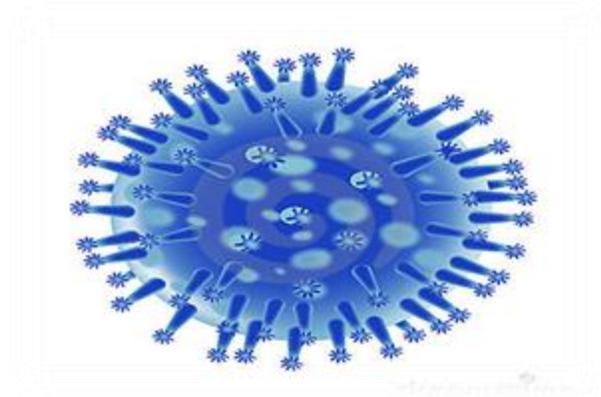


General Microbiology

Diagnosis of viral Infections

2023-2024



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Diagnosis of viral infections

1. Clinical signs.

2. Virus detection:

- a) Direct examinations.
- b) Indirect examinations.

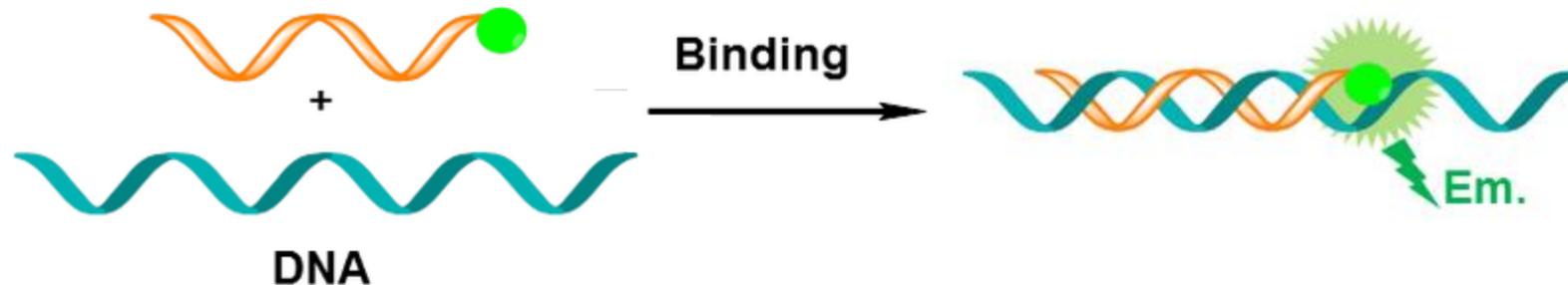
Diagnostics of viral diseases

Virus detection

1. Direct examination:

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)

hybridization with specific nucleic acid probes



Diagnosics of viral diseases

Virus detection

2. Indirect examination:

Cell Culture	cytopathic effect (CPE) hemadsorption
Serology	Hemagglutination inhibition test
Animals	disease or death

Diagnosics of viral diseases

Direct methods

Serology

- Most used lab method
- Detection of antigen

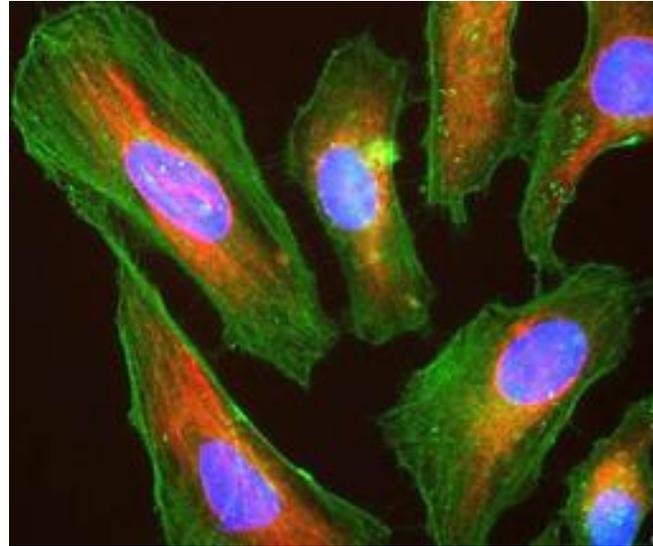
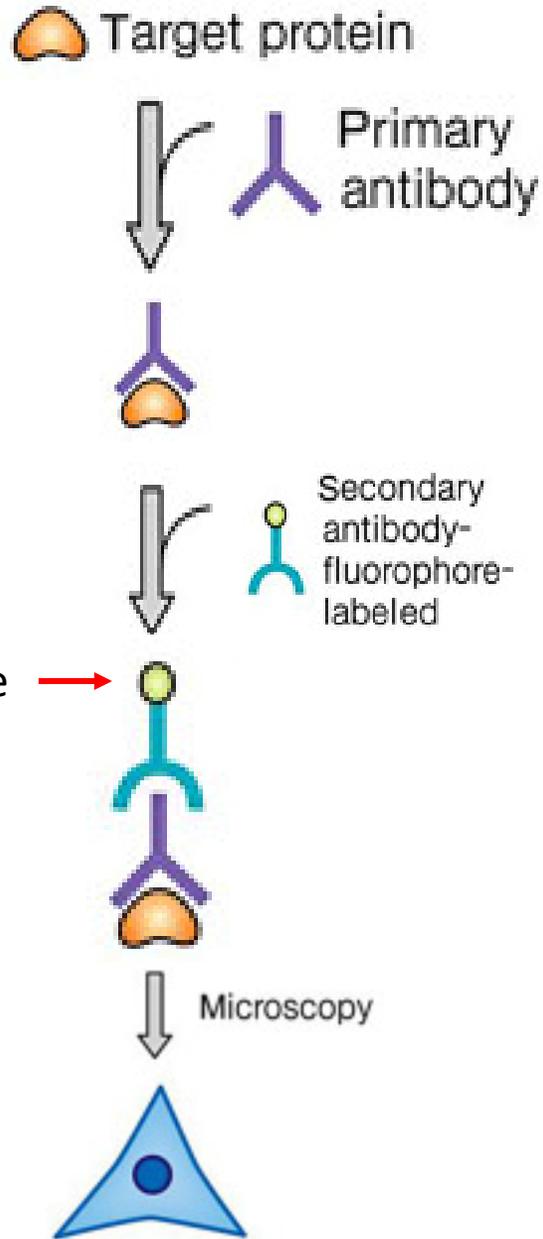
Classical Techniques

