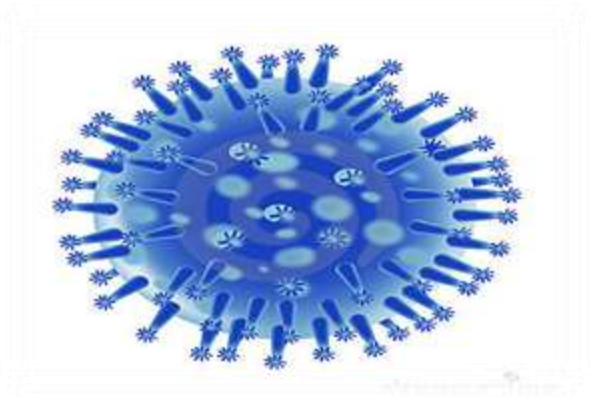
General Microbiology
Diagnosis of Viral Infections
2021-2022



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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)**

Diagnostics of viral diseases

Virus detection

2. Indirect examination:

Cell Culture	cytopathic effect (CPE) hemadsorption
Serology	Direct and indirect ELISA **Hemagglutination inhibition test**
Animals	disease or death

Diagnostics of viral diseases

Direct methods

Serology

- Most used lab method
- Detection of antigen

Classical Techniques

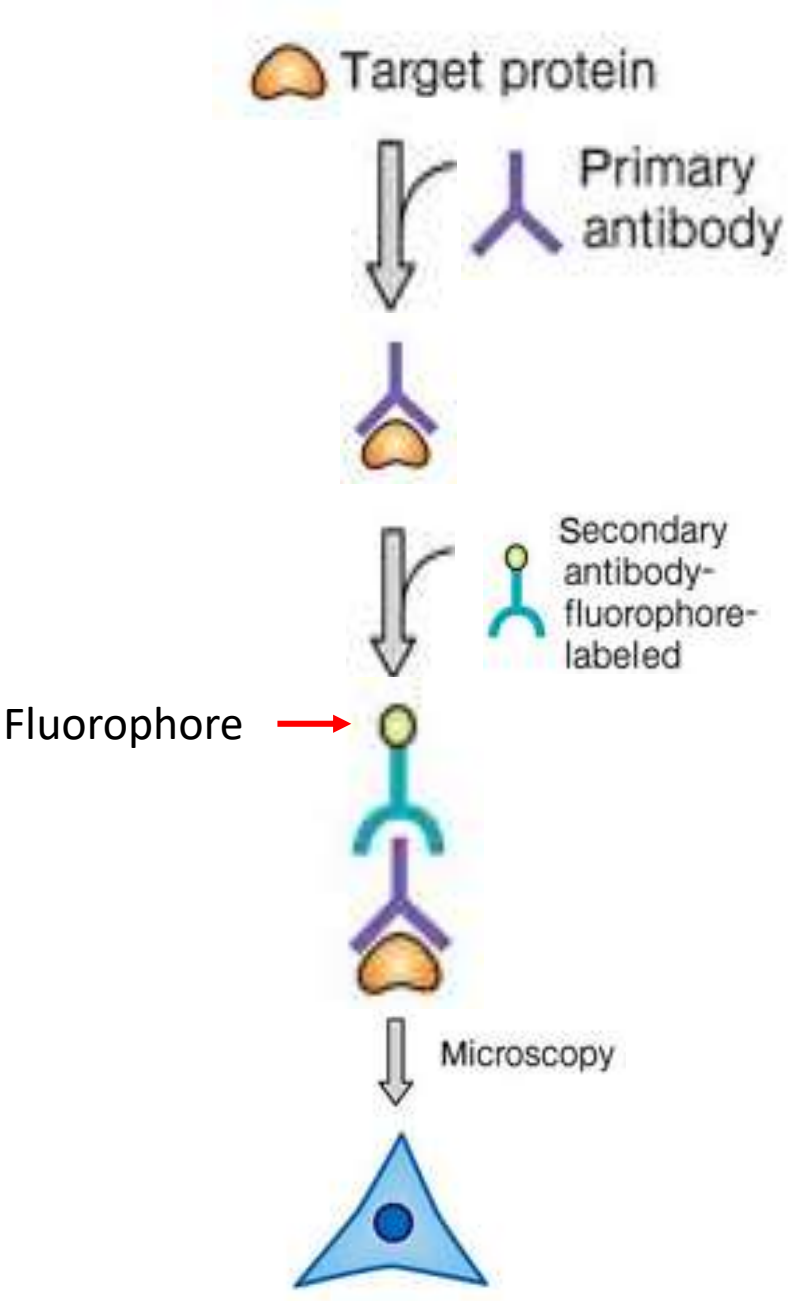
1. Complement fixation tests (CFT)
2. Immunofluorescence techniques (IF)
3. Neutralization tests

