Diagnosis of viral Infections

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Diagnosis of viral infections

1. Clinical signs
2. Laboratory Diagnosis (Virus detection)
   - Direct examinations
   - Indirect examinations

Laboratory Diagnosis

1. Direct Methods

Antigen detection	• Serology (immunofluorescence, ELISA etc.)
Electron microscopy	• morphology of virus particles
Viral genome detection	• Hybridization with specific nucleic acid probes • polymerase chain reaction (PCR)

2. Indirect Methods

Cell Culture	• Cytopathic effect (CPE) • Hemadsorption
Serology	• Hemagglutination inhibition test
Animals	• disease or death

Laboratory Diagnosis - Direct Methods

Serology

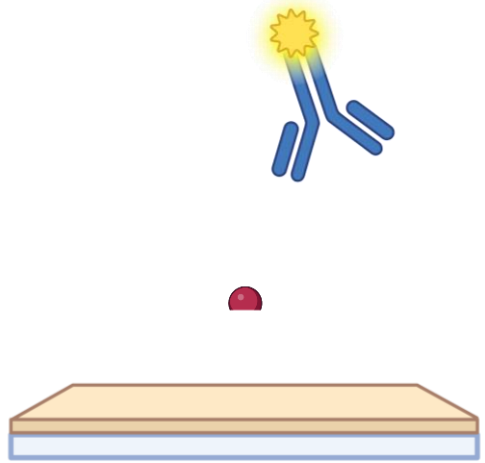
- Most used lab method
- Detection of antigen
- Examples
  - Immunofluorescence
  - ELISA
  - Complement fixation test

Laboratory Diagnosis - Direct Methods

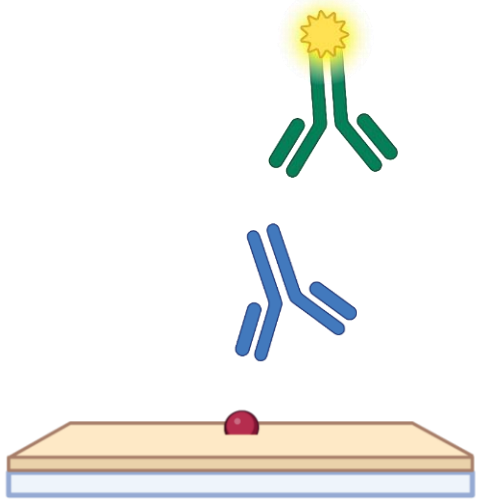
Serology - Immunofluorescence

Immunofluorescence Techniques:

- Direct Immunofluorescence:
  - Fluorescent-labeled specific antibodies
  - Direct detection of viral antigens
  - Quick results (2-4 hours)