1. Complement fixation tests (CFT)
2. Immunofluorescence techniques (IF)
3. Neutralization tests

Newer Techniques

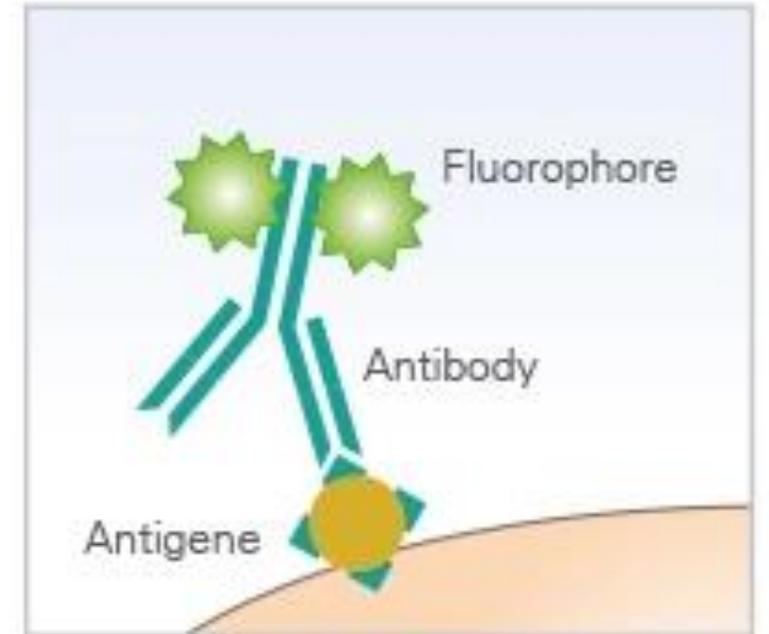
1. Radioimmunoassay (RIA).
2. Enzyme linked immunosorbent assay (ELISA).
3. Particle agglutination.
4. Western Blot (WB).

Indirect immunofluorescence



Immunofluorescence techniques (IF)

Direct Immunofluorescence



Diagnosics of viral diseases

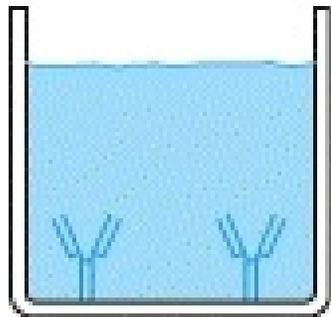
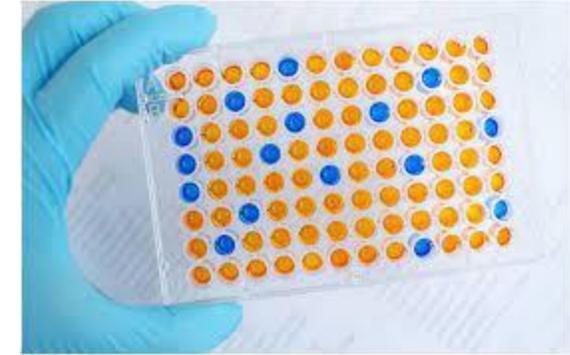
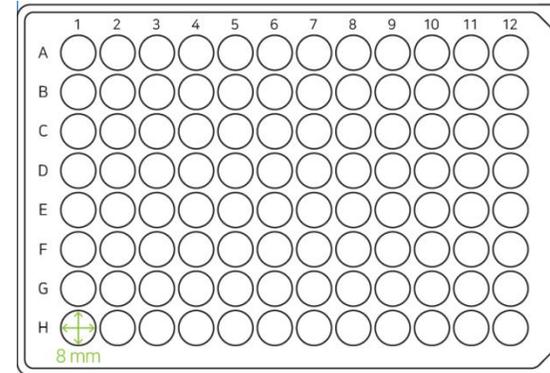
Direct methods

Serology

Enzyme Linked Immunosorbent Assay (ELISA).