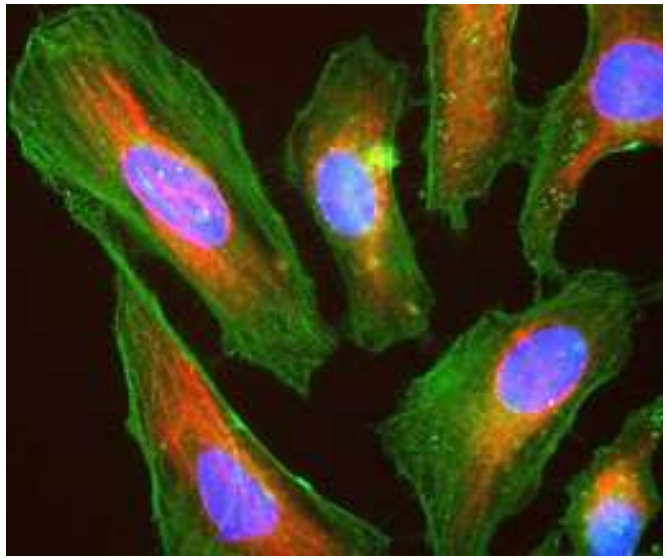
Newer Techniques

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

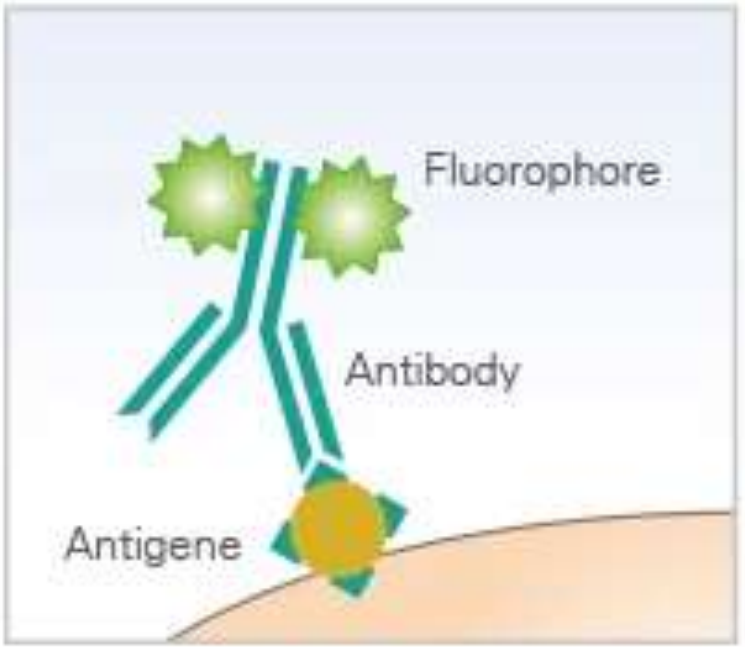
Immunofluorescence techniques (IF)

