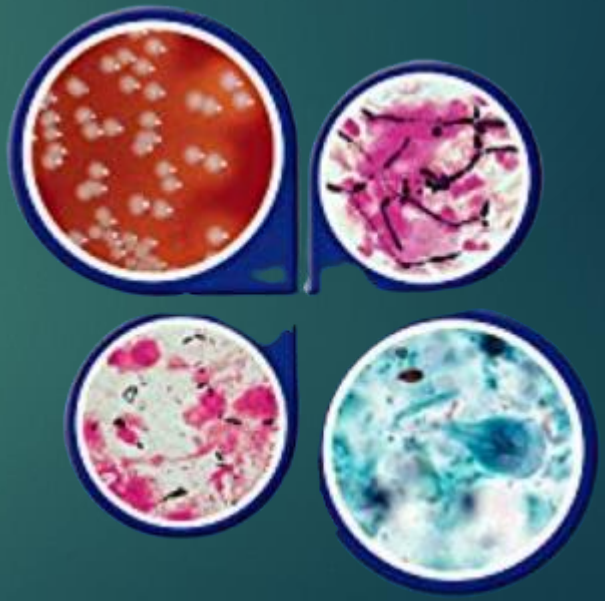
Diagnostic Microbiology

By
Dr. Shilait Barua



Diagnostic medical microbiology is concerned with the etiologic diagnosis of infection.

Identifying the organism causing an infectious process is usually essential for effective antimicrobial and supportive therapy. Initial treatment may be empiric, based on the microbiologic epidemiology of the infection and the patient's symptoms. However, definitive microbiologic diagnosis of an infectious disease usually involves one or more of the following five basic laboratory techniques, which guide the physician along a narrowing path of possible causative organisms: 1) direct microscopic visualization of the organism, 2) cultivation and identification of the organism, 3) detection of microbial antigens, 4) detection of microbial DNA or RNA, and 5) detection of an inflammatory or host immune response to the microorganism (Figure 4.1).

The methods employed in the laboratory for diagnosing infectious (bacterial, viral, fungal, parasitic) and non infectious diseases fall broadly into following categories

Phenotypic methods
Serological analysis
Nucleic acid tests
Biologic assays

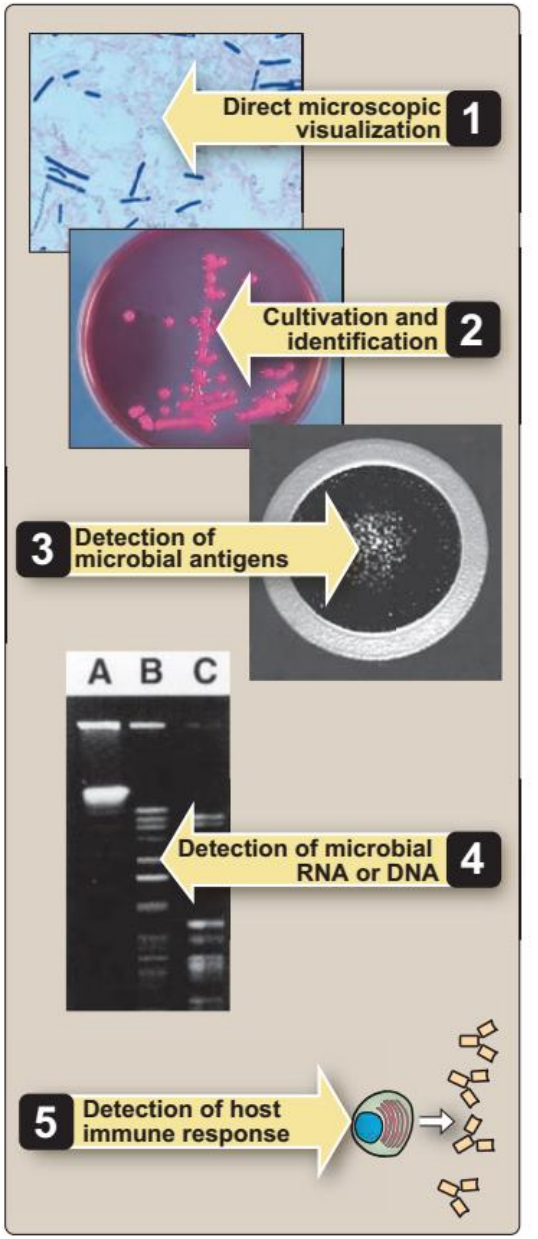
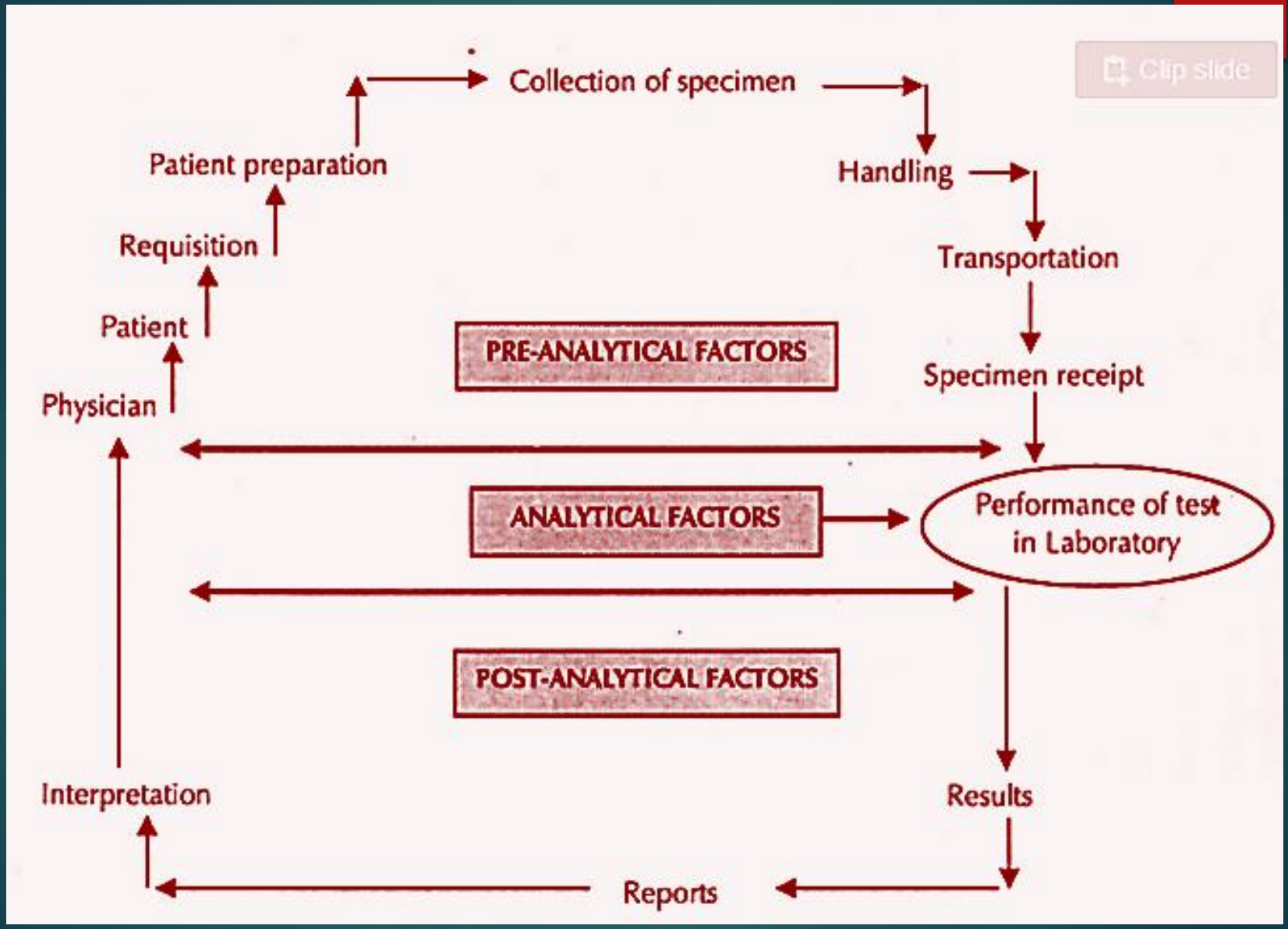


Figure 4.1
Laboratory techniques that are useful in diagnosis of microbial diseases.

Stages of Clinical Diagnosis



Specimens Collections & Specimen Processing

Specimen Definition

Any substance which is taken from body of a person for testing in the laboratory is called specimen.

For example:

- **Blood**
- **Urine**
- **Pus**
- **Saliva**
- **Sputum**
- **Nasal discharge etc**
- **Cerebro Spinal Fluid (CSF)**

Significance of Specimen Collection

- Specimen collection in microbiology is done in order to isolate and identify the causative agent of a disease.
- Without successful isolation and identification of microorganisms none of disease can be precisely diagnosed nor treated properly.
- This forms the back bone of all the investigative procedures.

Items that should be considered in collection of any specimen:

1. What to collect? **(Type of specimen).**
2. How to collect **(Procedure).**
3. When to collect specimen **(Best time of specimen collection)**
4. Use the proper aseptic collection technique.
5. Need for any preservative or transport medium.
6. Labeling of the specimen container.

General rules for collection

- Use aseptic technique for specimen collection.
- Start collection of specimens for all cultures before starting an Antibiotic treatment.
- Prevent contamination from normal flora.
- Specimen should be adequate in amount.
- Need for transport medium or any preservative.

- Collection should be done in a sterile, leak proof, properly labeled tightly closed container.
- Specimen should be accompanied with information paper/request form.
- Information paper has name, age, sex, address of patient, type of specimen, investigation required, date and time of specimen collection, name and signature of the doctor.
- Prompt processing of specimen and transportation to the laboratory after collection.

Type of specimen

- The correct type of specimen to collect will ***depend on the pathogens to be isolated.***
- Cervical (from the cervix) not vaginal swab is required for the most successful isolation of *N. gonorrhoeae from a woman.*
- *Sputum not saliva is essential for the isolation of respiratory pathogens.*

Time of Specimen Collection

- Best time to collect **urine and sputum** is early in the morning when patient wakes up (because microorganisms have had the opportunity to multiply for several hours).

- For collection of **blood** for culture best time is when the temperature of patient is rising (because at that time maximum number of microorganisms are present in the blood).

Use of preservative/transport medium

- When facilities are not available to perform the desired tests at the place of collection or laboratory is located far away.
- Then we use some suitable preservative or transport culture medium in order to keep suspected microorganisms **alive** and also to **suppress growth** of unwanted organisms.

Labeling specimens

- Each specimen must be clearly labeled with:

1.The **date.**

2.Timeof collection.

3.The patient's **name, number, ward *or health centre.***

Label High Risk Specimens

- Sputum with suspected *Mycobacterium tuberculosis.*
- Fecal specimens suspected with *Vibrio cholera* or typhoid bacterium.
- Serum suspected with AIDS and Hepatitis causing viruses.
- Pus or fluid from ulcers that may contain Anthrax causing bacterium.

**A HIGH RISK specimen should be seal inside a plastic bag or in a container with a tight-fitting lid.*

WHO specimen collection kit used for material suspected of containing varicella, monkeypox or smallpox viruses

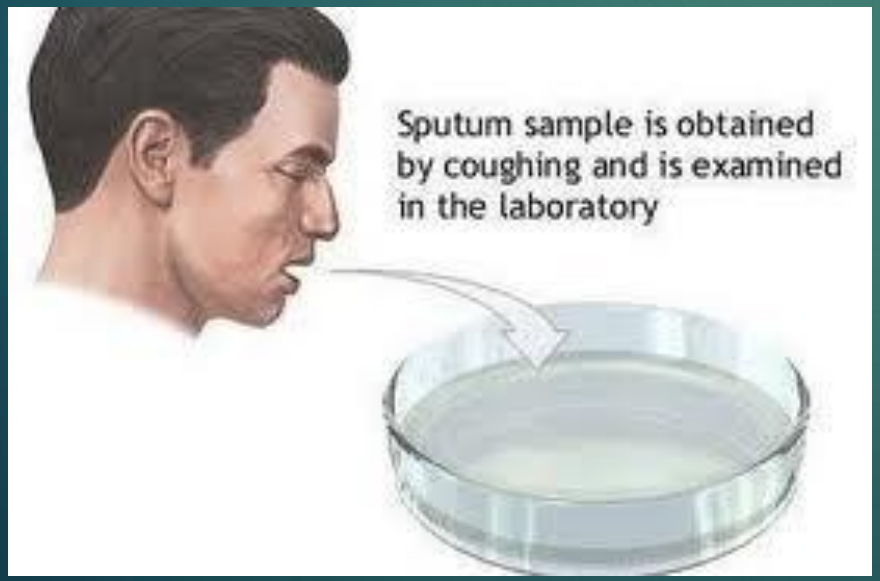
1. Lancet (sterile); 2. Sterile swabs; 3. Plastic specimen collection container; 4. Metal tin; 5. Outer cardboard mail[illegible]container.