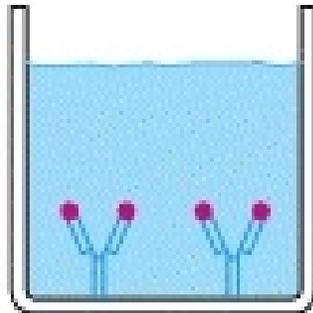
Sandwich ELISA

96 well plate



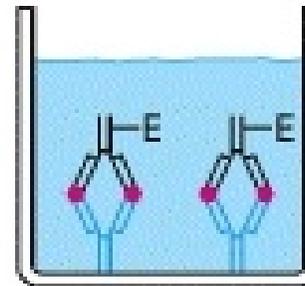
Monoclonal antibody-coated well

Wash



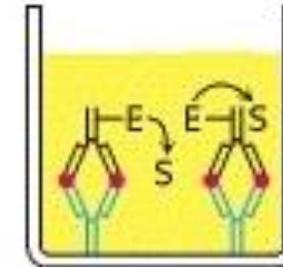
Antigen binds to antibody

Wash



A second monoclonal antibody, linked to enzyme, binds to immobilized antigen

Wash



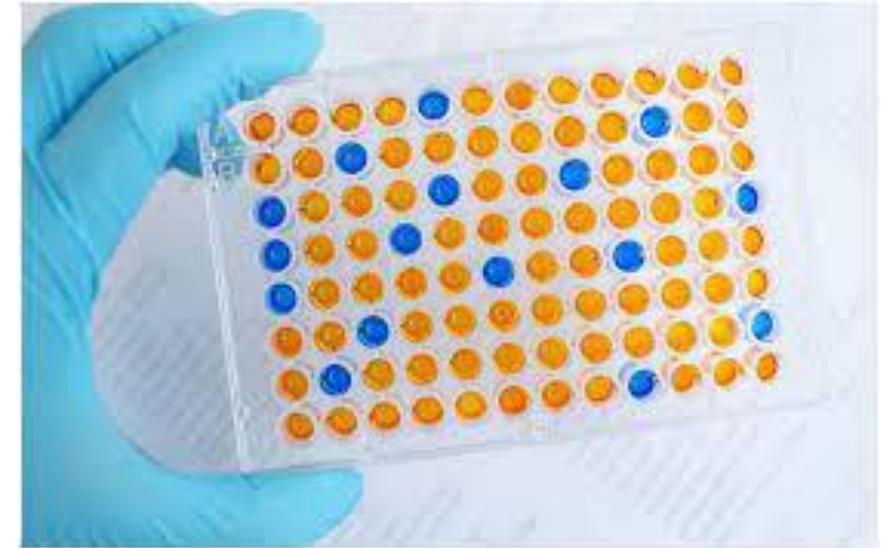
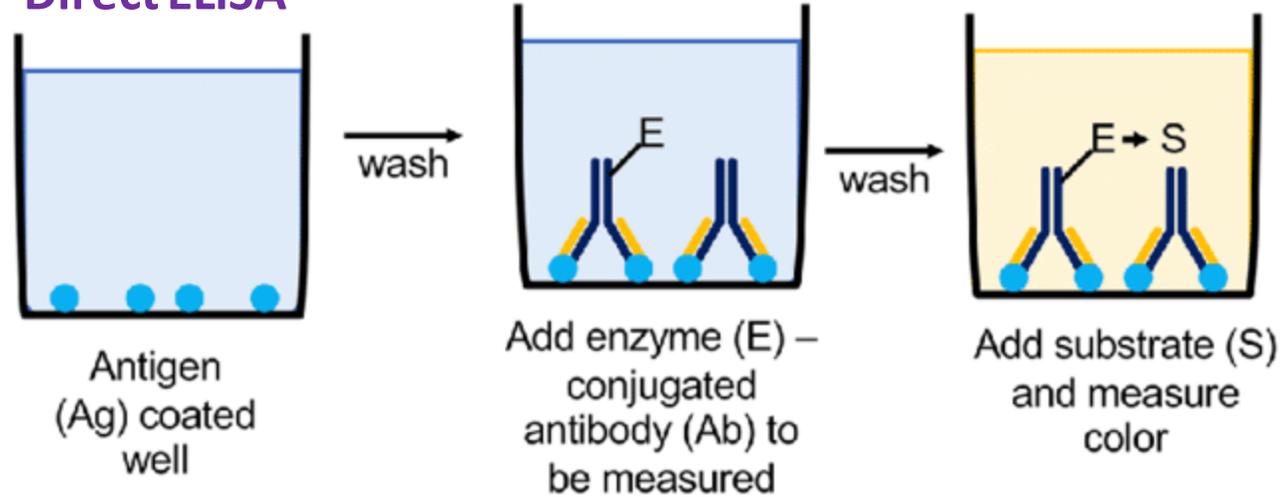
Substrate is added and converted by enzyme into colored product; the rate of color formation is proportional to the amount of antigen