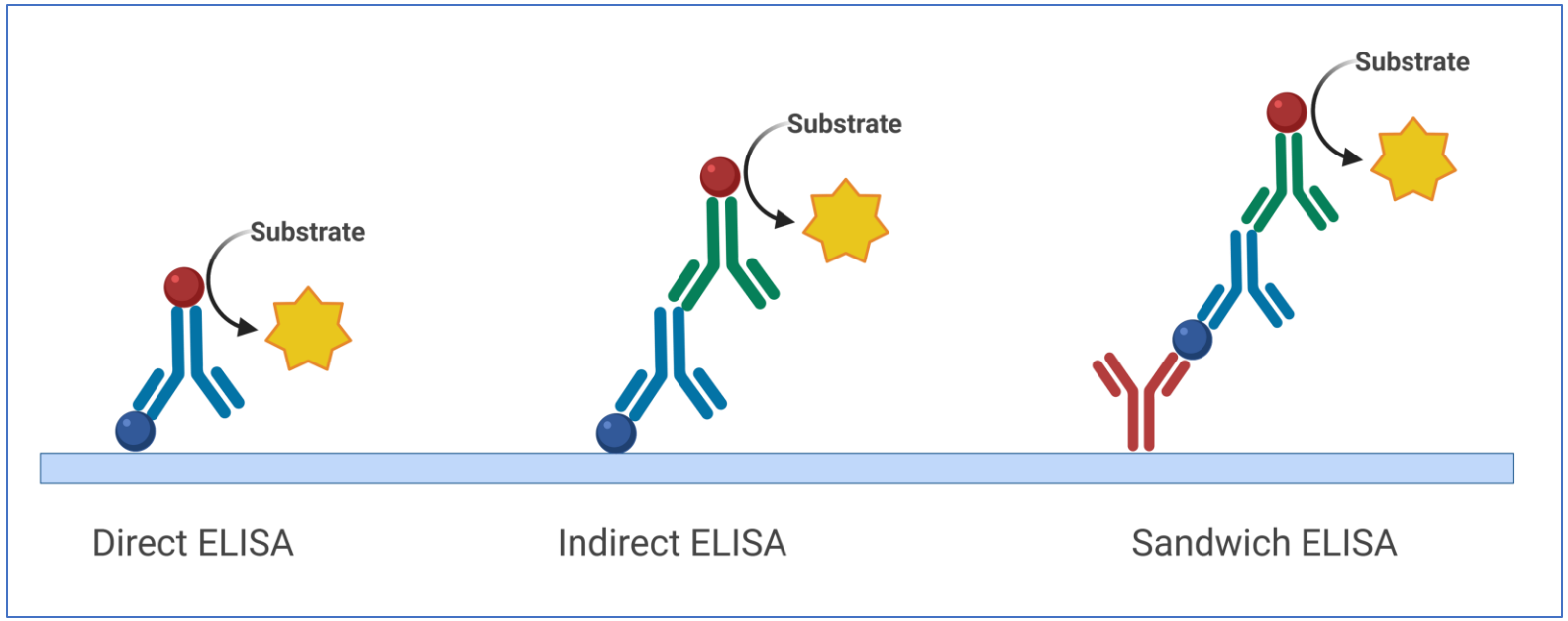
- Indirect Immunofluorescence:
  - Primary unlabeled antibody
  - Secondary fluorescent-labeled antibody
  - Higher sensitivity
  - More steps required



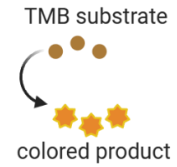
Laboratory Diagnosis - Direct Methods

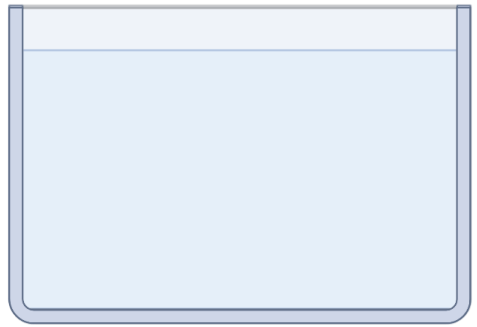
Serology - ELISA

- Enzyme Linked Immunosorbent Assay (ELISA)
- Overview:



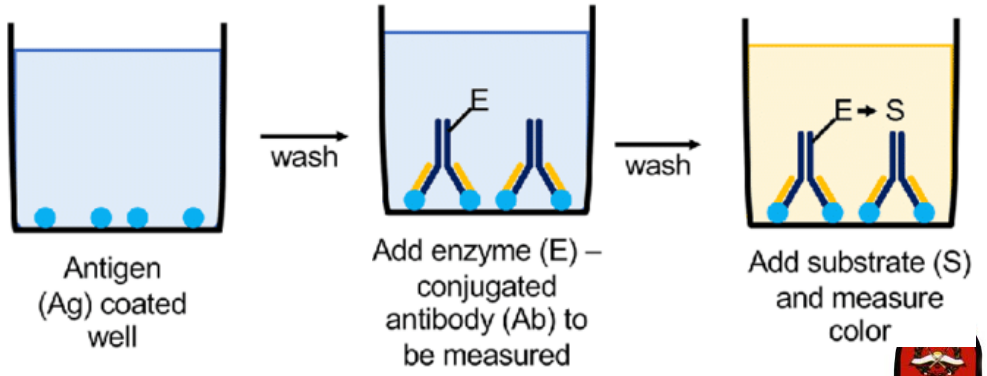
Laboratory Diagnosis - Direct Methods
Serology - ELISA