Collection Techniques For Different Specimens

1. Sputum

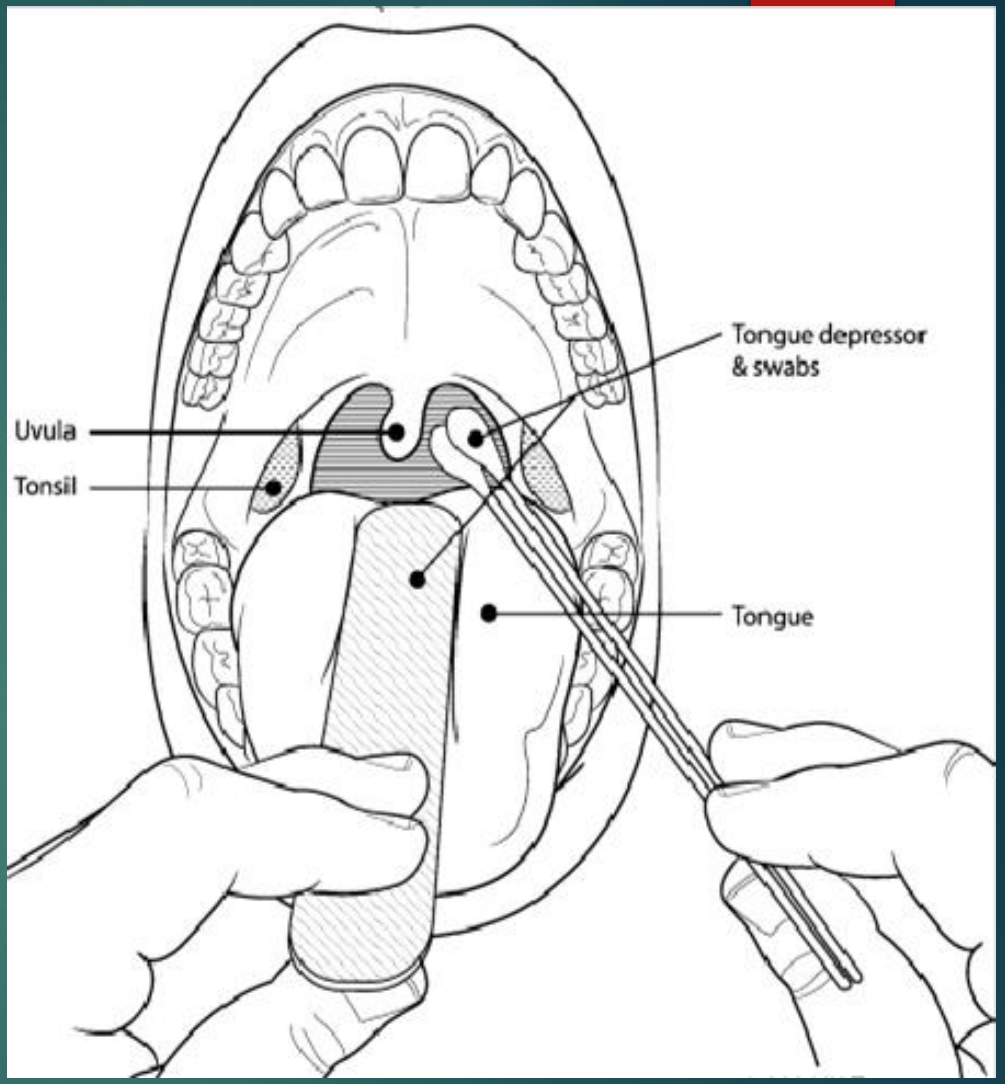
- A clean, dry, wide neck leak-proof container to be used.
- Patient is instructed to cough deeply.
- ***Specimen must be SPUTUM not Saliva.***
- Sputum is best collected in the morning soon after the patient wakes and before any mouth-wash is used.
- Must reach laboratory with in 6hrs.

- Use a phenol-containing disinfectant to wipe the outside of the container after collecting the specimen to prevent the spread of infectious organisms and to avoid contaminating the outside of the container.



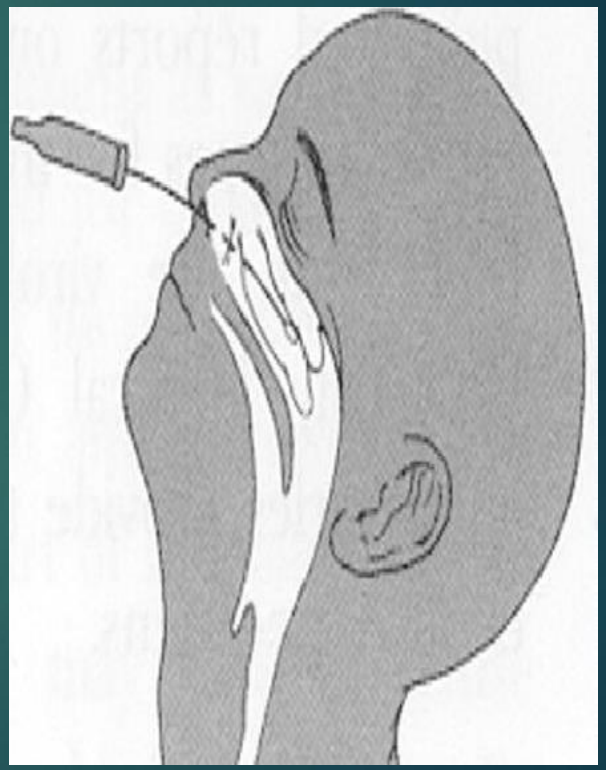
2. Throat and Mouth

- In good-light and using a tongue-depressor examine the inside of mouth.
- Look for inflammation, presence of membrane/ exudate /pus.
- Take specimen with a sterile cotton-wool swab.
- Avoid contamination with saliva (don't touch any-other part of mouth).
- *For 8 hours before swabbing, the patient must not be treated with antibiotics or antiseptic mouth-washes (gargles).*


Tongue depressor
& swabs
Uvula
Tonsil
Tongue

3. Nasal Swabs

- Use sterile cotton wool swab, gently swab the inside surface of the nose.



- Take care not to contaminate the swab,

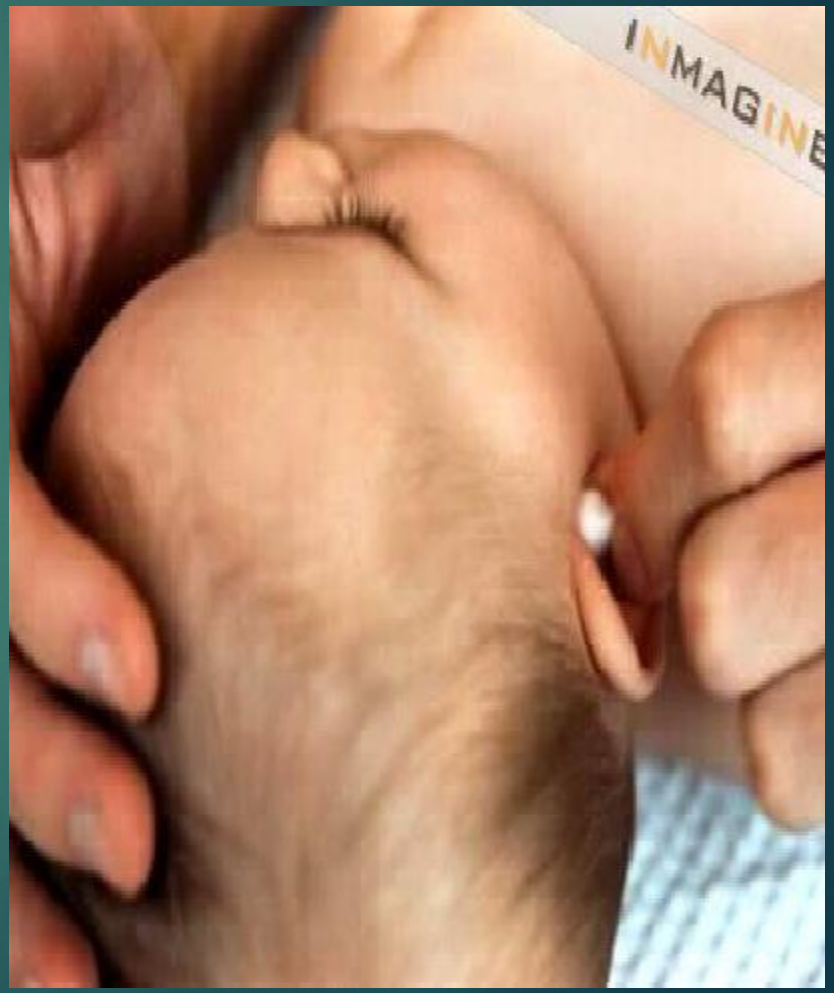
  replace in its sterile container

- Label and within 2 hrs.

  deliver to the lab.

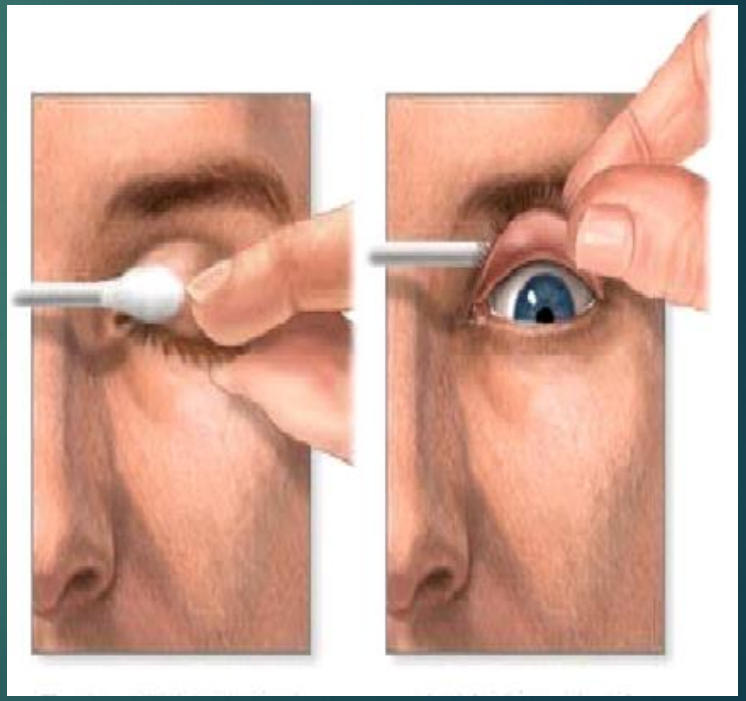
4. Ear Discharges

- Collect the specimen of the discharge on cotton wool swab
- Do not contaminate it.
- Transport within 2 hrs.
  to the lab



5. Eye Specimen

- Should be collected by an experienced person.
- With cotton wool swab, collect any discharge from the eye.
- Deliver immediately to the lab.



6. Collection of Pus from an Ulcer or Skin

- Pus from an abscess is best collected at the time the abscess is incised and drained, or after it has ruptured naturally.
- Avoid contamination from skin commensals.
- Specimen should be collected before Anti-septic dressing is applied.
- Take 5ml of pus with the help of a drainage tube/syringe **or** if pus cannot be drained then use a cotton-wool swab to collect sample.
- Transfer pus to a leak proof sterile container.

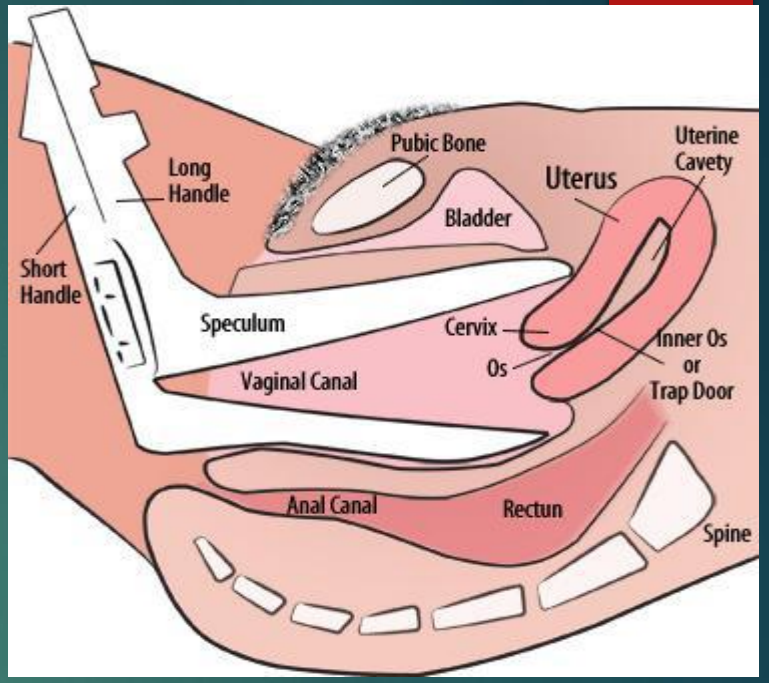
7. Collection of Urogenital Specimen

a) Urethral discharge from male

- Take urethral specimens at least 2–4 hours after urination after the patient has urinated.
- Clean the urethral opening using a swab moistened with sterile physiological saline (0.9% NaCl solution).
- Gently massage the urethra from above downwards.
- Using a sterile swab, collect a sample of discharge.

b) Cervical specimen from female

- Clean the cervix using a swab moistened with sterile saline (0.9% NaCl solution).
- By using a clean speculum (use sterile warm water and not a bactericidal material) containing a sterile cotton wool swab, collect the specimen.
- Endocervical canal is recommended for the isolation of *N. gonorrhoeae*.
- Take specimen by gently rotating the swab against the endocervical wall.



c) **Collection of vaginal discharge to detect T. vaginalis, C. albicans and G. vaginalis**

- *Use a sterile swab to collect a specimen from the vagina.*

*Use only a sterile saline solution or one that is checked daily by the laboratory to exclude contamination.

8. Urine collection

- Whenever possible, the first urine passed by the patient at the beginning of the day should be sent for examination. This specimen is the most concentrated.
- Midstream urine (MSU) for microbiological examination is collected as follows:
- ***Female patients:*** *Wash the hands. Cleanse the area around the urethral opening with clean water, dry the area with a sterile gauze pad, and collect the urine with the labia held apart.*
- ***Male patients:*** Wash the hands before collecting a specimen (middle of the urine flow).