Indirect immunofluorescence





Direct Immunofluorescence



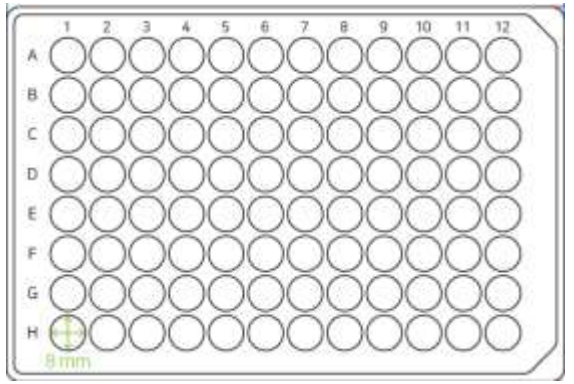
Diagnostics of viral diseases

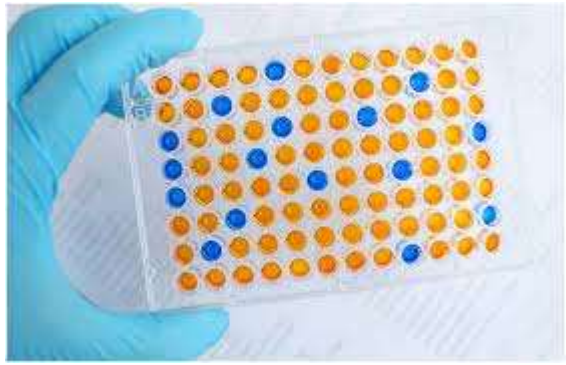
Direct methods

Serology

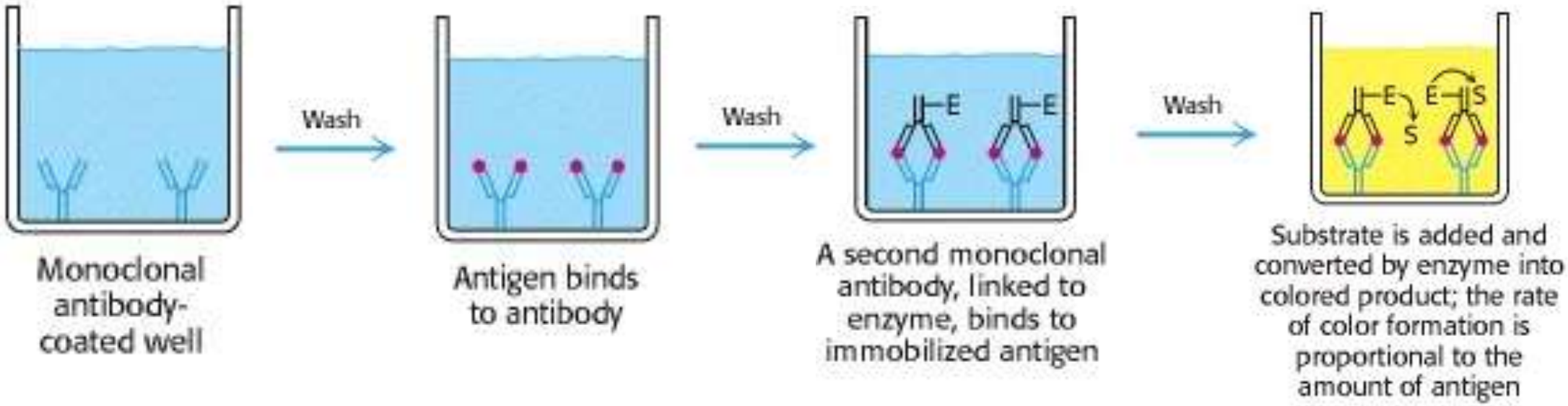
Enzyme Linked Immunosorbent Assay (ELISA).

