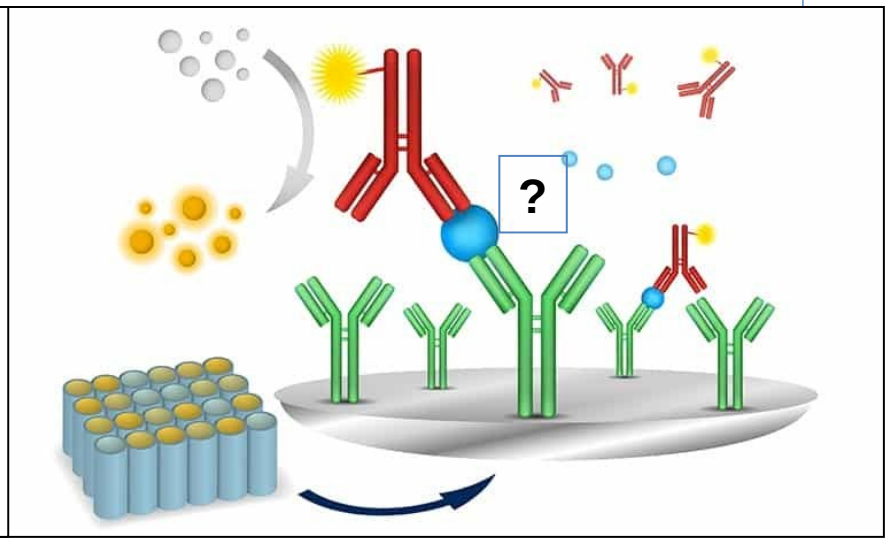


Enzyme-Linked Immunosorbent Assay

- In this test, a enzyme linked specific antibody is used to detect the presence of the homologous antigen.

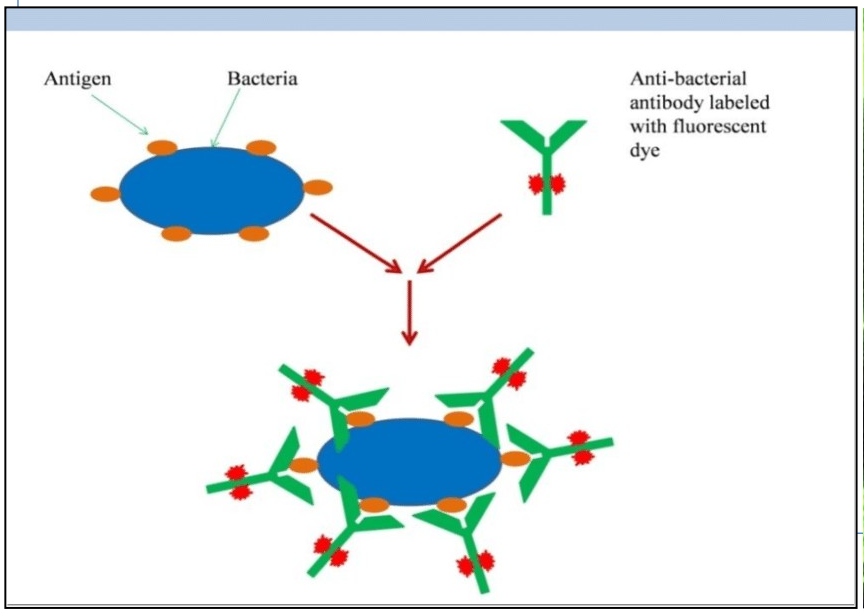
  Because several techniques have been devised. This test is useful in detecting a wide variety of bacterial, viral, and fungal infections.