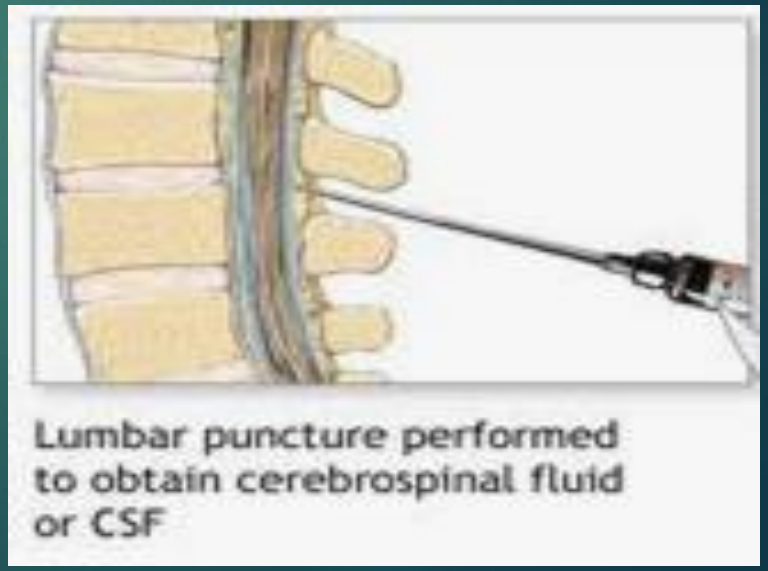
- Collect in a sterile, dry, wide-necked, leak proof container.
- Important: Explain to the patient the need to collect the urine with as little contamination as possible, i.e. a 'clean-catch' specimen.
- **Infant patients:** use the sterile urine collection bags adhered to the skin surrounding the urethral area.
- **Catheter collection specimen** when a patient is bedridden or cannot urinate independently. Specimens may be collected directly from a foley tube.

- Immediate delivery, if not possible refrigerate at 4-6^{0}C **OR**
- Add boric acid as preservative.

Which allows bacteria to remain viable without multiplying.

9. Collection of Cerebrospinal Fluid (CSF)

- CSF must be collected *aseptically to prevent organisms being introduced into the central nervous system.*
- The fluid is usually collected from the arachnoid space.
- A sterile wide-bore needle is inserted between the fourth and fifth lumbar vertebrae and the CSF is allowed to drip into a dry sterile container.



10. Collection of semen

- Semen specimens must be collected 3–7 days of sexual abstinence.
- Collection should be in a clean, dry, leak-proof container.

11. Blood Culture

- Blood culture is required when bacteremia (presence of bacteria in blood) is suspected.
- Whenever possible blood should be collected before antimicrobial treatment has started.
- When the patient has recurring fever, collect the blood as the temperature begins to rise.

Requirements

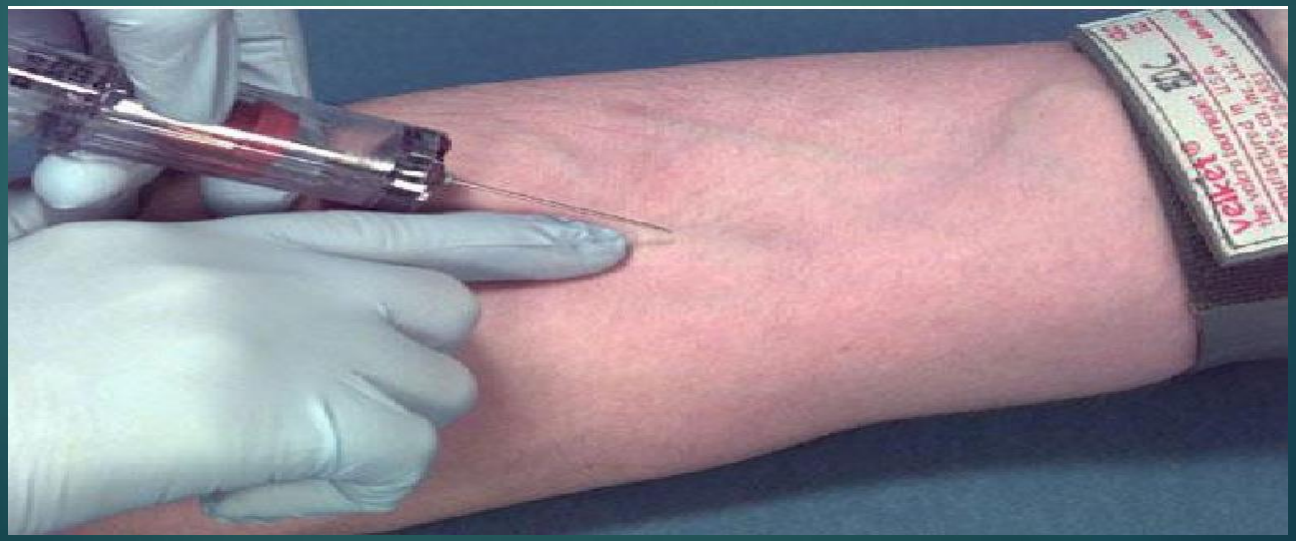
- Gloves.
- Sterile syringes.
- Alcohol swabs.
- Tincture iodine.
- *Oxoid Signal Blood culture system.*
- Incubator.

A strict aseptic technique must be used:

- Wearing gloves, thoroughly disinfect the venepuncture site as follows:
- 70% ethanol, cleanse an area about 50 mm in diameter and allow to air-dry.
- Apply 2% tincture of iodine in a circular action, swab the area beginning at the point where the needle will enter the vein.
- Allow the iodine to dry on the skin for at least 1 minute.
- Remove the protective cover from the top of the culture bottle(s). Wipe the top of the bottle using an ethanol swab.

Procedure

1. Inject 10 ml of blood.
2. Place inoculated bottle at 37°C for one hour before inserting signal device.
3. Continuously shake for 24 hrs at 37°C.
4. Incubate for at least 7 days before recording negative.



STOOL SAMPLE COLLECTION

- Position sample collection paper across the rim of toilet bowl.
- Make a bowel movement onto the collection paper.
- Avoid mixing with urine or water from toilet.
- Poke onto stool at six different sites.
- Collect in a neat, clean, wide mouthed jar.
- Do not clump, scoop, or fill the tube.
- Screw it tightly and level it.
- Store between 2-8 degree or room temperature

- Transport within 1 hour of collection or refrigerate upto 24 hours.
- Warm stools are best for detecting ova and parasites.
- Not recommended on patients who have been hospitalisd for >3 days & not admitted with a diagnosis of gastroenteritis.
- Specimen should be collected before antibiotic therapy is initiated.
- Diarrhoeal stool will always give good results.

Transport of Specimens

1. RAPID, OPTIMALLY IN LESS THAN 2 HOURS.
2. FOR DELAYS IN TRANSPORT, MOST SPECIMENS SHOULD BE REFRIGERATED.
3. EXCEPTIONS: BLOOD, CEREBROSPINAL FLUID (CSF), AND SPECIMENS TO BE EXAMINED FOR ANAEROBES, FASTIDIOUS ORGANISMS SUCH AS *Neisseria gonorrboeae* AND *Bordetella pertussis*, AND *Trichomonas vaginalis*, ALL OF WHICH SHOULD BE MAINTAINED AT ROOM TEMPERATURE.

- **Transport medium** is a safe and an appropriate way of carrying the clinical specimens from distances (long or short) for transporting to the lab for examination.
  - It allows organisms to survive.
  - It does not allow organisms to proliferate.
- **Examples:**
  - For bacteria – **Cary Blair**
  - For viruses – **virus transport media (VTM)**

Specimen rejection criteria(1)

1. IMPROPER TRANSPORT TEMPERATURE
2. IMPROPER TRANSPORT CONTAINER OR MEDIUM
3. PROLONGED TRANSPORT TIME
4. UNLABELED OR MISLABELED SPECIMEN
5. BROKEN OR CRACKED CONTAINER
6. LEAKING SPECIMEN

Specimen rejection criteria(2)

7. DRIED-OUT SPECIMEN
8. INAPPROPRIATE SPECIMEN FOR TEST REQUESTED
9. INADEQUATE VOLUME
10. SPECIMEN IN FIXATIVE (FOR CULTURE)
11. DUPLICATE SAMPLE IN 24-HR PERIOD (FOR URINE, SPUTUM, FECES CULTURE)

Specimen rejection criteria(3)

1. WHEN SPECIMENS ARE REJECTED, THE HEALTH CARE PROVIDER IS NOTIFIED SO THAT ANOTHER SPECIMEN MAY BE PROPERLY SUBMITTED.
2. IF INFORMATION ON THE REQUISITION IS INCOMPLETE, LABORATORY PERSONNEL SHOULD ASK A RESPONSIBLE PERSON TO PROVIDE THE INFORMATION BEFORE PROCESSING THE SPECIMEN FURTHER.
3. IF A SPECIMEN IS MISLABELED, THE SAMPLE SHOULD BE RECOLLECTED. RELABELING OF A SPECIMEN IS ACCEPTABLE ONLY FOR DIFFICULT TO COLLECT SPECIMENS, SUCH AS TISSUE OBTAINED DURING A SURGICAL PROCEDURE OR CSF.

Specimen not routinely accepted for anaerobic culture(1)

1. THROAT, NASOPHARYNGEAL, OR GINGIVAL SWABS
2. SPUTA
3. BRONCHIAL WASH, LAVAGE, OR BRUSH (EXCEPT WHEN COLLECTED WITH A PROTECTED DOUBLE LUMEN CATHETER)
4. GASTRIC AND BOWEL CONTENTS

Specimen not routinely accepted for anaerobic culture(2)

5. ILEOSTOMY AND COLOSTOMY EFFLUENT
6. VOIDED OR CATHETERIZED URINE
7. FEMALE GENITAL TRACT SPECIMENS COLLECTED THROUGH THE VAGINA
8. SURFACE SWABS OF ULCERS, WOUND, AND ABSCESSES

Standard Precautions

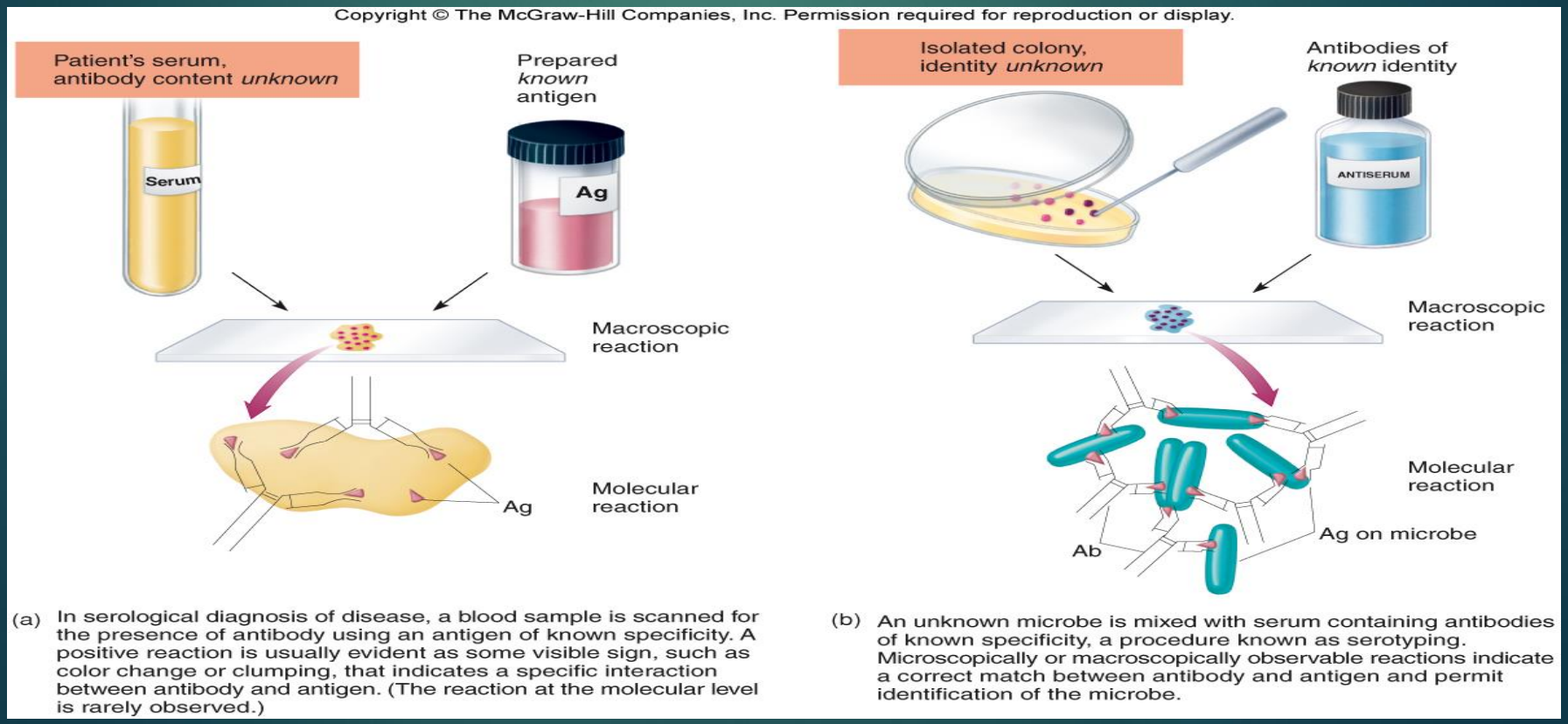
1. ALL SPECIMENS SHOULD BE PRESUMED TO CONTAIN TRANSMISSIBLE AGENTS AND THEREFORE SHOULD BE COLLECTED AND HANDLED USING STANDARD PRECAUTIONS.
2. USE OF GLOVES, GOWN, MASK, AND PROTECTIVE EYEWEAR WHEN THERE IS A RISK OF COMING IN CONTACT WITH THE SPECIMEN
3. IN MOST CLINICAL LABORATORIES, A SPECIAL AREA IS DESIGNED FOR PROCESSING CLINICAL SAMPLES FOR CULTURE.