Diagnosics of viral diseases

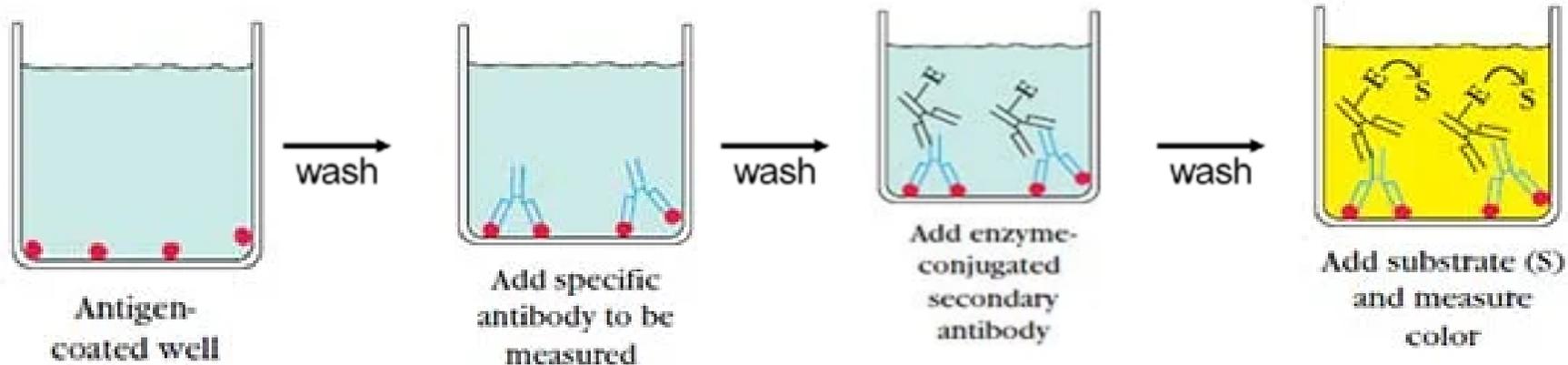
Direct methods

Serology

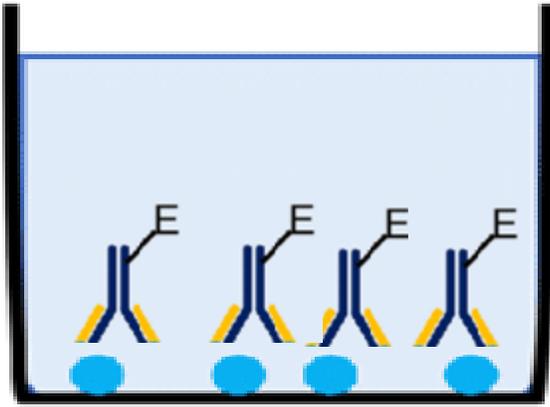
Direct ELISA



Indirect ELISA

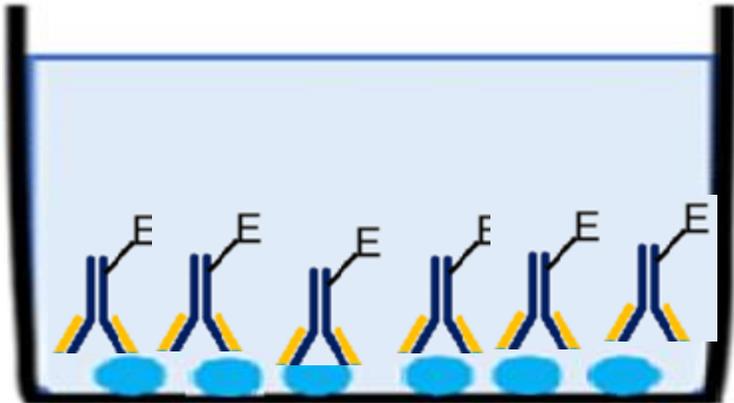


The idea behind ELIZA



Antigen
(Ag) coated
well

+ Substrate = Absorbance

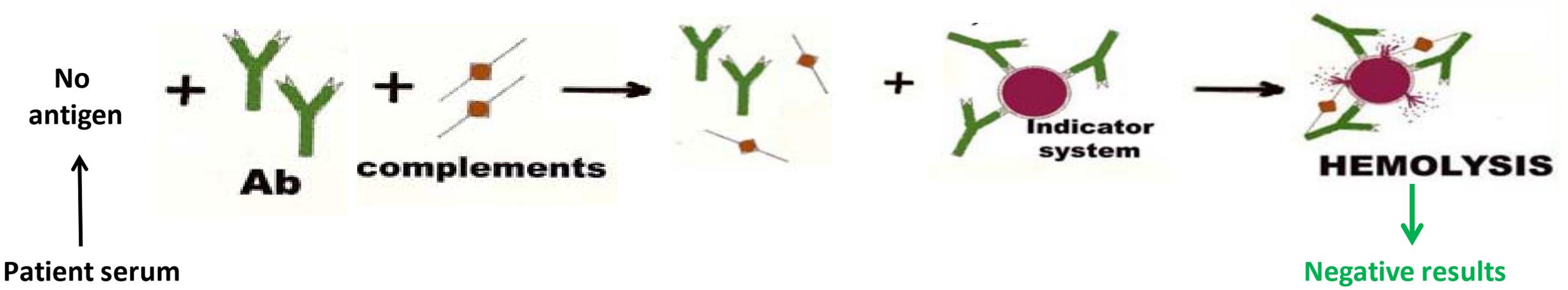
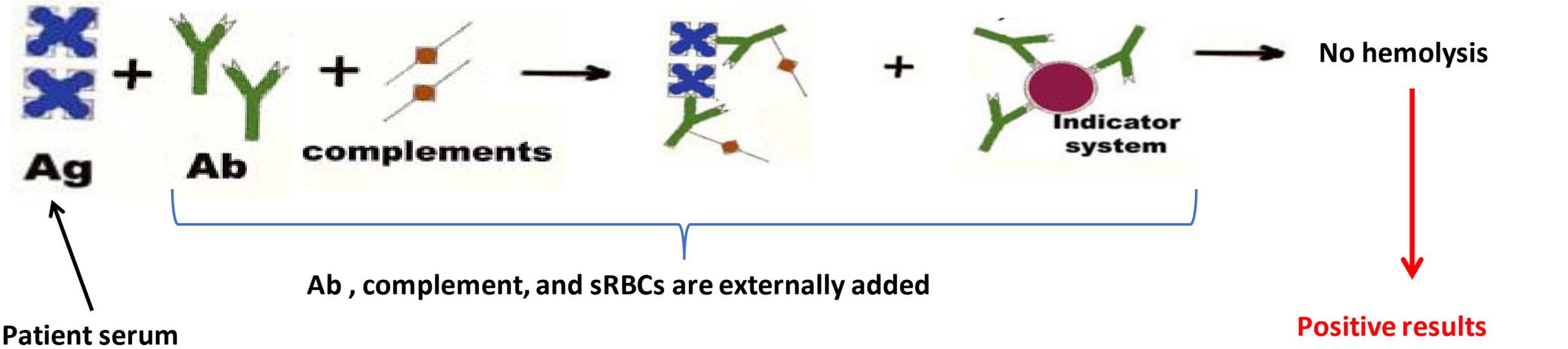