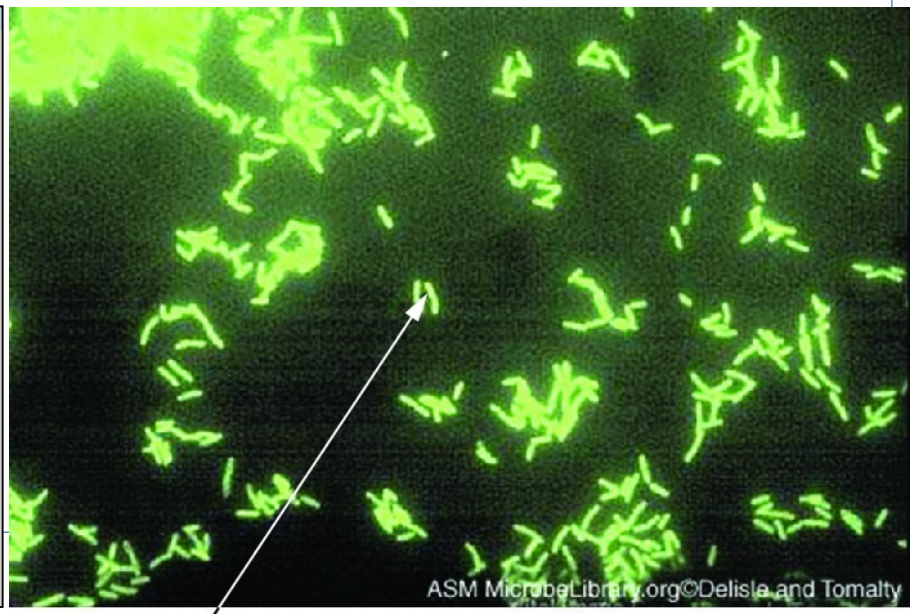




Fluorescent Antibody Tests

- A variety of bacteria can be identified by exposure to known antibody-labeled with fluorescent dye, which is detected visually in the ultraviolet microscope.





Fluorecein-labeled antibody attached to *Legionella* bacilli

Identification of serum antibodies with known antigens

- **Slide or Tube Agglutination Test**

  serial two-fold dilutions of a sample of the patient's serum are mixed with standard bacterial suspensions. The highest dilution of serum capable of agglutinating the bacteria is the titer of the antibody. It is used for diagnosis of typhoid fever, brucellosis, tularemia, plague, leptospirosis, and rickettsial diseases.

- **Serologic Tests for Syphilis**

  Flocculation (clumping) of the cardiolipin occurs in the presence of antibody to *T. pallidum*. The VDRL and RPR tests are the examples.

- **Cold Agglutinin Test**

  Patients with *Mycoplasma pneumoniae* infections develop autoimmune antibodies that agglutinate human red blood cells in the cold (4°C) but not at 37°C

Specific antibody detection

- **Seroconversion** occurs when antibody is produced in response to a primary infection.

- IgM: early in infection (2-3 weeks)
  transient (3-6 months)

Persists for short time

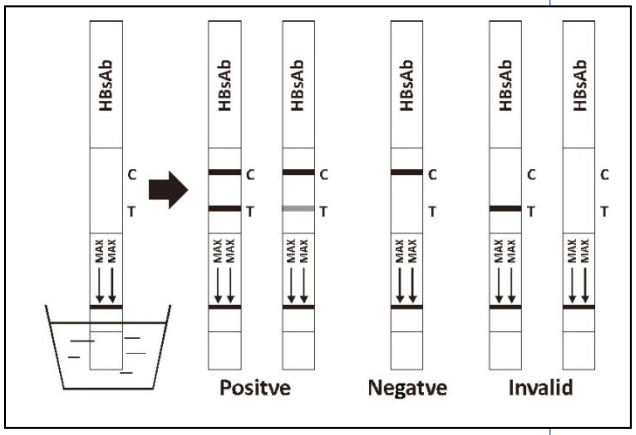
*sometimes persists longer

- IgG: forms later (immunity)
  highest in 4-6 months
  usually persists during the whole life