Serologic tests for Identification of Pathogens

Serologic tests

- Tests based on the in vitro interaction of antigens and antibodies
- Two approaches

1. Using known antibody to identify an unknown antigen
2. Using known antigens to detect antibody in patient's serum



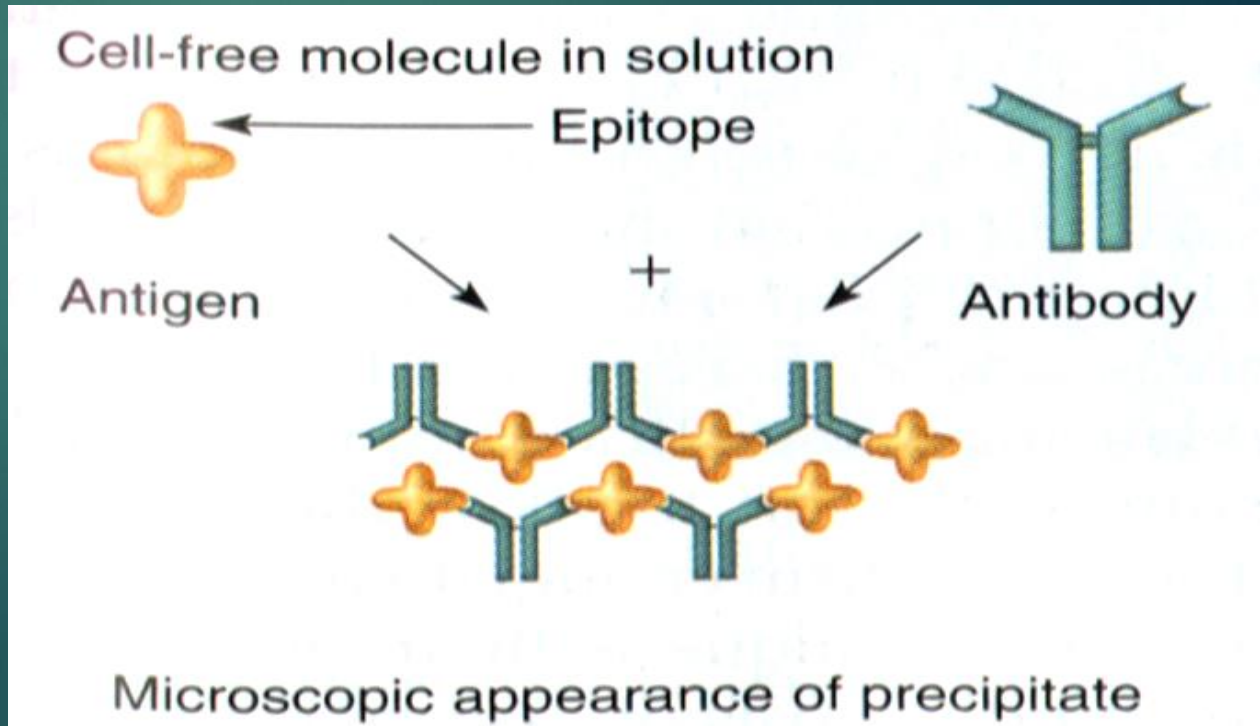
How to detect antigen antibody reactions

Various techniques detect antigen antibody reactions in the laboratory for diagnosis:

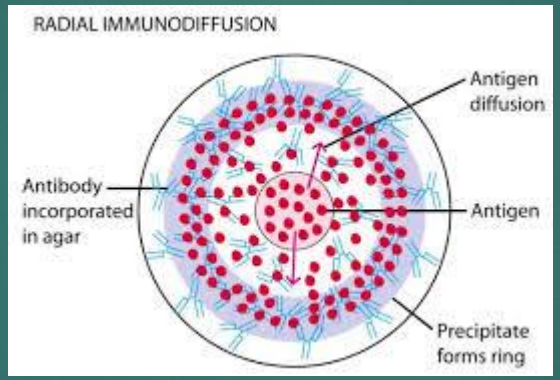
- Precipitation
- Agglutination
- Complement fixation
- Immunochromatography
- Label immunoassays

Precipitation Reactions

- Precipitation is the interaction of a soluble antigen with an antibody resulting in the formation of an **insoluble complex**
- The complex formed is an aggregate of antigen and antibody

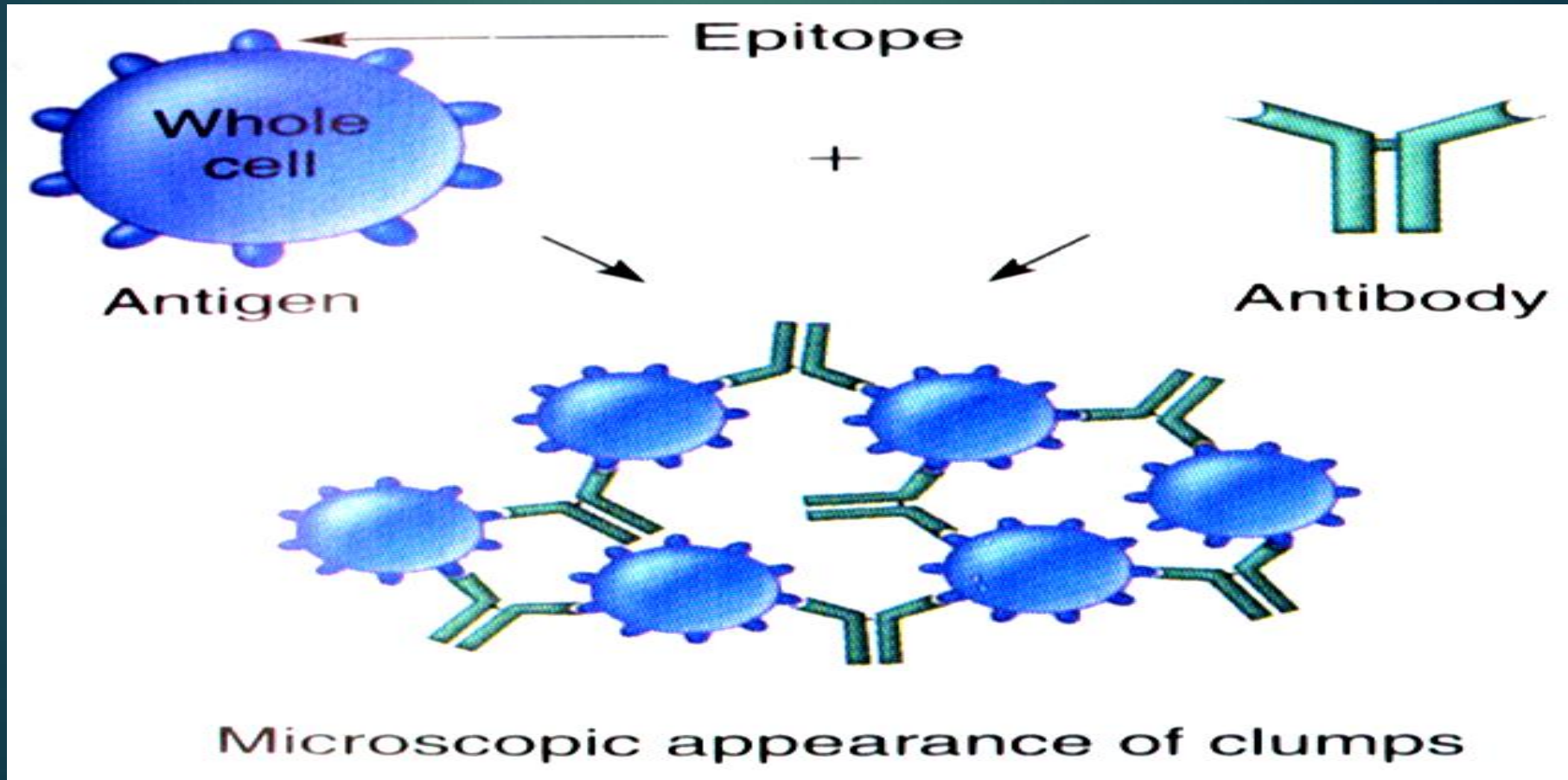


Immunodiffusion



Agglutination Reactions

- Agglutination is the clumping of an antigen when mixed with a specific antibody



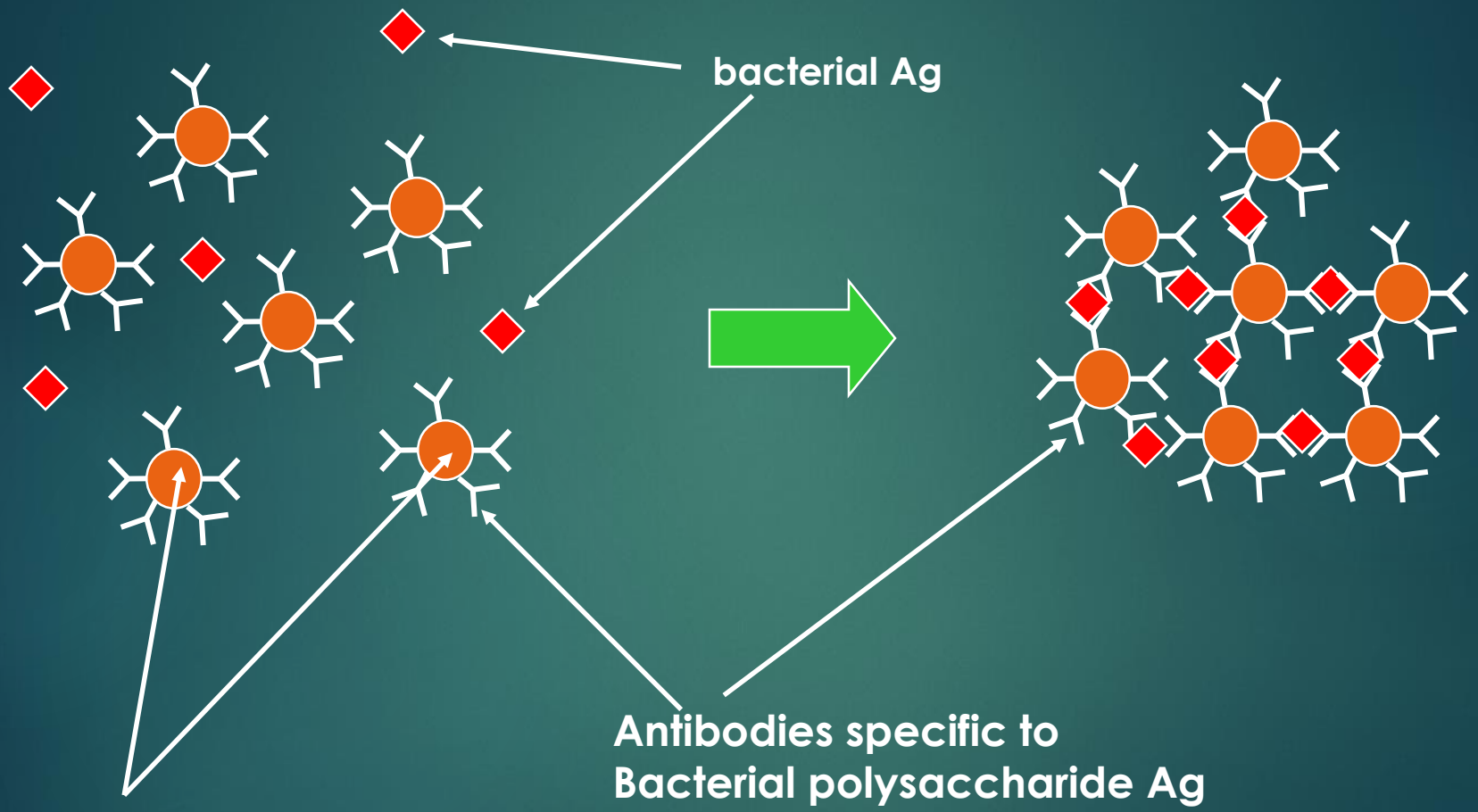
Applications

1. Identification using known antibodies

- Slide agglutination test

  Antisera can be used to identify Salmonella or Shigella antigen in the sample

Latex agglutination test



Latex agglutination test:

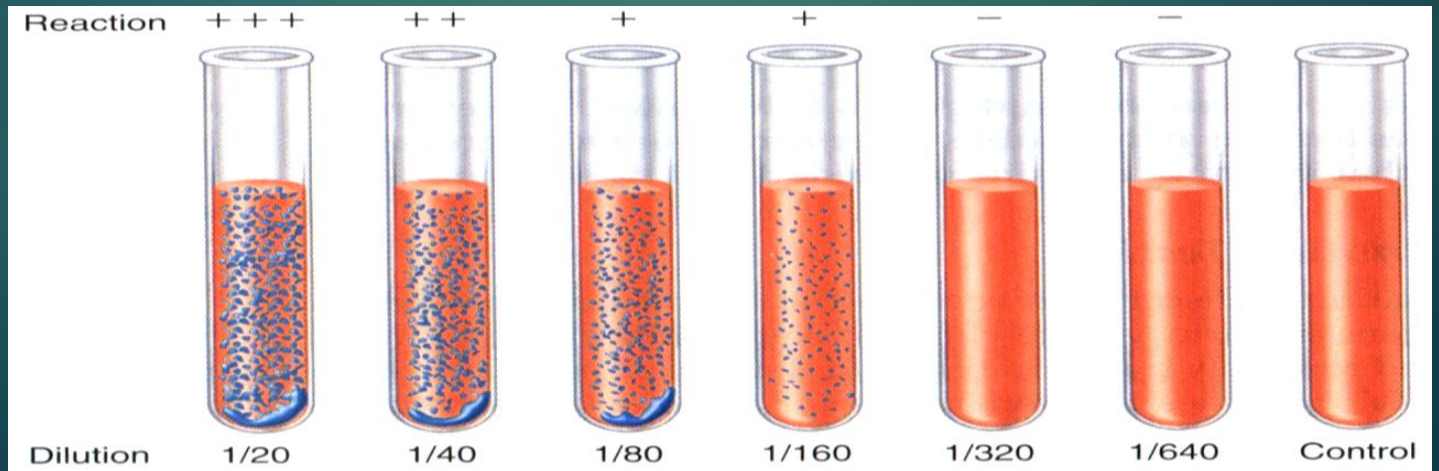
Latex beads coated with specific antibody are agglutinated in the presence of specific antigens. This test is used to determine the presence of H. influenzae, N. meningitidis, several species of streptococci and Cryptococcus neoformans.