96 well plate





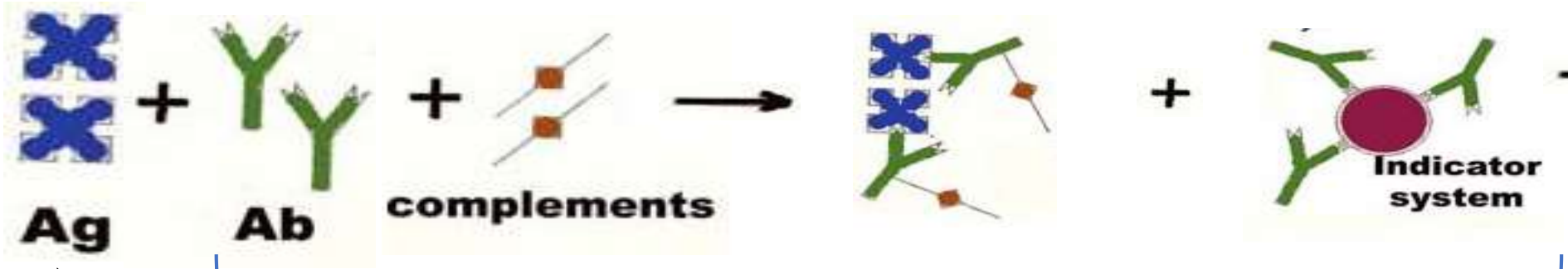
Sandwich ELISA



Complement fixation test

Procedure

Looking for antigens



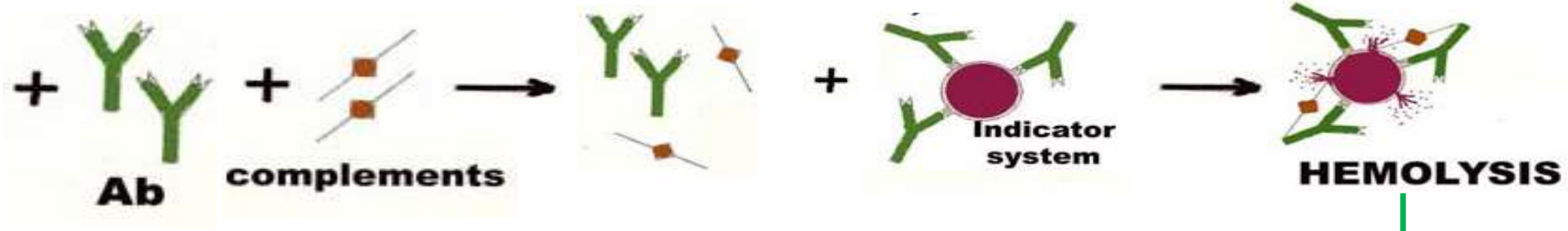
Patient serum

Ab , complement, and sRBCs are externally added

No hemolysis

Positive results

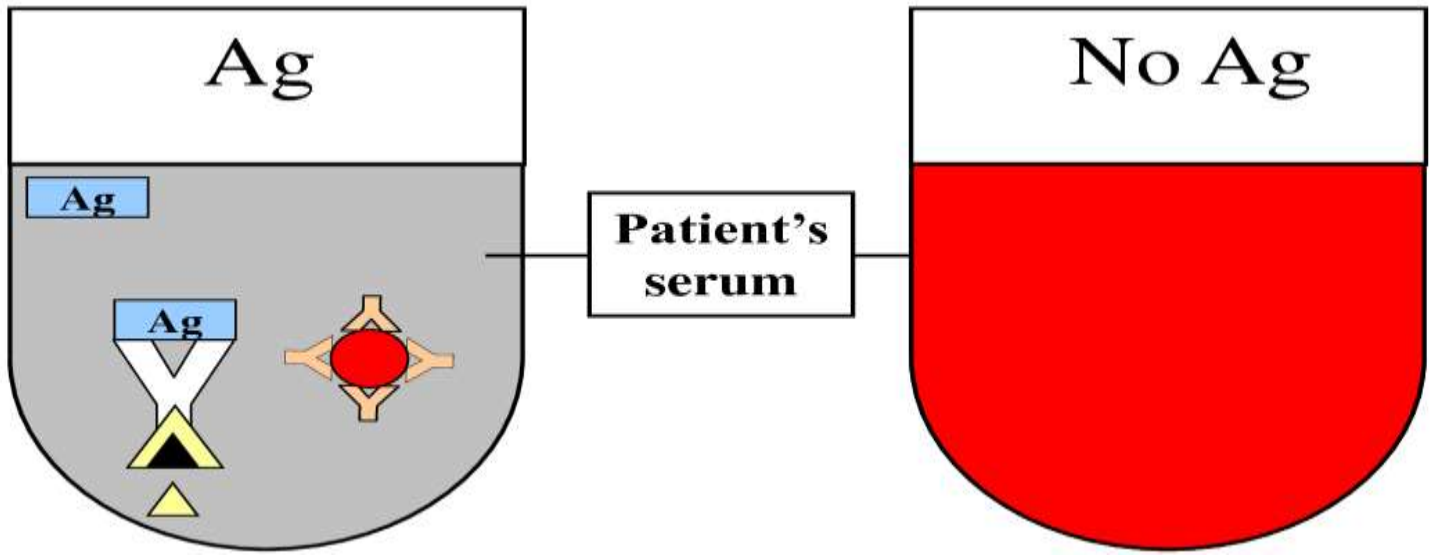