+ Substrate = Higher Absorbance

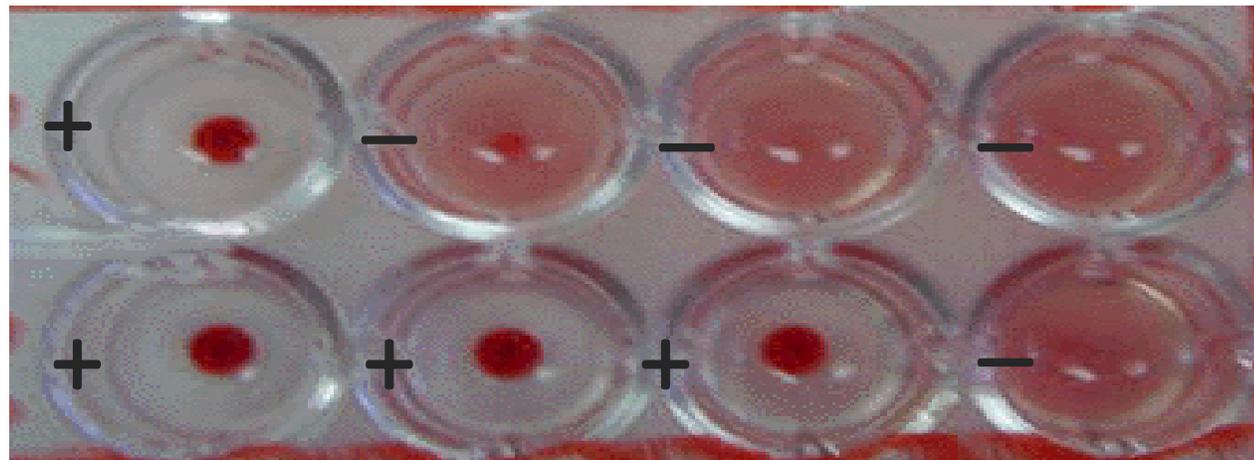
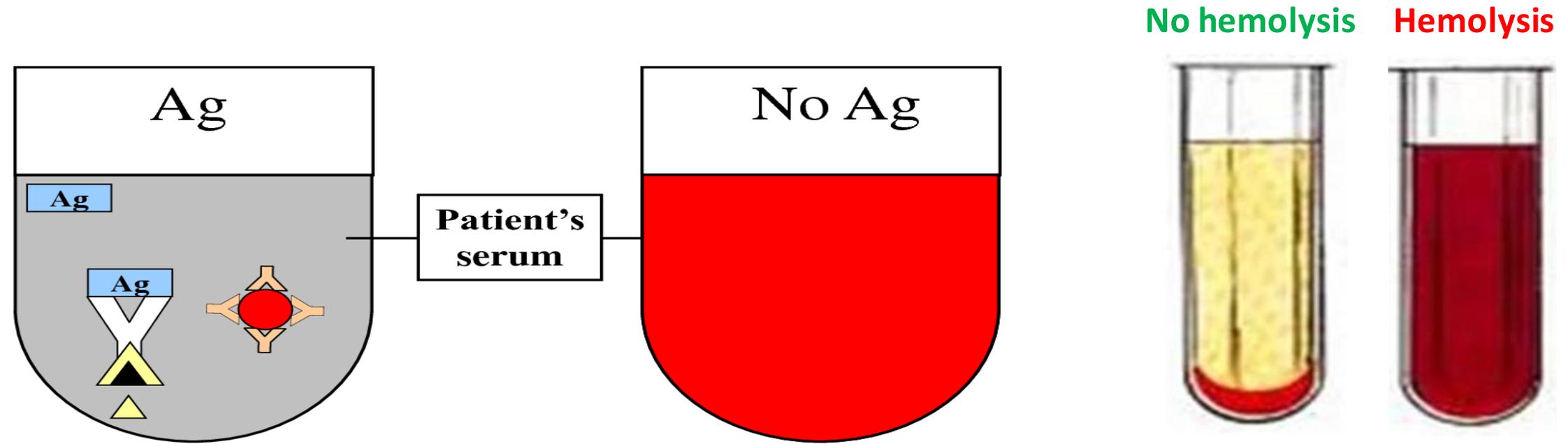
Complement fixation test