2. Identification of serum antibodies with known antigens

Tube agglutination: Patients serum mixed with standard bacterial suspensions example: detection of agglutinins (antibodies) for H and O antigen for Salmonella in patients with enteric fever.

Procedure: Serial dilutions of the patients serum is taken from 1:10 to 1:640. To each equal volumes of Salmonella antigens are added. The tubes are incubated overnight and read.

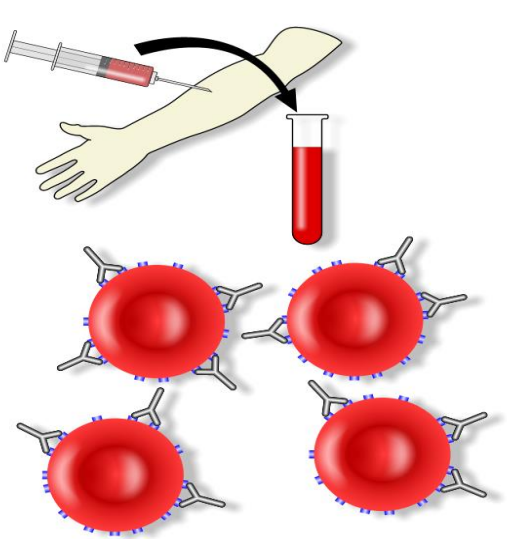
Result: The highest dilution of the serum in which agglutinations occurs is noted.



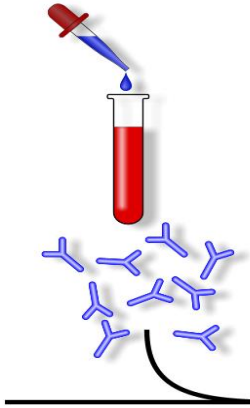
Agglutination reactions also employed in hematology labs for:

- Blood grouping
- Cross match
- hemolytic anemias diagnosis

Direct Coombs test / Direct antiglobulin test

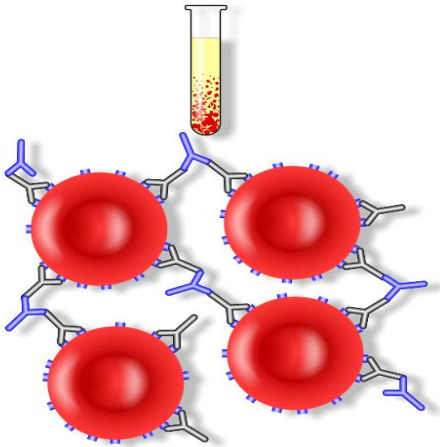


Blood sample from a patient with immune mediated haemolytic anaemia: antibodies are shown attached to antigens on the RBC surface.

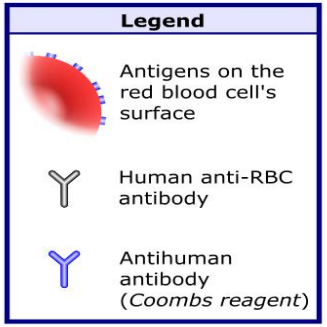


The patient's washed RBCs are incubated with antihuman antibodies (*Coombs reagent*).

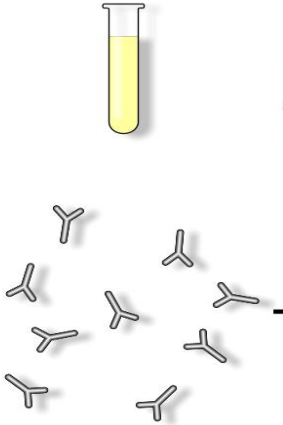
Positive test result



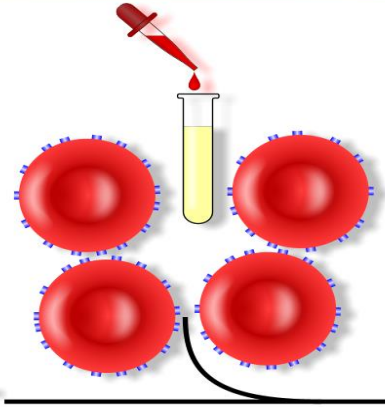
RBCs agglutinate: antihuman antibodies form links between RBCs by binding to the human antibodies on the RBCs.



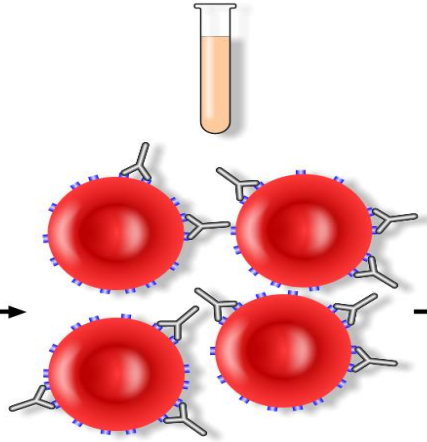
Indirect Coombs test / Indirect antiglobulin test



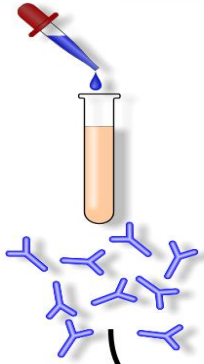
Recipient's serum is obtained, containing antibodies (Ig's).



Donor's blood sample is added to the tube with serum.

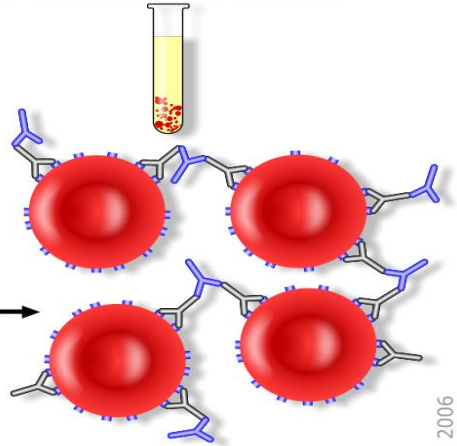


Recipient's Ig's that target the donor's red blood cells form antibody-antigen complexes.



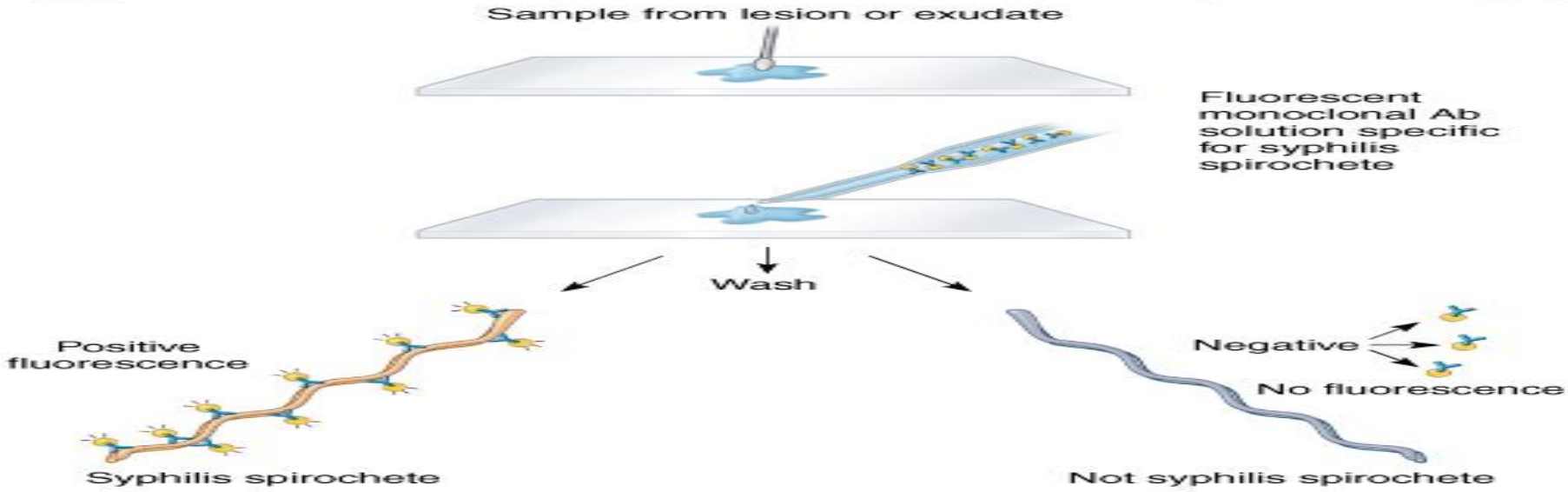
Anti-human Ig's (*Coombs antibodies*) are added to the solution.

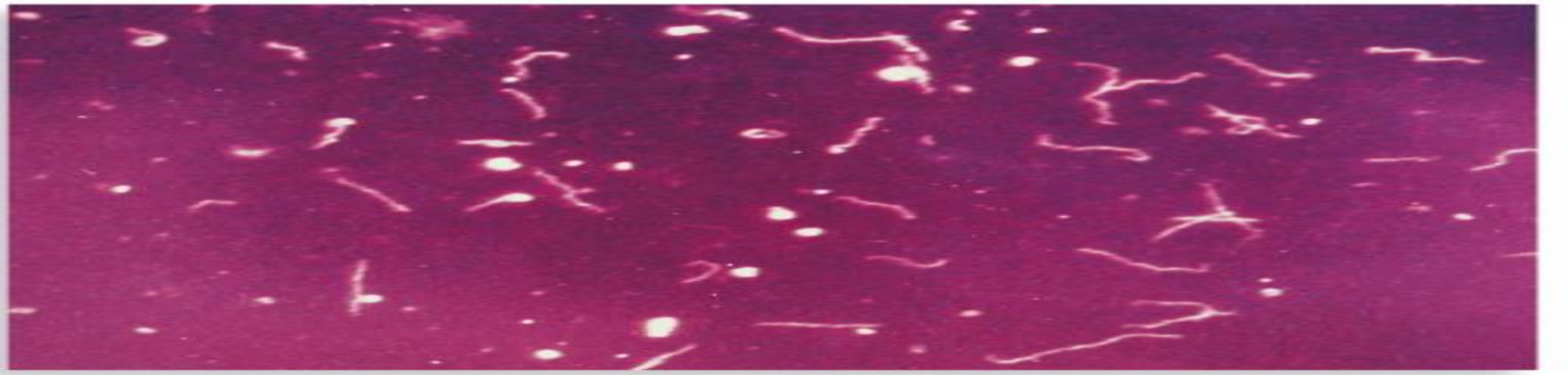
Positive test result



Agglutination of red blood cells occurs, because human Ig's are attached to red blood cells.

Fluorescent Antibodies and Immunofluorescence Testing





Visible fluorescence

Fluorescent microscopy

Unknown antigen (usually cell or tissue)

+

Antibody labeled with fluorescent dye

(a) Direct Testing

Ab2 fluorescently labeled; specific for Ab1

Ab1 in serum

Known Ag

No Ab in serum

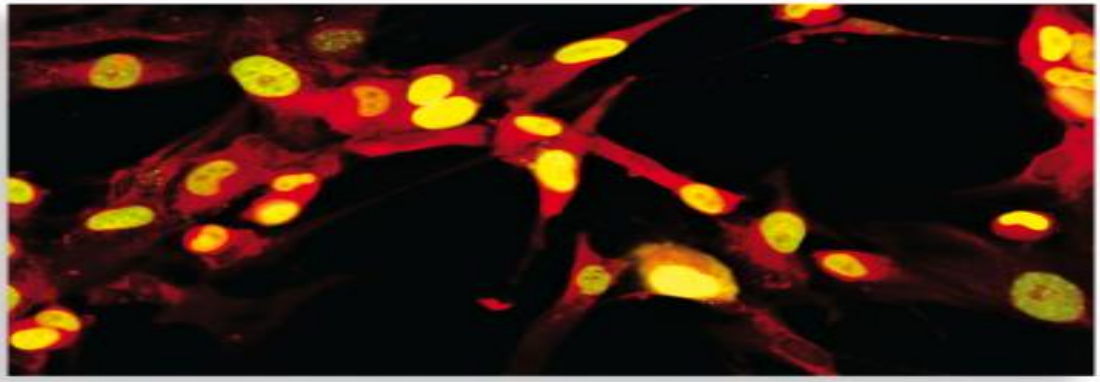
Ab2 attaches to Ab1— visible fluorescence