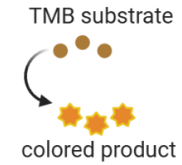
Direct ELISA

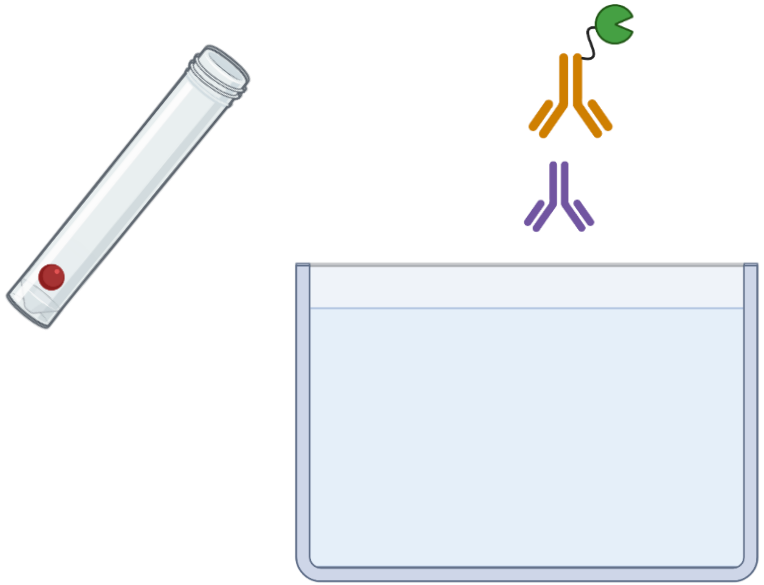
- The antigen-containing sample is directly adsorbed onto a plastic microwell plate (usually made of polystyrene)
- Primary Antibody Addition → An enzyme-conjugated primary antibody (specific to the target antigen) is added to the wells → The antibody is directly labeled with an enzyme
- Substrate Addition → A specific substrate for the enzyme is added → The enzyme converts the substrate into a colored product
- The intensity of the color development is proportional to the amount of antigen present → Can be measured using a spectrophotometer/plate reader



Laboratory Diagnosis - Direct Methods

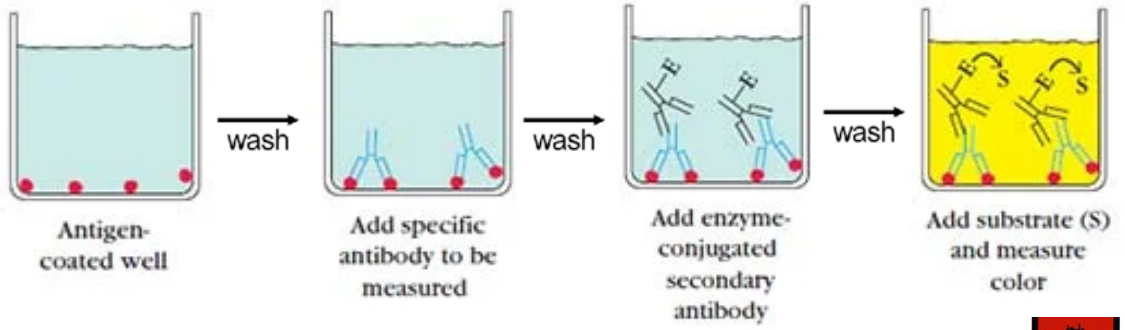
Serology - ELISA





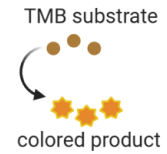
Indirect ELISA

- Similar to Direct ELISA, antigen is immobilized on a microwell plate
- Primary Antibody Addition (**First Key Difference**) → **Unlabeled** primary antibody specific to target antigen is added
- Secondary Antibody Addition (**Major Difference**) → Enzyme-conjugated secondary antibody is added
- Substrate Addition → A specific substrate for the enzyme is added → The enzyme converts the substrate into a colored product