Procedure

Looking for antigens



Complement fixation test



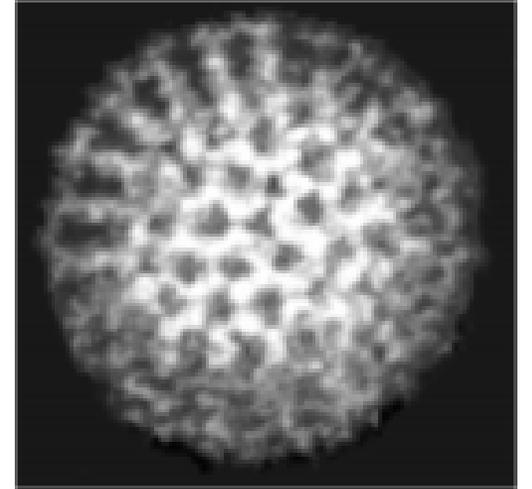
Diagnostics of viral diseases

Direct methods

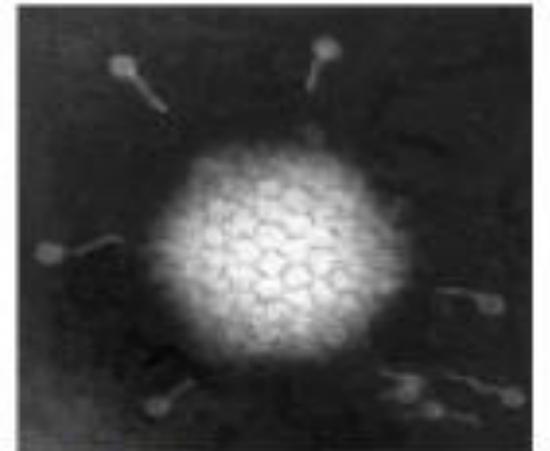
Electron Microscopy

- 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

Electronmicrographs



Rotavirus



Adenovirus

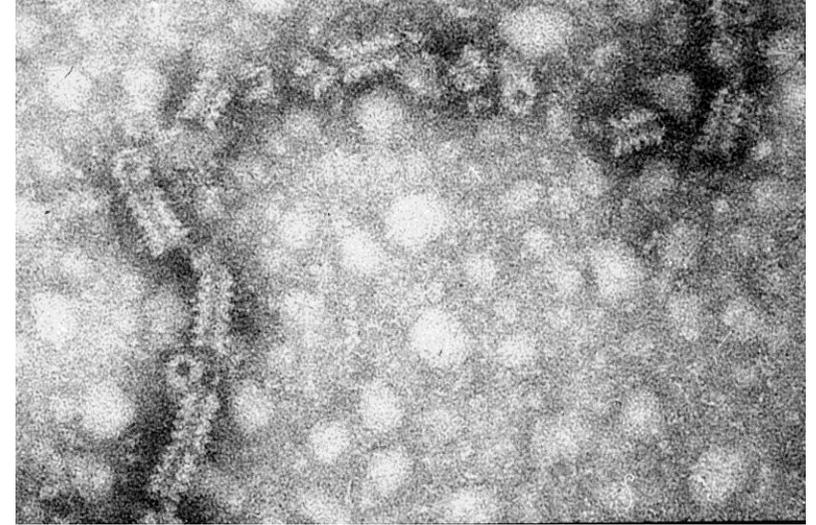
Diagnositics of viral diseases

Direct methods

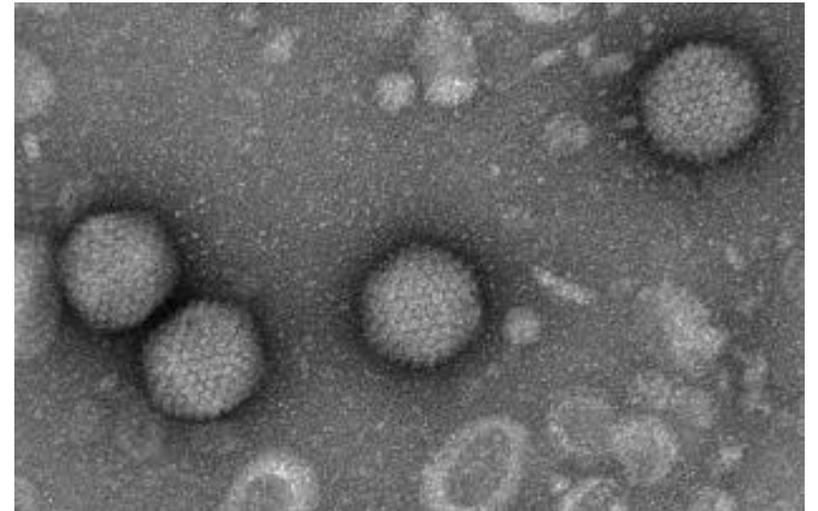
Electron Microscopy

Problems with Electron Microscopy

- Expensive equipment
- Expensive maintenance
- Require experienced observer



Cylindrical (Mumps virus)



Icosahedral (poliovirus)

Diagnostics of viral diseases

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.
- Easy to set up.
- Fast turnaround time

Disadvantages of PCR

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

Diagnosics of viral diseases

Virus detection

2. Indirect examination:

Cell Culture	cytopathic effect (CPE) hemadsorption
Serology	Hemagglutination inhibition test
Animals	disease or death

Diagnosics of viral diseases

Indirect methods

Cell Culture

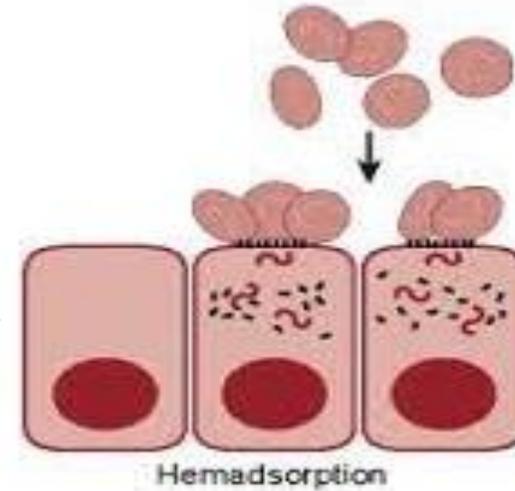
Are used for virus isolation. However, they are very expensive and it is often difficult to obtain a reliable supply.