Positive

Ab2 cannot attach— no fluorescence

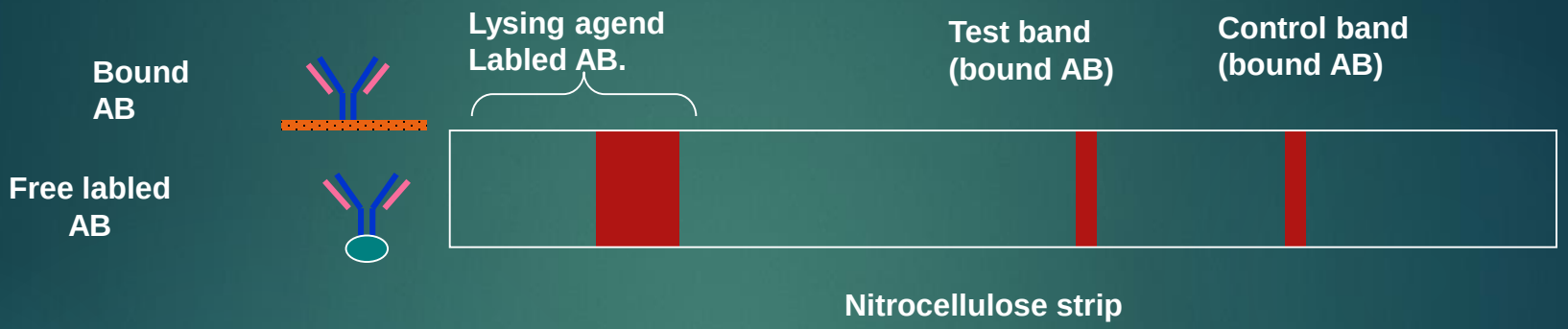
Negative

(b) Indirect Testing



(c) Indirect Immunofluorescence Testing

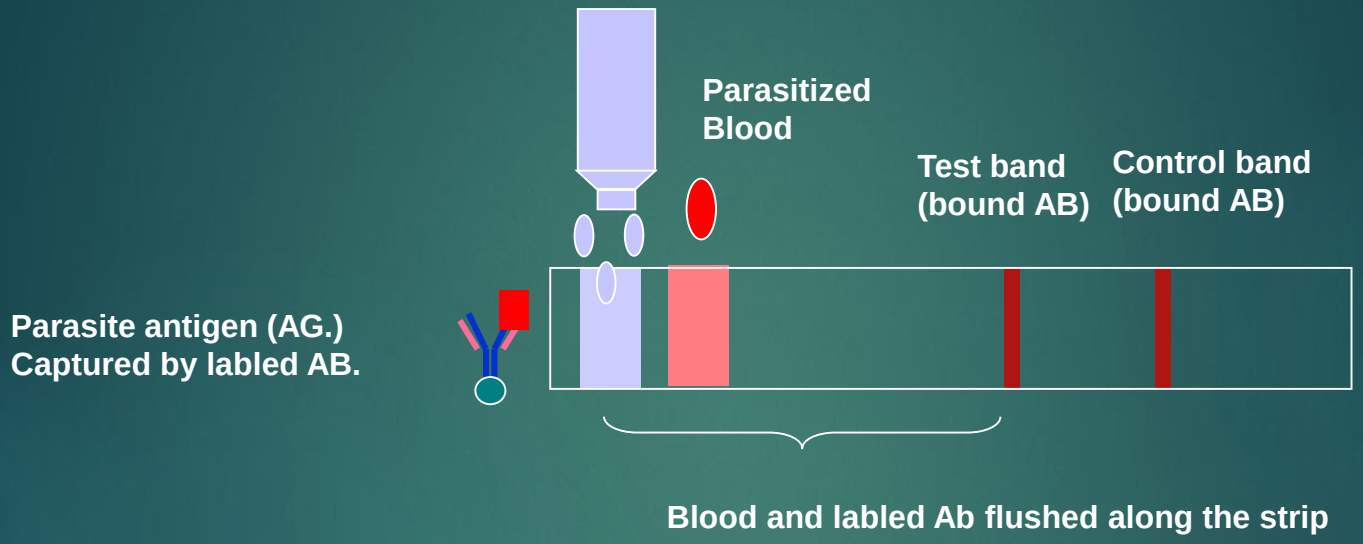
Immunochromatography



Dye-labelled antibody, specific for target antigen, is present on the lower end of nitrocellulose strip

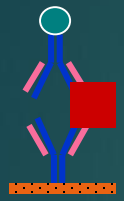
Antibody, also specific for the target antigen, is immobilised to the strip in a test line, and antibody specific for the labelled antibody is immobilised at the control line

Immunochromatography



Blood sample is placed on the strip, the antigen binds with labelled antibody and the complex is drawn up the strip by capillary action

Immunochromatography

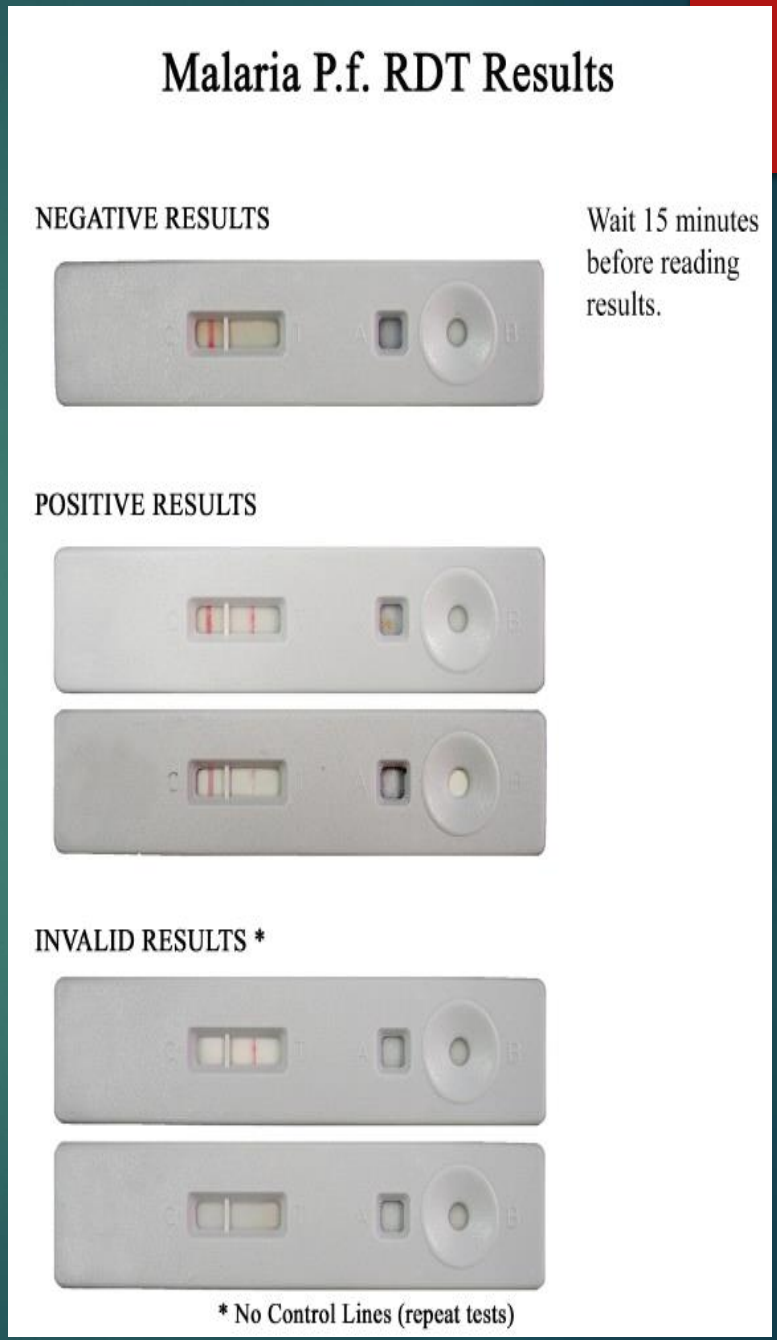


Labled AB-AG-complex Captured by bound AB of test band

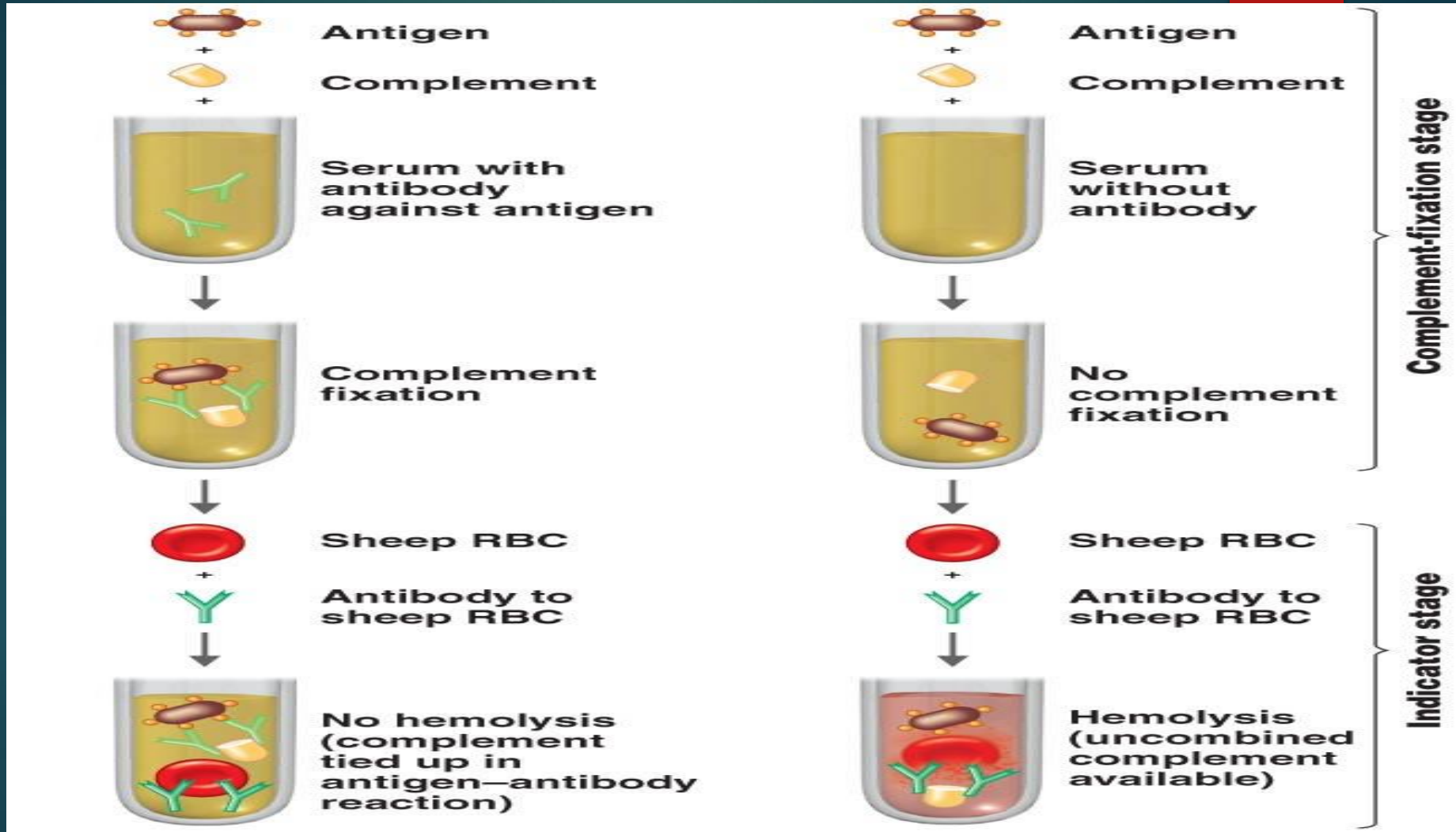
Labled AB-AG-complex Captured by bound AB of control band

If antigen is present, some labelled antibody will be trapped on the test line. Excess-labelled antibody is trapped on the control line

Pregnancy test



Complement Fixation:



(a) Positive test. All available complement is fixed by the antigen–antibody reaction; no hemolysis occurs, so the test is positive for the presence of antibodies.

(b) Negative test. No antigen–antibody reaction occurs. The complement remains, and the red blood cells are lysed in the indicator stage, so the test is negative.