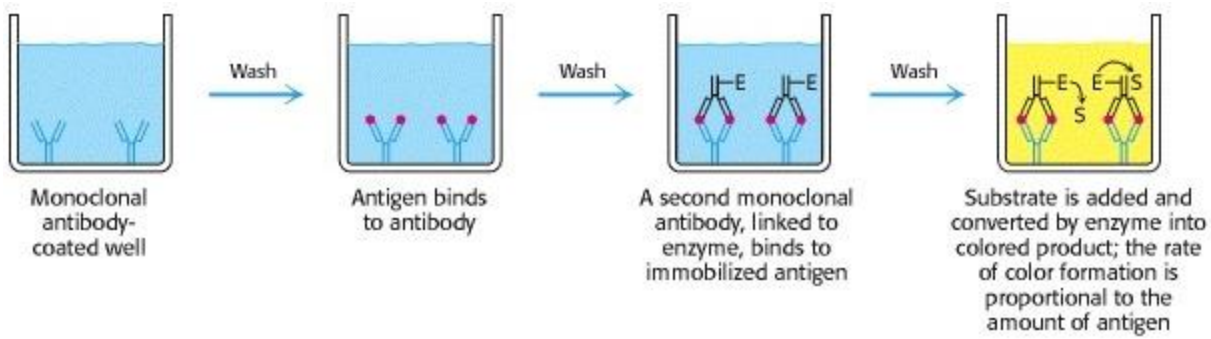
Laboratory Diagnosis - Direct Methods

Serology - ELISA



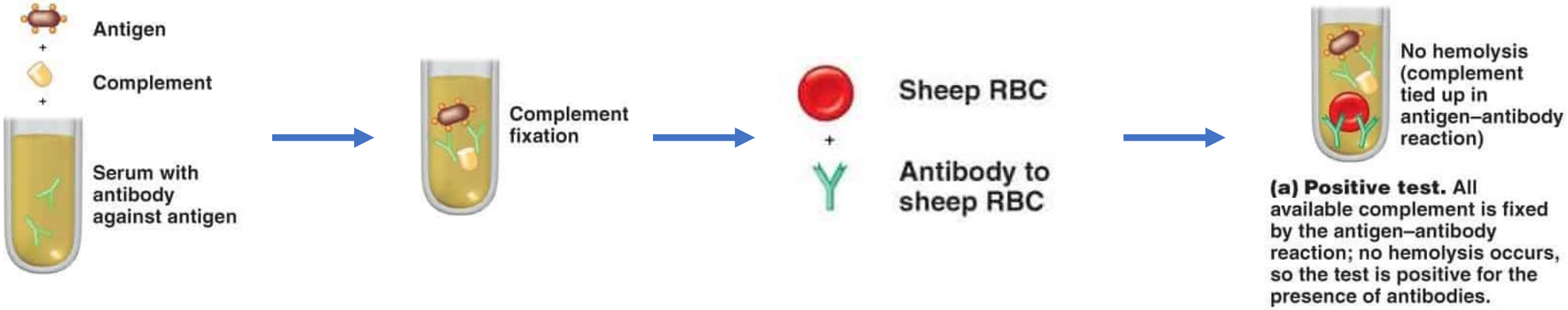
Sandwich ELISA

- A capture antibody specific to target antigen is immobilized on plate
- Add sample containing target antigen → Antigen binds specifically to capture antibody → Creates first part of the "sandwich"
- Add enzyme-conjugated secondary antibody specific to different epitope of antigen
- Substrate Addition → A specific substrate for the enzyme is added → The enzyme converts the substrate into a colored product