Problems with cell culture

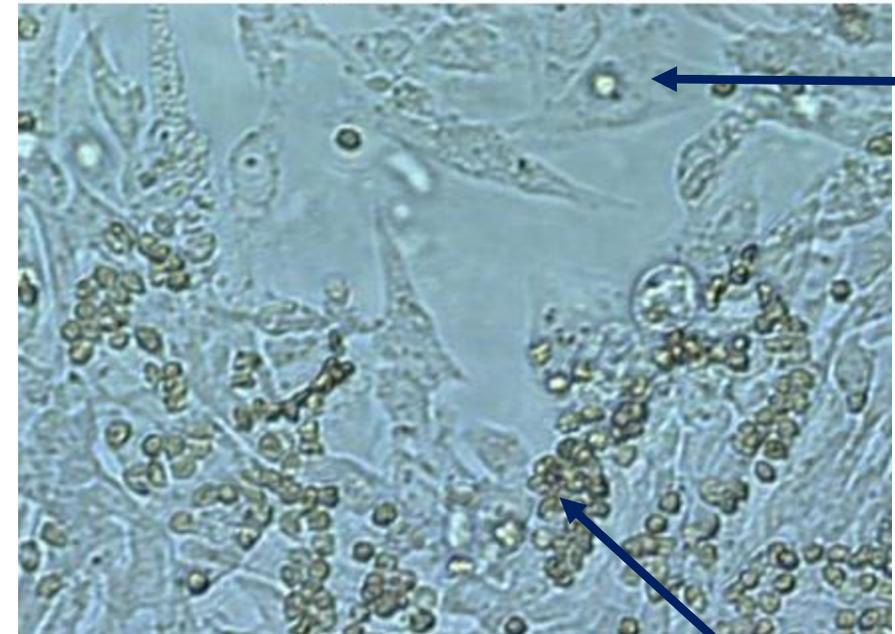
- Long period (up to 4 weeks) required for result.
- Often very poor sensitivity, sensitivity depends on a large extent on the condition of the specimen.
- Susceptible to bacterial contamination.
- Susceptible to toxic substances which may be present in the specimen.

Hemadsorption

- To detect the presence of certain viruses, the hemadsorption test is commonly used.
- Influenza and parainfluenza viruses express a viral hemagglutinin on the surface of infected cells.
- By the hemadsorption test, the **culture medium is removed and replaced with a 0.5% dilute solution of guinea-pig red blood cells.**



Microscopic view of hemadsorption



Culture cell

Hemadsorption inhibition

Patient serum with suspected Influenza infection + Cultured cells + Red Blood Cells

= No hemadsorption = Positive infection

Adsorbed RBCs on the culture cell

Diagnosics of viral diseases

Indirect methods

Serology

Detection of antibodies against the virus.

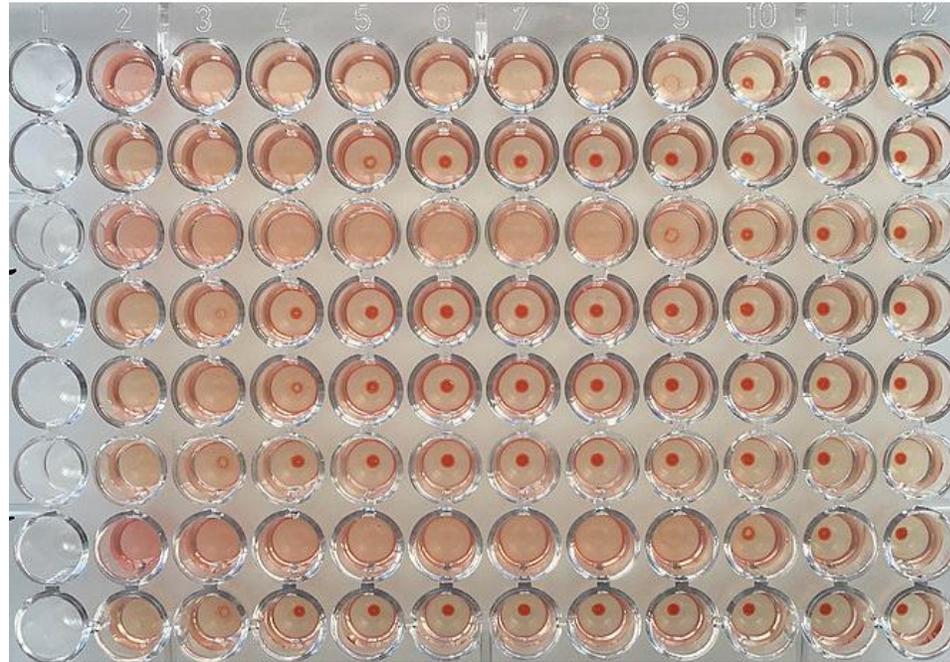
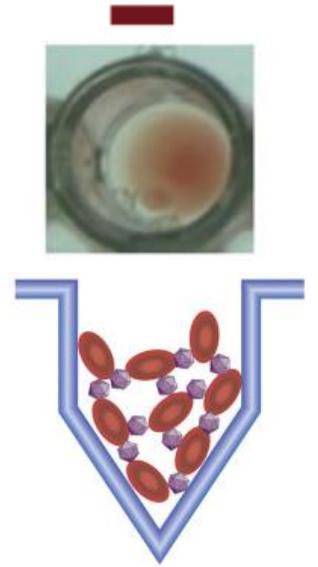
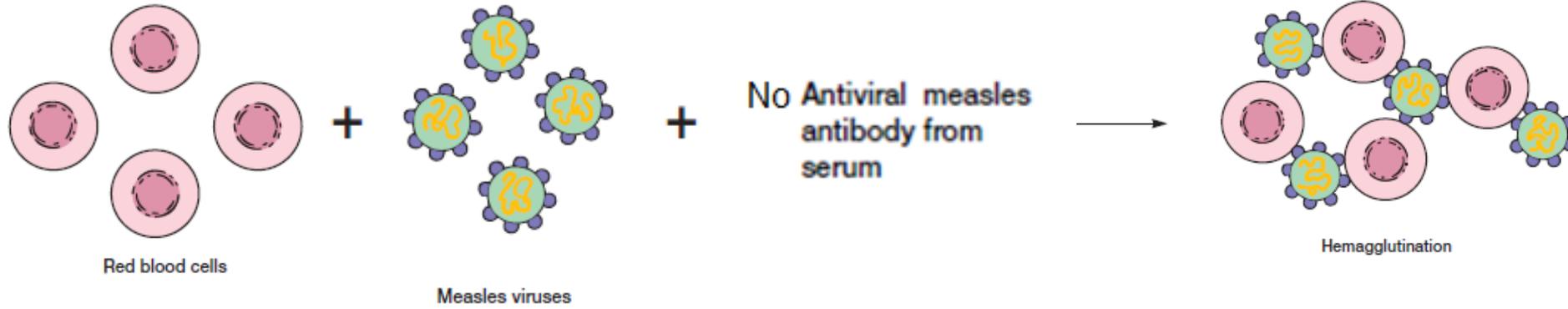
Criteria for diagnosing primary infection