Applications:

- Diagnosis of fungal, viral and parasitic infections
- Studying complement levels

Immunofixation (Western Blot)

- Involves electrophoretic separation of proteins followed by an immunoassay to detect those proteins
- Test material is electrophoresed in a gel to separate out particular bands
- Gel transferred to a special blotter that binds the reactants in place
- Blot developed by incubating it with a solution of antigen or antibody labeled with radioactive, fluorescent, or luminescent labels
- Used to confirm the presence of antibody to specific antigens (HIV)

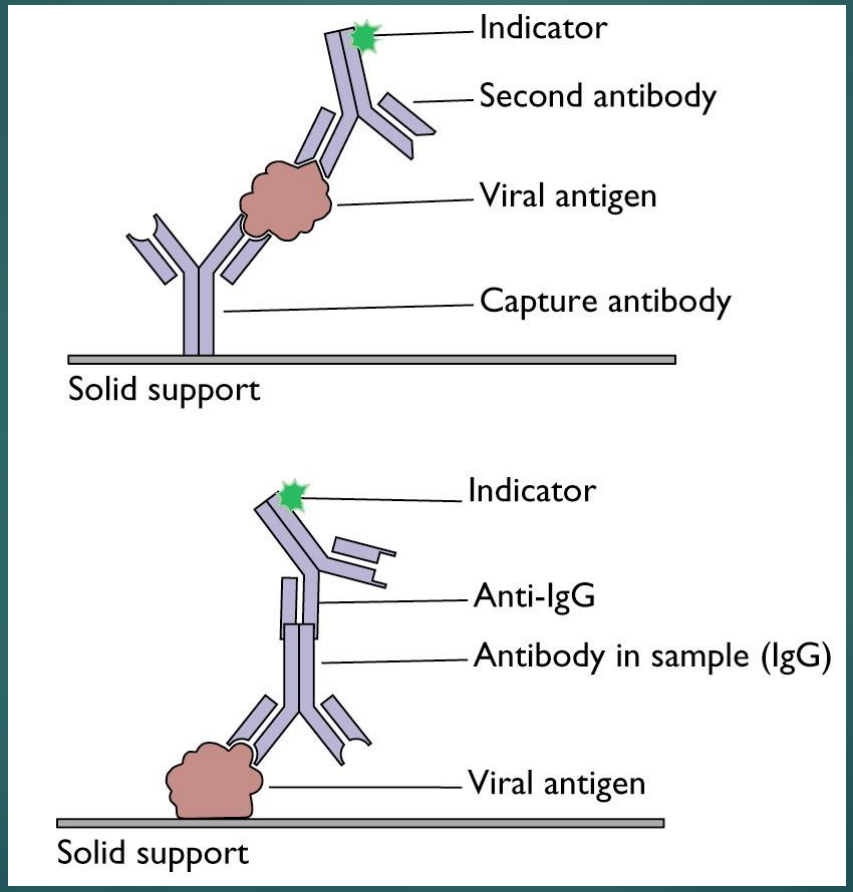
Indicator\ label Immunoassays

- This immunoassay system detects antigen antibody binding by a label /indicator bound antigen or antibody
- The indicators or labels used are enzymes, radioactive, chemiluminescent or florescent molecules
- Examples include

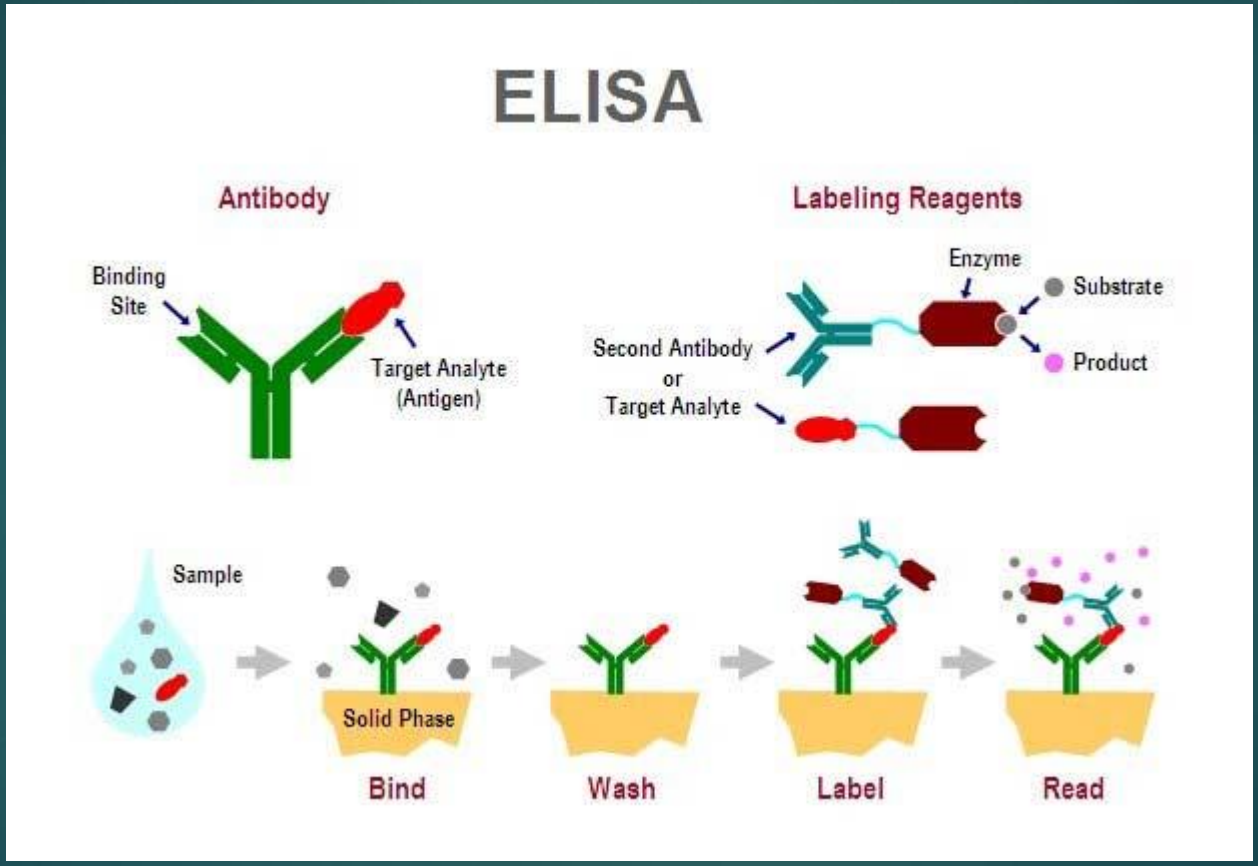
  Radioimmunoassay (RIA)

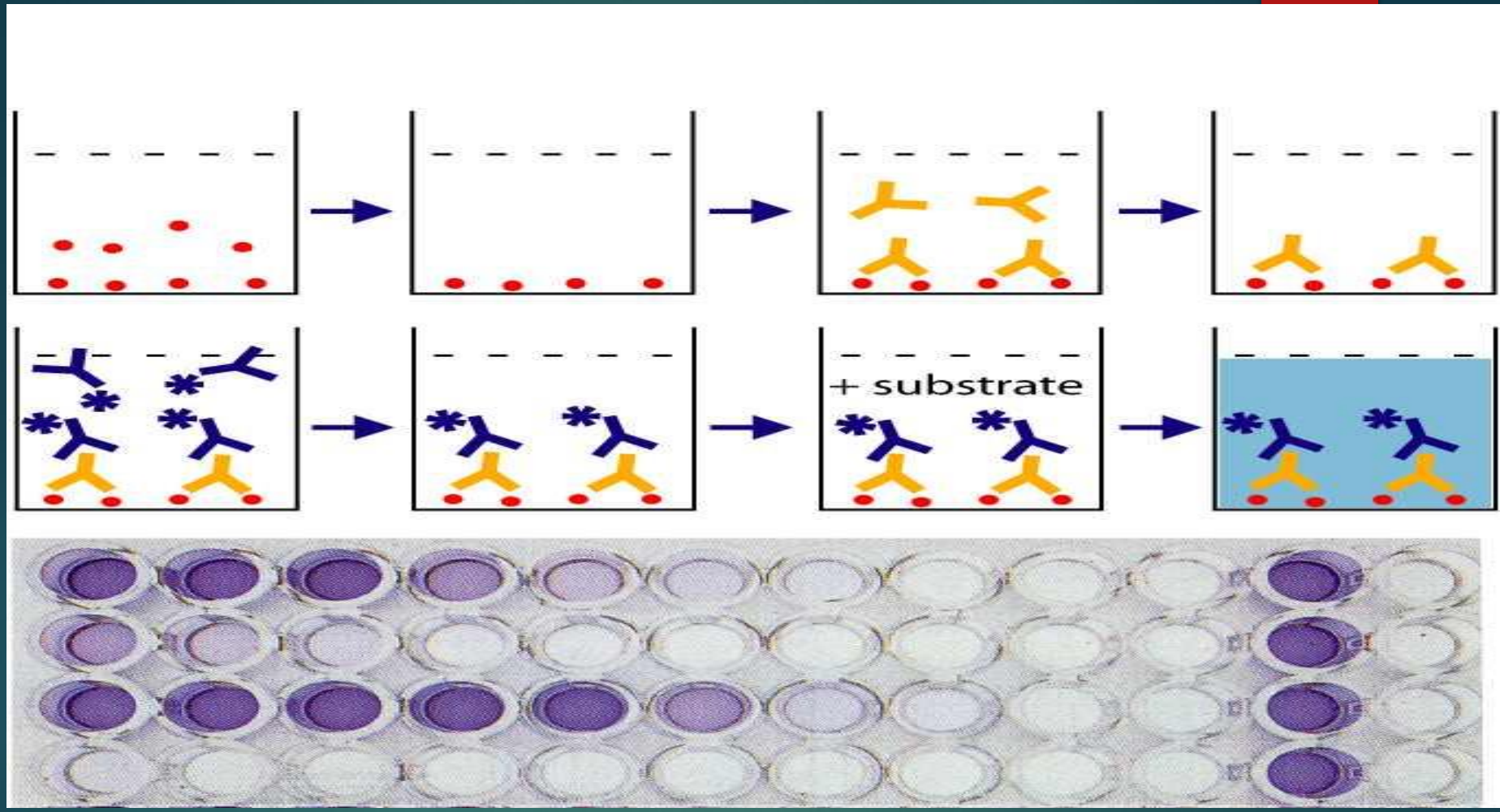
  ELISA

ELISA


Indicator
Second antibody
Viral antigen
Capture antibody
Solid support
Indicator
Anti-IgG
Antibody in sample (IgG)
Viral antigen
Solid support

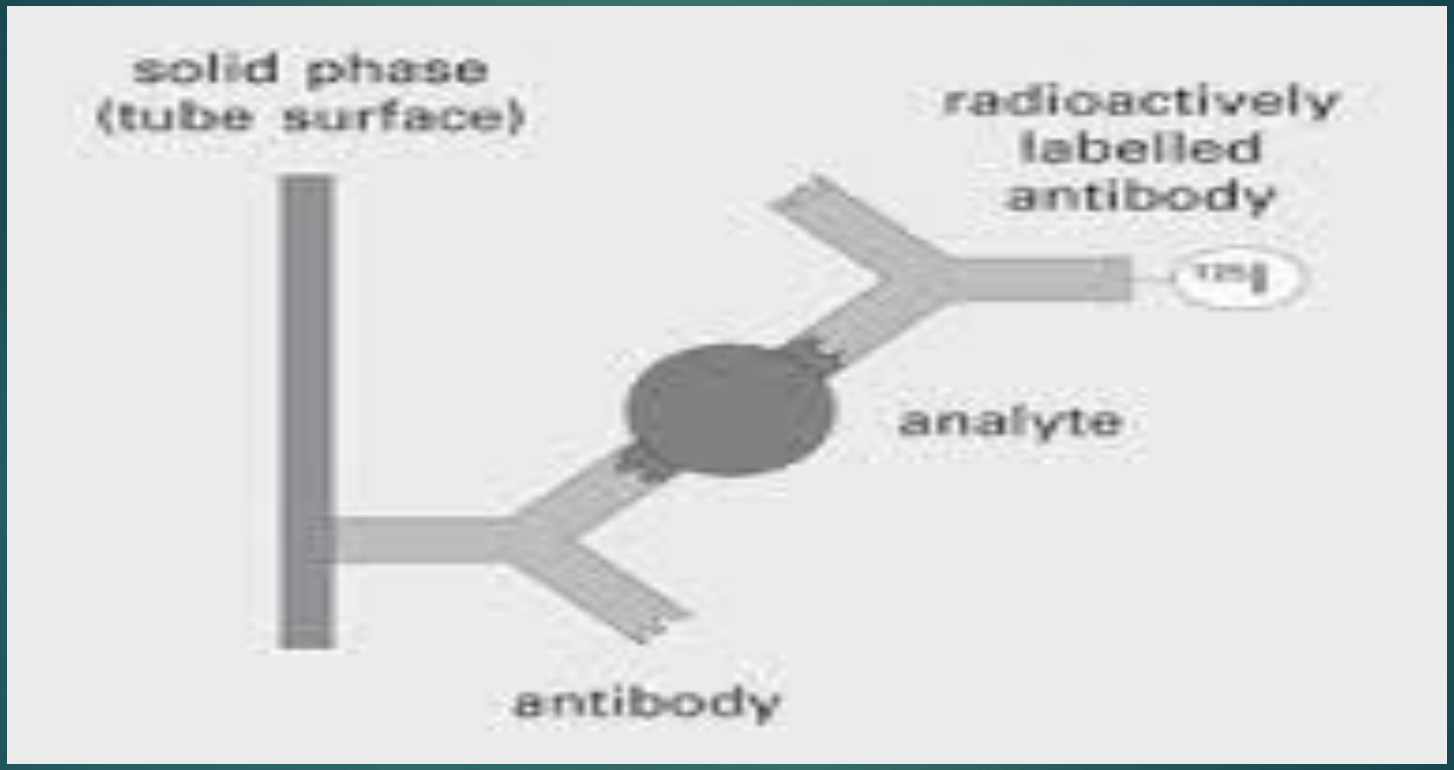
ELISA


ELISA
Antibody
Binding Site
Target Analyte (Antigen)
Labeling Reagents
Enzyme
Substrate
Product
Second Antibody or Target Analyte
Sample
Solid Phase
Bind
Wash
Label
Read



- A spectrophotometer is used to measure the color change

Radioimmunoassay



Applications:

Label immunoassays form the basis of highly sophisticated tests for both infectious and non infectious conditions.