- IgG avidity: High: past infection
  Low: new infection

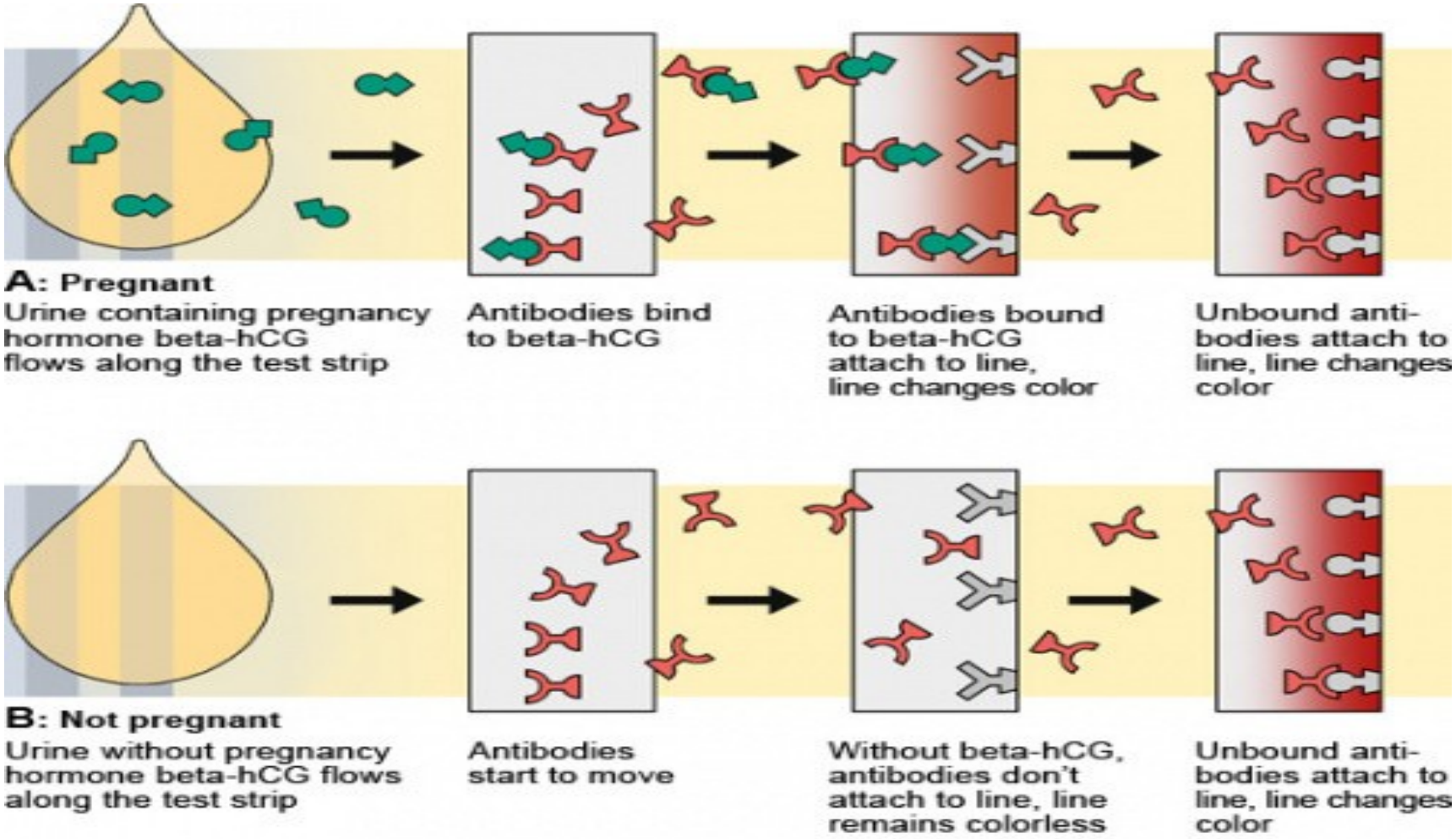
Immunologic tests for diagnosis of infections

- The **immunological tests** based on artificial antibodies that exactly "match" the suspected pathogen.
- When these antibodies come into contact with a sample of blood, urine or stool, they bind to the matching substance or germ if present in the sample i.e. ELISA & rapid tests.