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

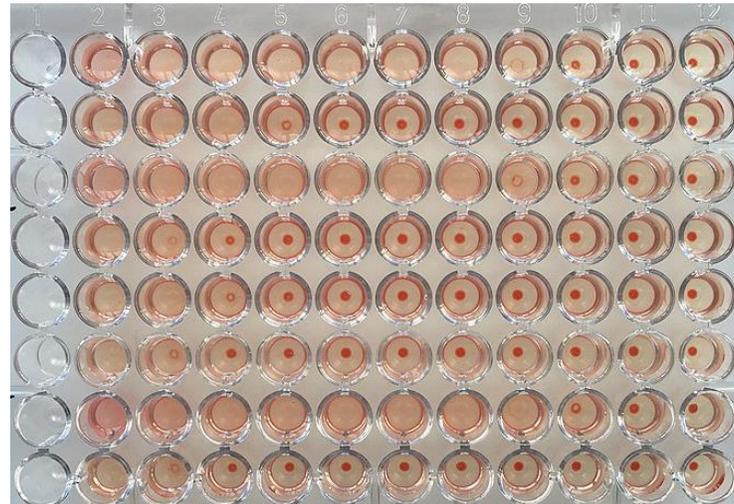
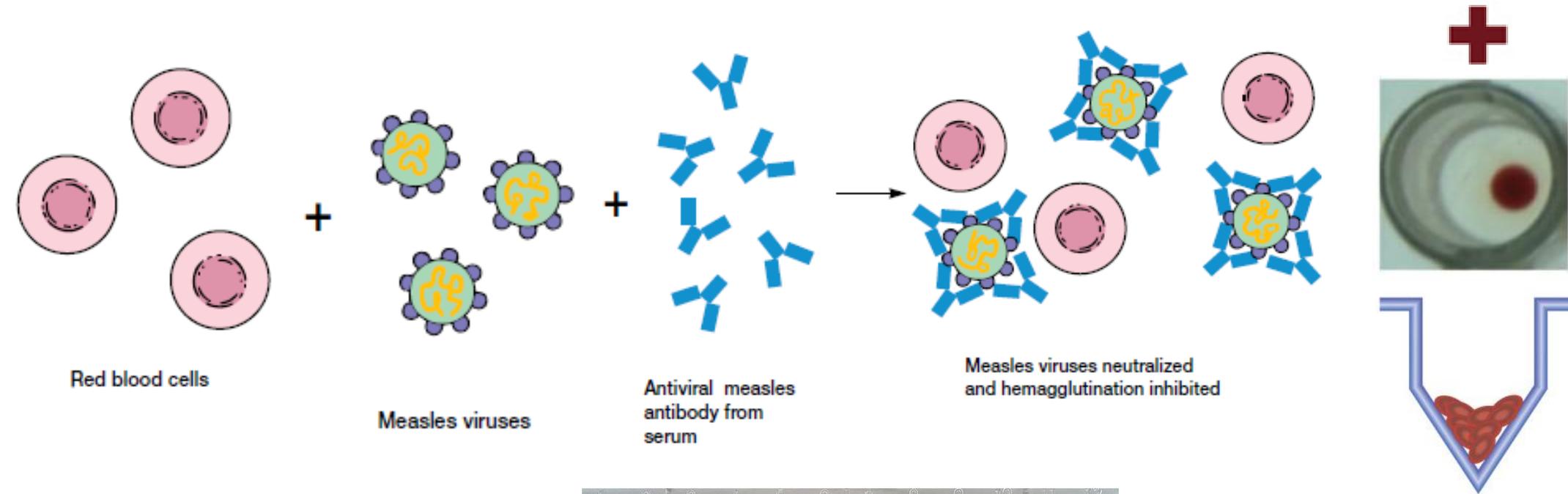
Criteria for diagnosing reinfection

- fold or more increase in titer of IgG or total antibody between acute and convalescent sera
- Absence or slight increase in IgM

Haemagglutination inhibition test



Haemagglutination inhibition test



Diagnosics of viral diseases

Indirect methods

Serology

Problems with Serology:

- Long period of time required for diagnosis for paired acute and convalescent sera.
- Mild local infections such may not produce a detectable Abs.
- Immunocompromised patients often give a reduced or absent Abs.
- Patients with infectious mononucleosis and those with connective tissue diseases such as SLE may react non-specifically giving a false positive result.
- Patients given blood or blood products may give a false positive result due to the transfer of antibody