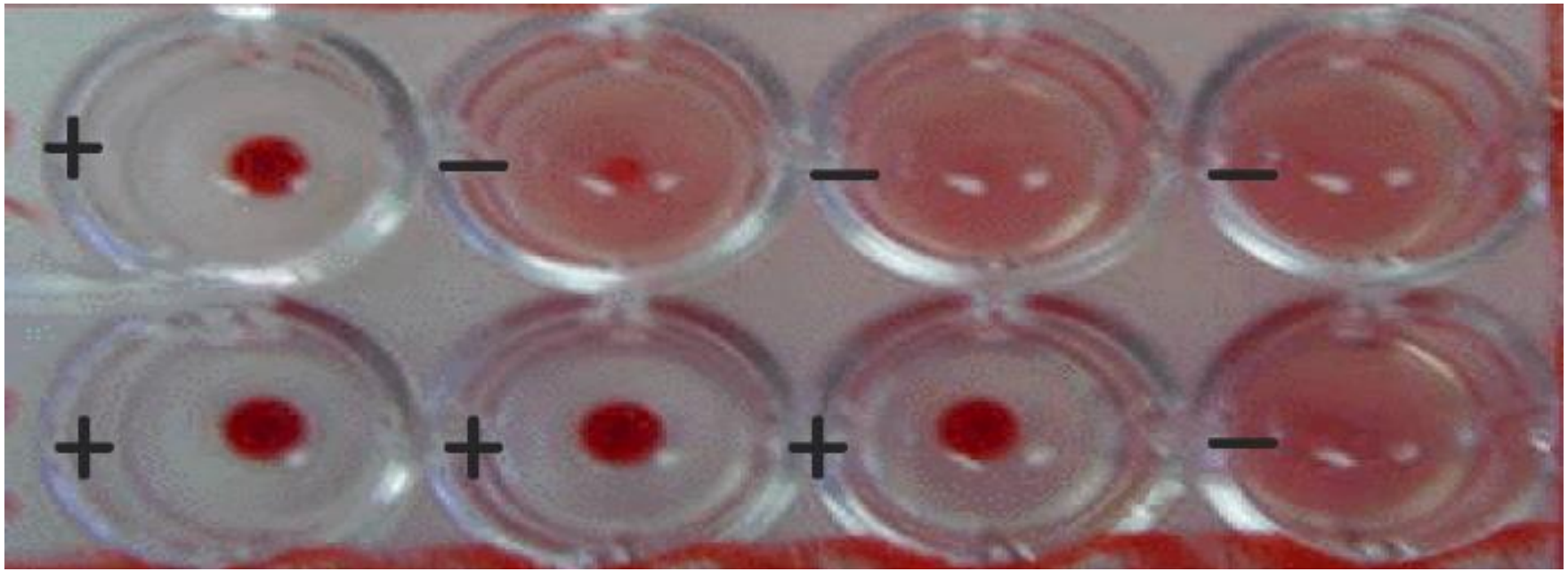
Laboratory Diagnosis - Direct Methods

Serology – Complement fixation test



Laboratory Diagnosis - Direct Methods

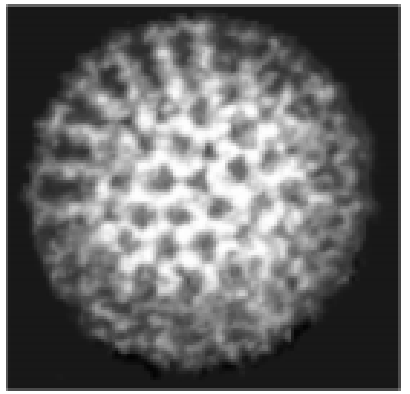
Serology – Complement fixation test



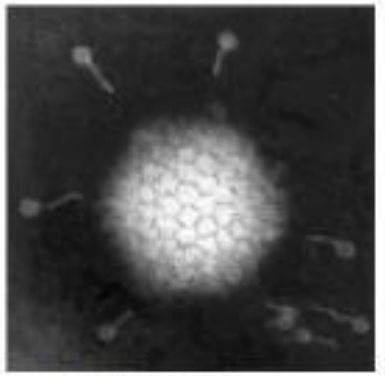
Laboratory Diagnosis - Direct Methods

Electron Microscopy

- 10^6 virus particles per ml required for visualization.
- 50,000 - 60,000 magnification normally used.
- Viruses may be detected in the following specimens:
  - **Faeces:** Rotavirus, Adenovirus, Norwalk like viruses, Astrovirus, Calicivirus
  - **Vesicle Fluid:** HSV, VZV
  - **Skin scrapings:** papillomavirus, molluscum contagiosum
- Problems with Electron Microscopy
  - Expensive equipment
  - Skilled operators needed
  - High maintenance costs