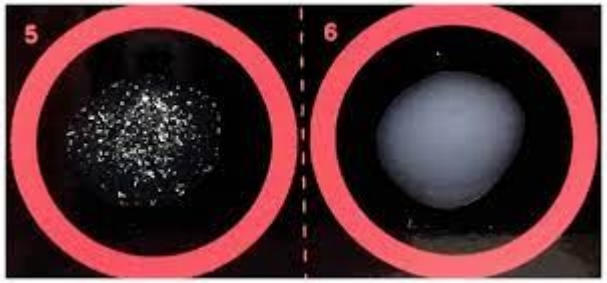
- Other technique used are Antibody generation, Antibody isolation and purification, ELISPOT, Immunoblotting, Immunohistochemistry, Immunoprecipitation, Immune cell isolation.

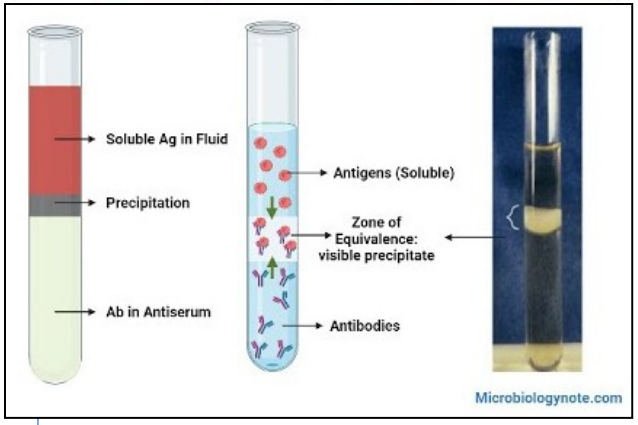
Rapid Tests


A: Pregnant
Urine containing pregnancy hormone beta-hCG flows along the test strip
Antibodies bind to beta-hCG
Antibodies bound to beta-hCG attach to line, line changes color
Unbound anti-bodies attach to line, line changes color
B: Not pregnant
Urine without pregnancy hormone beta-hCG flows along the test strip
Antibodies start to move
Without beta-hCG, antibodies don't attach to line, line remains colorless
Unbound anti-bodies attach to line, line changes color

Classical Serologic methods

- **Agglutination test:** Clumping of antibodies with antigens i.e Widal test for *Salmonella,* Wright test *for Brucella,* Weil-Felix test for *Rickettsia)*
- **Precipitation test**: Soluble antigen + sol antibody= insoluble clumps
- **Immunofluorescent techniques**.
- **Complement fixation test**
- **Heamagglutination inhibition test**
- **Neutilization test**