No antigen

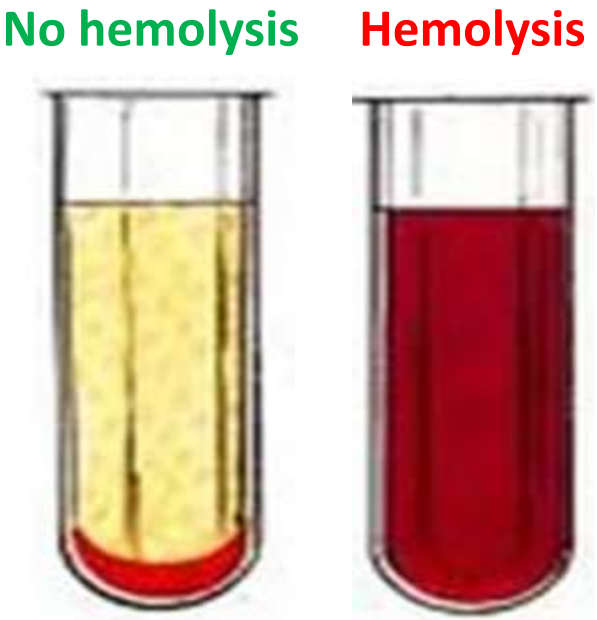


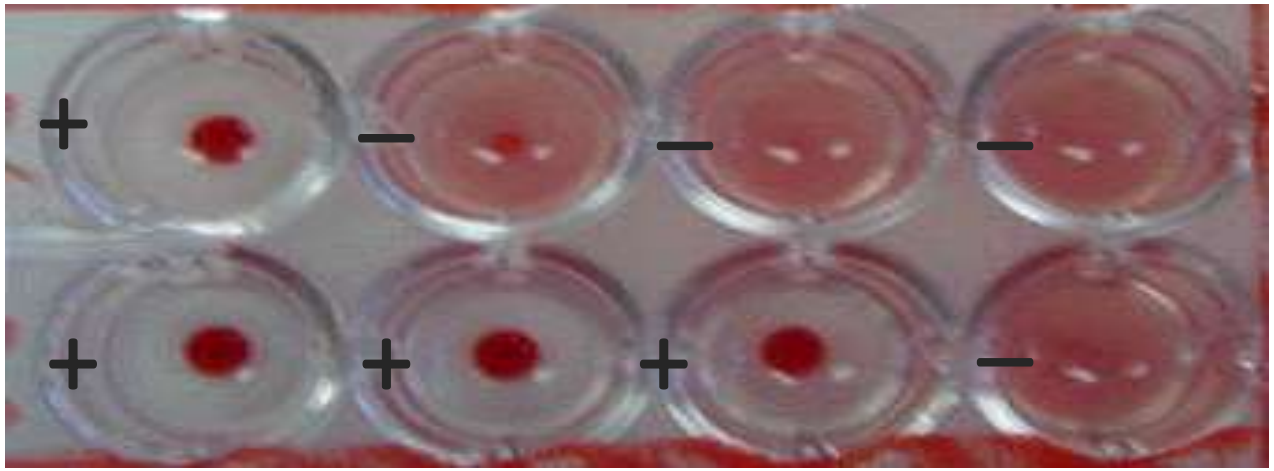
Patient serum

Negative results

Complement fixation test







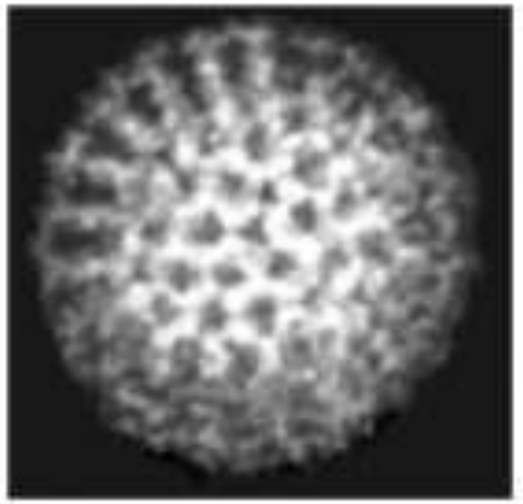
Diagnostics of viral diseases

Direct methods

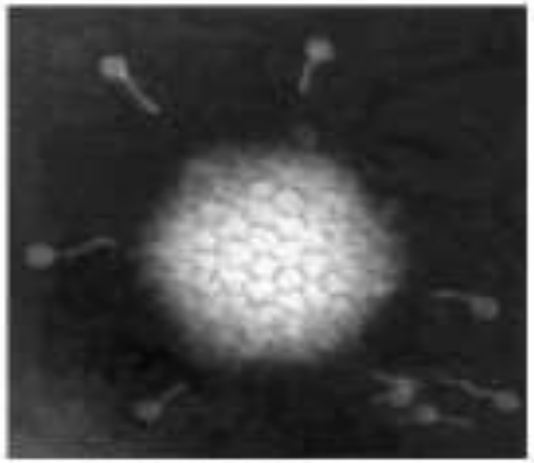