Rotavirus



Adenovirus

Laboratory Diagnosis - Direct Methods

Molecular Methods

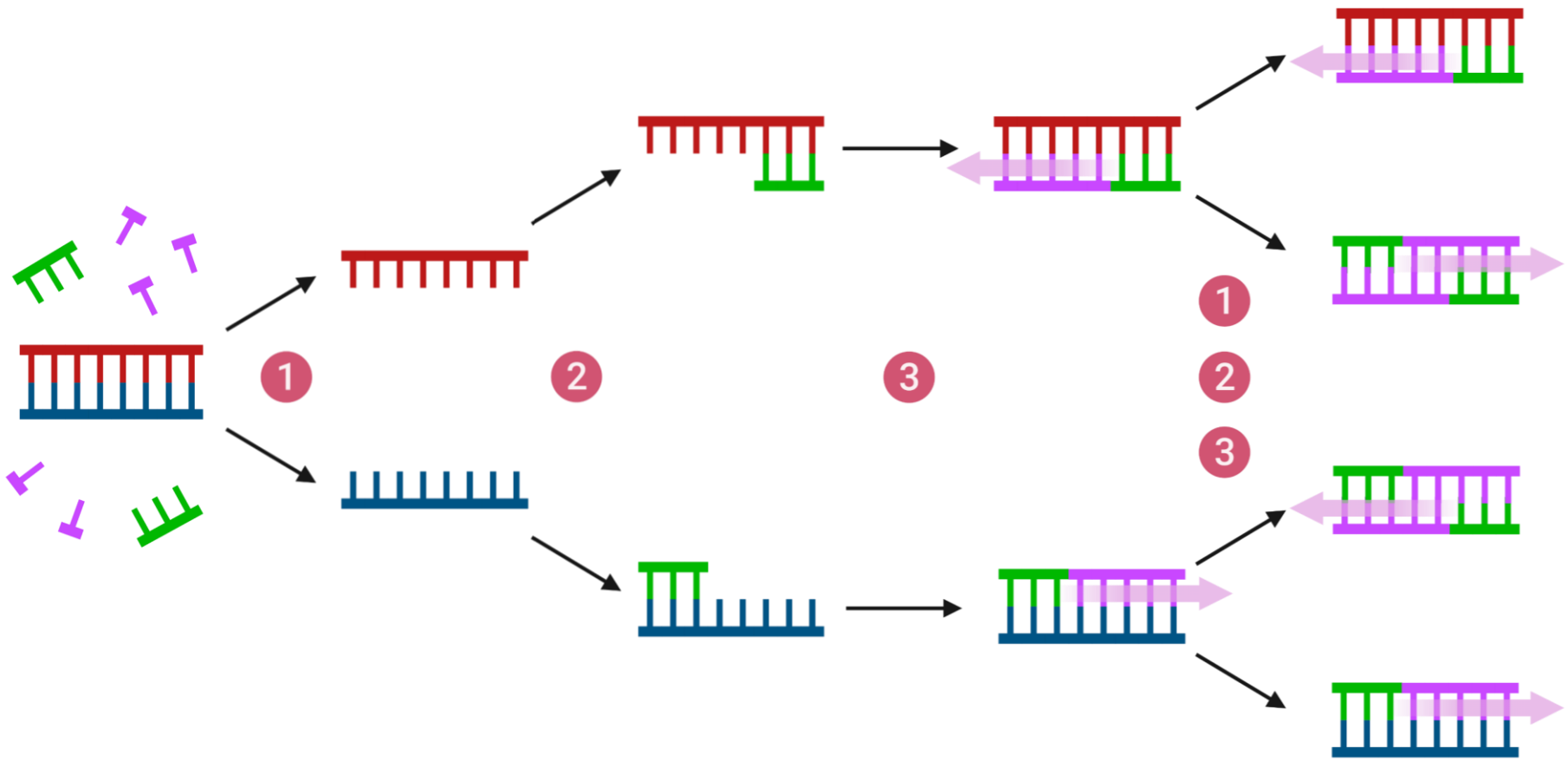
- Methods based on the detection of viral genome.
- By Polymerase Chain Reaction (PCR)
- However, in practice, although the use of these methods is indeed increasing, the role played by molecular methods in a routine diagnostic virus laboratory is still small compared to conventional methods.

Advantages of PCR:

- Extremely high sensitivity, may detect down to one viral genome per sample volume.
- Fast turnaround time
- Early diagnosis possible
- Easy to set up.

Disadvantages of PCR:

- Extremely liable to contamination.
- High degree of operator skill required
- Not easy to set up a quantitative assay.



1 Denaturation at 95-96°C

2 Annealing at 68°C

3 Elongation at 72°C

Laboratory Diagnosis - Indirect Methods

Cell Culture	• Cytopathic effect (CPE) • Hemadsorption
Serology	• Hemagglutination inhibition test
Animals	• disease or death

Laboratory Diagnosis - Indirect Methods
Cell Culture

- Are used for virus isolation. However, they are very expensive, and it is often difficult to obtain a reliable supply.

Problems with cell culture

- Long period (up to 4 weeks) required for result.
- Often very poor sensitivity. Sensitivity depends on a large extent on the condition of the specimen.
- Susceptible to bacterial contamination.