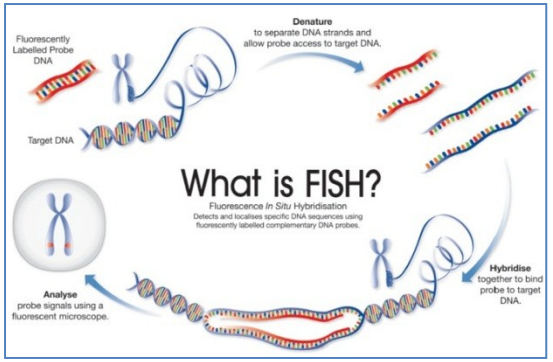


NUCLEIC ACID BASED METHODS

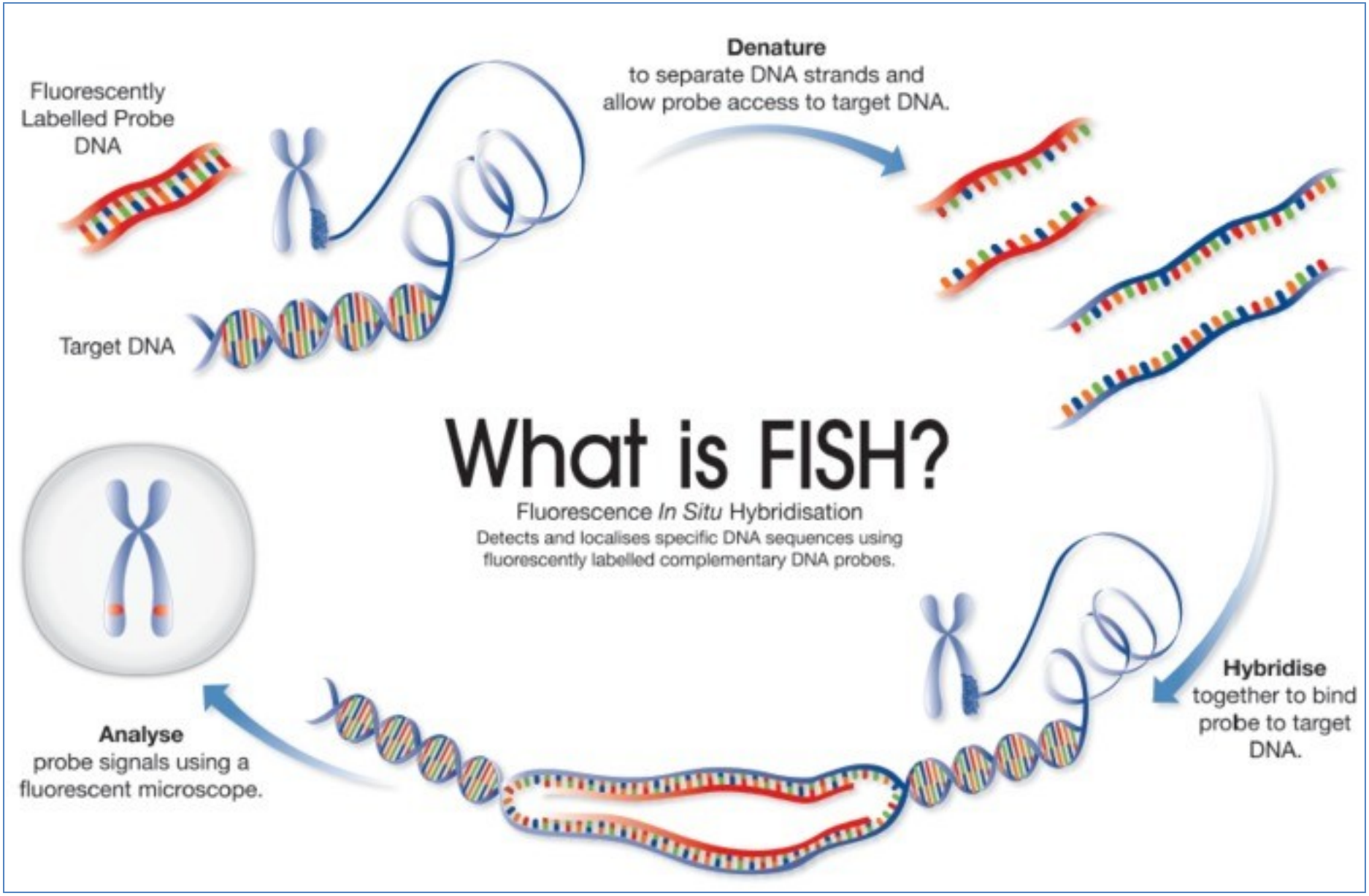
- Molecular based techniques such as PCR detects and amplifies the specific nucleic acids of pathogens.
- In combination with the advanced methods in genome sequencing, PCR is the gold-standard for diagnosis of microbial disease (Nucleic acid amplification technique NAT or NAAT)

Genetic probes

- Genetic probes **identify** genus or species-specific DNA or RNA sequences.



Genetic Probes is a fragment of **DNA** or RNA of variable length (usually 100-1000 bases long) which is radioactively labeled. These are used in **DNA** or RNA samples to detect the presence of nucleotide sequences (the **DNA** target) that are complementary to the sequence in the **probe**.


Denature
to separate DNA strands and
allow probe access to target DNA.
Fluorescently
Labelled Probe
DNA
Target DNA
What is FISH?
Fluorescence *In Situ* Hybridisation
Detects and localises specific DNA sequences using
fluorescently labelled complementary DNA probes.
Hybridise
together to bind
probe to target
DNA.
Analyse
probe signals using a
fluorescent microscope.

Treatment selection:

Ideal antibiotic therapy is based on determination of the etiological agent and its sensitive antibiotics.

Thanks