Electron Microscopy

- 106 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

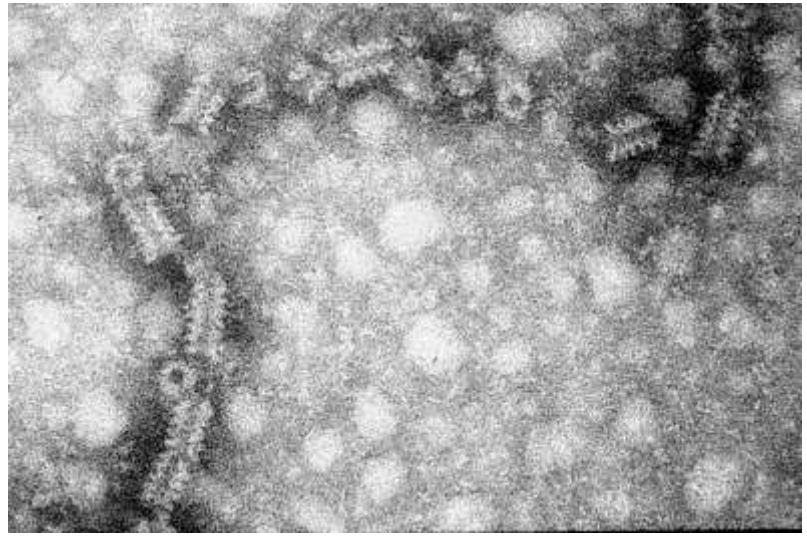
Diagnostics of viral diseases

Direct methods

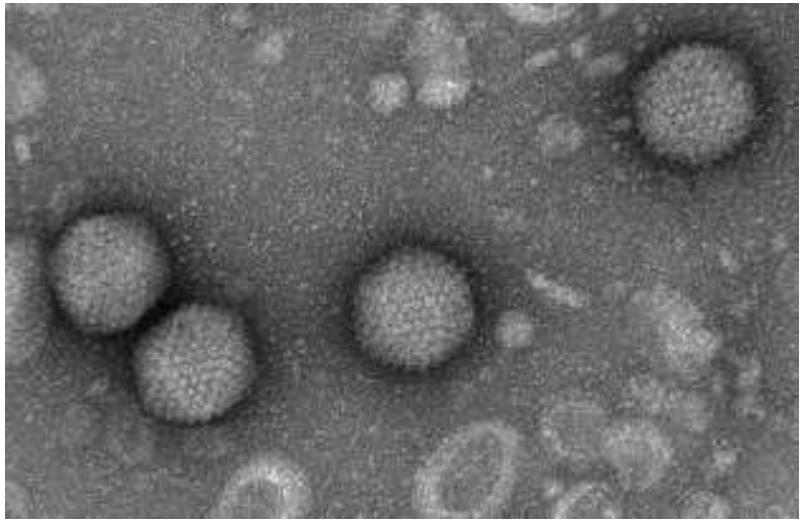
Electron Microscopy

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.