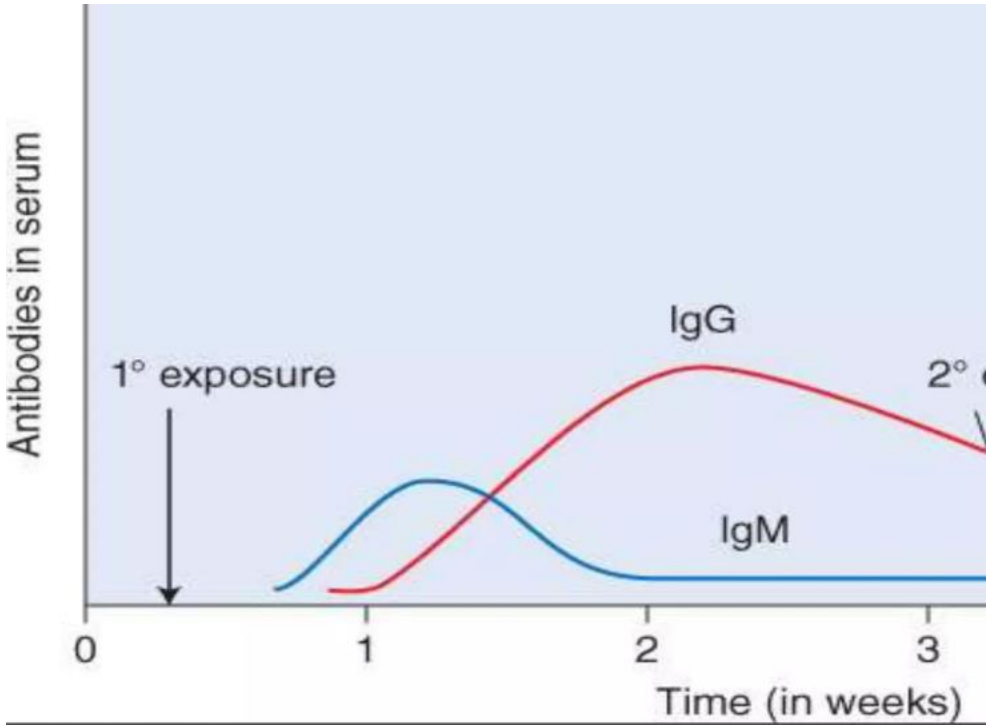
Laboratory Diagnosis - Indirect Methods

Serology

- Detection of antibodies against the virus.

Criteria for diagnosing primary infection

- 4-fold or more increase in titer of IgG or total antibody between acute and convalescent sera
- Presence of IgM
- Seroconversion