Some of the applications include:

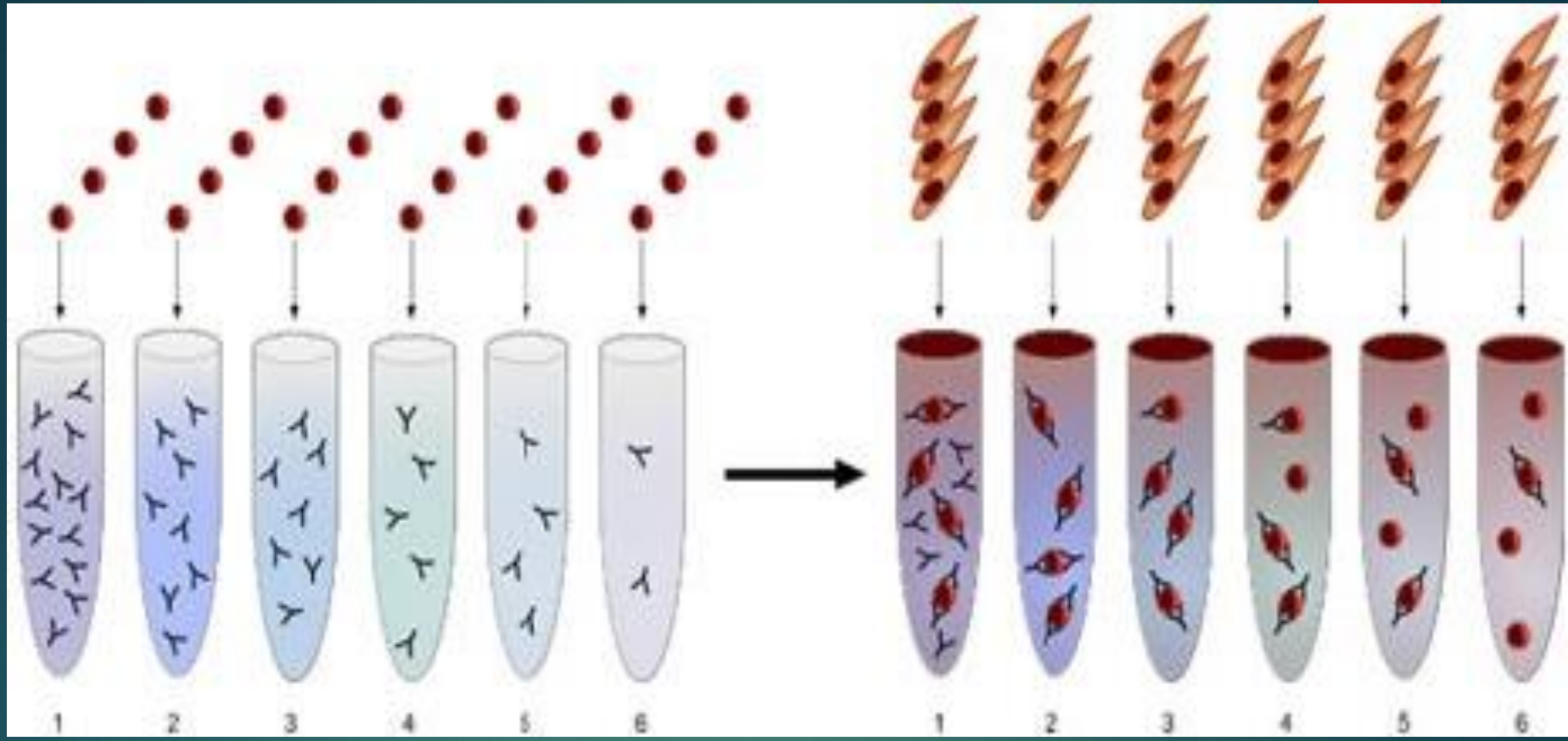
- Diagnosis of viral infections like hepatitis, dengue fever
- Diagnosis of bacterial infections
- Parasitic infections
- Hormone analysis
- Therapeutic drug monitoring
- Tumor markers etc

Immunohistochemistry

- Immunohistochemistry (IHC) combines histological, immunological and biochemical techniques for the identification of specific tissue components by means of a specific antigen/antibody reaction tagged with a visible label.

Neutralization tests

- These tests use the ability of the antibodies to block the effect of toxins or infectivity of viruses
- A serum sample is diluted in steps and each is incubated with the same quantity of virus
- If there are enough antibodies in the serum the viruses are neutralized. In order to make the neutralization effect visible, susceptible cells are added to the samples. If neutralization is successful the cells remain intact, i.e. a cytopathic effect is not recognized or an immune staining remains negative.



Serum neutralisation test

1. A specific amount of virus is added to progressively diluted serum probes.
2. Susceptible cells are added to the virus-antibody mixture.
3. The neutralisation effect is made visible (cytopathic effect or immunostaining).

Nucleic Acid Tests

- Use of nucleic acid probes
- Tests that use nucleic acid probes
- Nucleic acid sequence analysis

Tests that use nucleic acid probes

- Tests that use nucleic acid probes are designed to detect DNA or RNA directly (without amplification) using a labeled DNA or RNA probe that will hybridize specifically to the target nucleic acid (Southern blot or northern blot)

- These tests are simpler to perform than amplification tests but are less sensitive

Southern Blot

- DNA is digested with a restriction enzyme and separated by gel electrophoresis.
- The DNA is transferred to a membrane which is a sheet of special blotting paper. The DNA fragments retain the same pattern of separation they had on the gel.
- The blot is incubated with many copies of a probe which is single-stranded DNA. This probe will form base pairs with its complementary DNA sequence and form a double-stranded DNA molecule.
- The probe is either radioactive or has an enzyme bound to it.

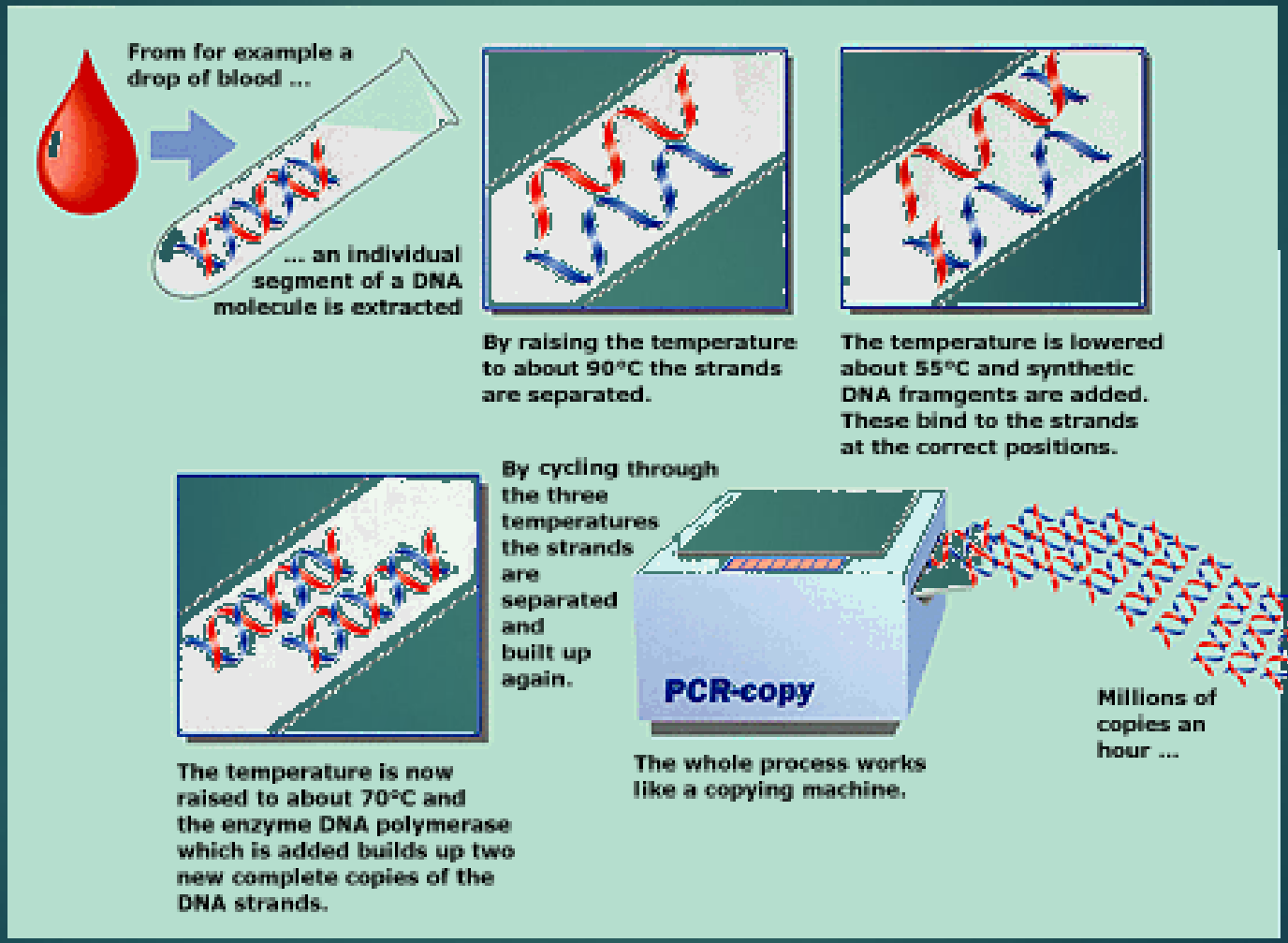
Polymerase Chain Reaction

The PCR requires the following components:

- **DNA template** - The sample DNA that contains the target sequence.
- **DNA polymerase** – an enzyme that synthesizes new strands of DNA complementary to the target sequence.
- **Primers -** short pieces of single-stranded DNA that are complementary to the target sequence. The polymerase begins synthesizing new DNA from the end of the primer.
- **Nucleotides** - single units of the bases A, T, G, and C which are the "building blocks" for new DNA strands.

RT-PCR (Reverse Transcription PCR) is PCR preceded by conversion of sample RNA into DNA with enzyme reverse transcriptase .

PCR


From for example a drop of blood ...
... an individual segment of a DNA molecule is extracted
By raising the temperature to about 90°C the strands are separated.
The temperature is lowered about 55°C and synthetic DNA framgents are added. These bind to the strands at the correct positions.
By cycling through the three temperatures the strands are separated and built up again.
PCR-copy
Millions of copies an hour ...
The temperature is now raised to about 70°C and the enzyme DNA polymerase which is added builds up two new complete copies of the DNA strands.
The whole process works like a copying machine.

Biological assays

- Biological assay (bioassay), or biological standardization is a type of scientific experiment by which the presence, nature or effect of a substance is estimated by studying its effects on living matter.

- A bioassay involves the use of a live animal (*in vivo*) or tissue (*in vitro*) to determine the biological activity of a substance, such as a hormone, drug or infectious agent.

Antimicrobial Susceptibility

Antimicrobial susceptibility tests are performed by either

- disk diffusion or
- dilution method

General tests

- Blood complete picture
- ESR
- Urine analysis
- CRP levels

TABLE 9–1 General Approach to the Diagnosis of a Bacterial Infection

1. Obtain a specimen from the infected site.
2. Stain the specimen using the appropriate procedure (e.g., Gram stain or acid-fast stain). If bacteria are seen in the Gram stain specimen, their shape (e.g., cocci or rods), size, arrangement (e.g., chains or clusters), and whether they are gram-positive or gram-negative should be observed. It is also important to determine whether only one or more than one type of bacteria is present. The microscopic appearance is *not* sufficient to speciate an organism, but it often allows an educated guess to be made regarding the genus of the organism and thereby guides empiric therapy.
3. Culture the specimen on the appropriate media (e.g., blood agar plates). In most instances, the plates should be streaked in such a manner to obtain isolated colonies (i.e., a "pure culture"). The plates should be incubated in the presence or absence of oxygen as appropriate.
4. Identify the organism using the appropriate tests (e.g., sugar fermentation, DNA probes, antibody-based tests such as agglutination, or immunofluorescence). Note special features such as hemolysis and pigment formation.
5. Perform antibiotic susceptibility tests.

TABLE 9–2 How to Diagnose a Bacterial Infection When the Culture Is Negative

1. Detect antibody in the patient's serum. Detection of immunoglobulin (Ig) M antibody indicates a current infection. A fourfold or greater rise in antibody titer between the acute serum sample and the convalescent serum sample also indicates a current infection. (A major drawback with the use of acute and convalescent serum samples is that the convalescent sample is usually taken 10–14 days after the acute sample. By this time, the patient has often recovered and the diagnosis becomes a retrospective one.) A single IgG antibody titer is difficult to interpret because it is unclear whether it represents a current or a previous infection. In certain diseases, a single titer of sufficient magnitude can be used as presumptive evidence of a current infection.
2. Detect antigen in the patient's specimen. Use known antibody to detect presence of antigens of the organisms (e.g., fluorescent antibody to detect antigens in tissue, latex agglutination to detect capsular polysaccharide antigens in spinal fluid).
3. Detect nucleic acids in the patient's specimen. Use polymerase chain reaction (PCR) and DNA probes to detect the DNA or RNA of the organism.

TABLE 9–3 Commonly Used Bacteriologic Agars and Their Function

Name of Agar[1]	Bacteria Isolated on the Agar	Function or Properties of the Agar
Blood	Various bacteria	Detect hemolysis
Bordet-Gengou	*Bordetella pertussis*	Increased concentration of blood allows growth
Charcoal-yeast extract	*Legionella pneumophila*	Increased concentration of iron and cysteine allows growth
Chocolate	*Neisseria meningitidis* and *Neisseria gonorrhoeae* from sterile sites	Heating the blood inactivates inhibitors of growth
Chocolate agar plus X and V factors	*Haemophilus influenzae*	X and V factors are required for growth
Egg yolk	*Clostridium perfringens*	Lecithinase produced by the organism degrades egg yolk to produce insoluble precipitate
Eosin-Methylene Blue	Various enteric gram-negative rods	Selects against gram-positive bacteria and differentiates between lactose fermenters and nonfermenters
Löwenstein-Jensen	*Mycobacterium tuberculosis*	Selects against gram-positive bacteria in respiratory tract flora and contains lipids required for growth
MacConkey	Various enteric gram-negative rods	Selects against gram-positive bacteria and differentiates between lactose fermenters and nonfermenters
Tellurite	*Corynebacterium diphtheriae*	Causes tellurite to become tellurium, which has black color
Thayer-Martin	*N. gonorrhoeae* from nonsterile sites	Chocolate agar with antibiotics to inhibit growth of normal flora
Triple sugar iron (TSI)	Various enteric gram-negative rods	Distinguishes lactose fermenters from nonfermenters and H_2S producers from nonproducers

[1]Names are listed in alphabetical order.