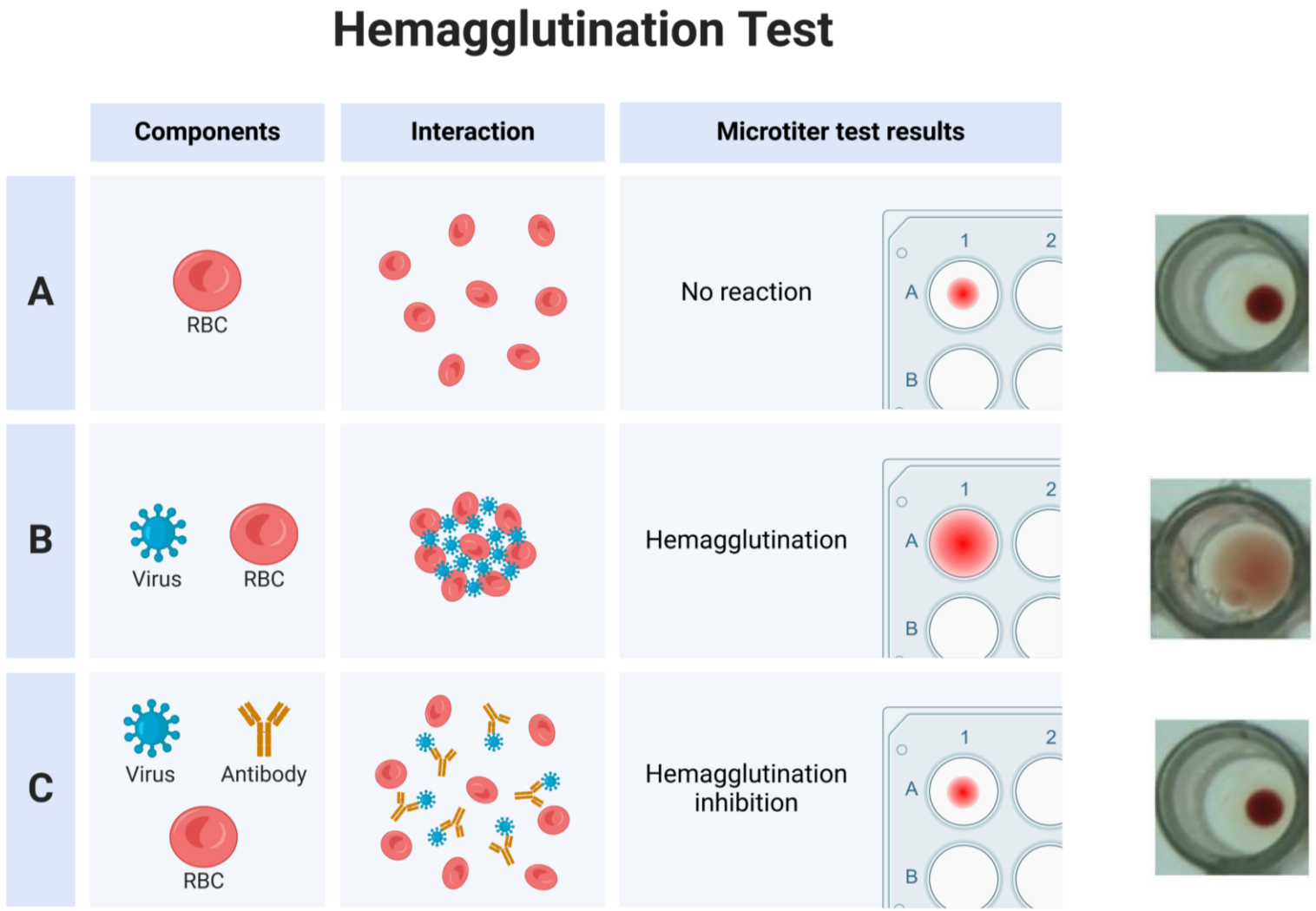


Laboratory Diagnosis - Indirect Methods
Serology - Haemagglutination test

- The Hemagglutination test is a diagnostic method used to detect and measure the presence of specific antigens or antibodies in a sample.
- It exploits the natural property of red blood cells (RBCs) to aggregate (or clump together) in the presence of certain viruses or antigens.
- **Mechanism:**
  - Many viruses, such as influenza and measles viruses, have proteins (e.g., hemagglutinin) on their surfaces that can bind to receptors on red blood cells.
  - This interaction causes the red blood cells to clump together, forming visible aggregates.
- **Results:**
  - **Positive:** Agglutination is observed, indicating the presence of the viral protein.
  - **Negative:** No agglutination, suggesting the absence of the virus or antigen.

Laboratory Diagnosis - Indirect Methods

Serology - Haemagglutination test



Laboratory Diagnosis - Indirect Methods
Serology – Haemagglutination Inhibition test

- The Hemagglutination Inhibition test is an extension of the Hemagglutination test and is primarily used to detect specific antibodies against a virus.
- **Principle:**
  - If a serum contains antibodies specific to the virus being tested, these antibodies will bind to the viral proteins and prevent them from interacting with red blood cells.
  - This inhibition of hemagglutination indicates the presence of antibodies.
- **Results:**
  - **Positive (Inhibition observed):** Indicates that the serum contains antibodies specific to the virus.
  - **Negative (No inhibition):** Suggests the absence of virus-specific antibodies.

Comparison of the Two Tests

Feature	Hemagglutination Test	Hemagglutination Inhibition Test
Purpose	Detect viral antigens/proteins.	Detect antibodies against the virus.
Mechanism	Exploits viral ability to agglutinate RBCs.	Uses antibodies to inhibit this process.
Result Interpretation	Agglutination indicates a positive result.	Inhibition of agglutination indicates a positive result.
Applications	Diagnosing presence of a virus.	Assessing immunity or confirming infection.

Test Type	Positive Test	Negative Test
HA Test	Diffuse, uniform reddish "mat."	Tight, compact "button."
HI Test	Tight, compact "button."	Diffuse, uniform reddish "mat."

Laboratory Diagnosis - Indirect Methods
Serology

Problems with Serology:

- Long period of time required for diagnosis for paired acute and convalescent sera.
- Mild local infections may not produce detectable Abs.
- Immunocompromised patients often give a reduced or absent Abs.
- Patients given blood or blood products may give a false positive result due to the transfer of antibody