Diagnostics of viral diseases

Virus detection

2. Indirect examination:

Cell Culture	cytopathic effect (CPE) hemadsorption
Serology	Direct and indirect ELISA **Hemagglutination inhibition test**
Animals	disease or death

Diagnostics of viral diseases

Indirect methods

Cell Culture

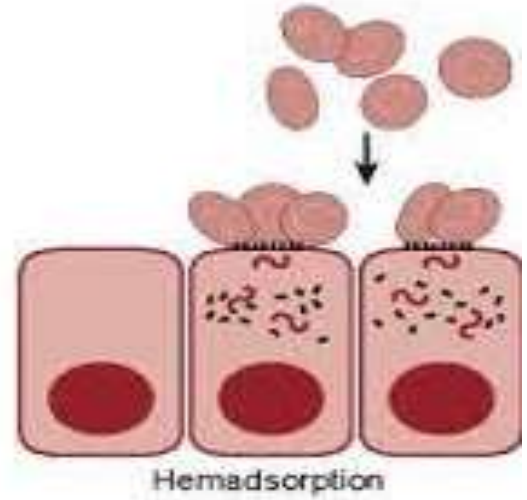
Are used for virus isolation. However, they are very expensive and it is often difficult to obtain a reliable supply.

Problems with cell culture

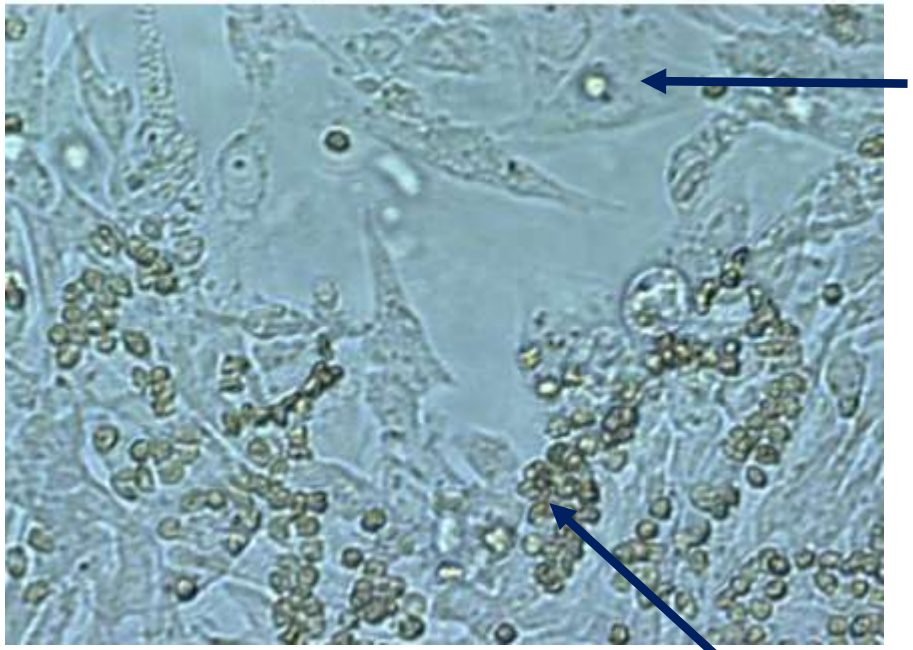
- Long period (up to 4 weeks) required for result.
- Often very poor sensitivity, sensitivity depends on a large extent on the condition of the specimen.
- Susceptible to bacterial contamination.
- Susceptible to toxic substances which may be present in the specimen.

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



Hemadsorption inhibition

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

= No hemadsorption = Positive infection

Diagnostics of viral diseases

Indirect methods

Serology

Detection of antibodies against the virus.

Criteria for diagnosing primary infection

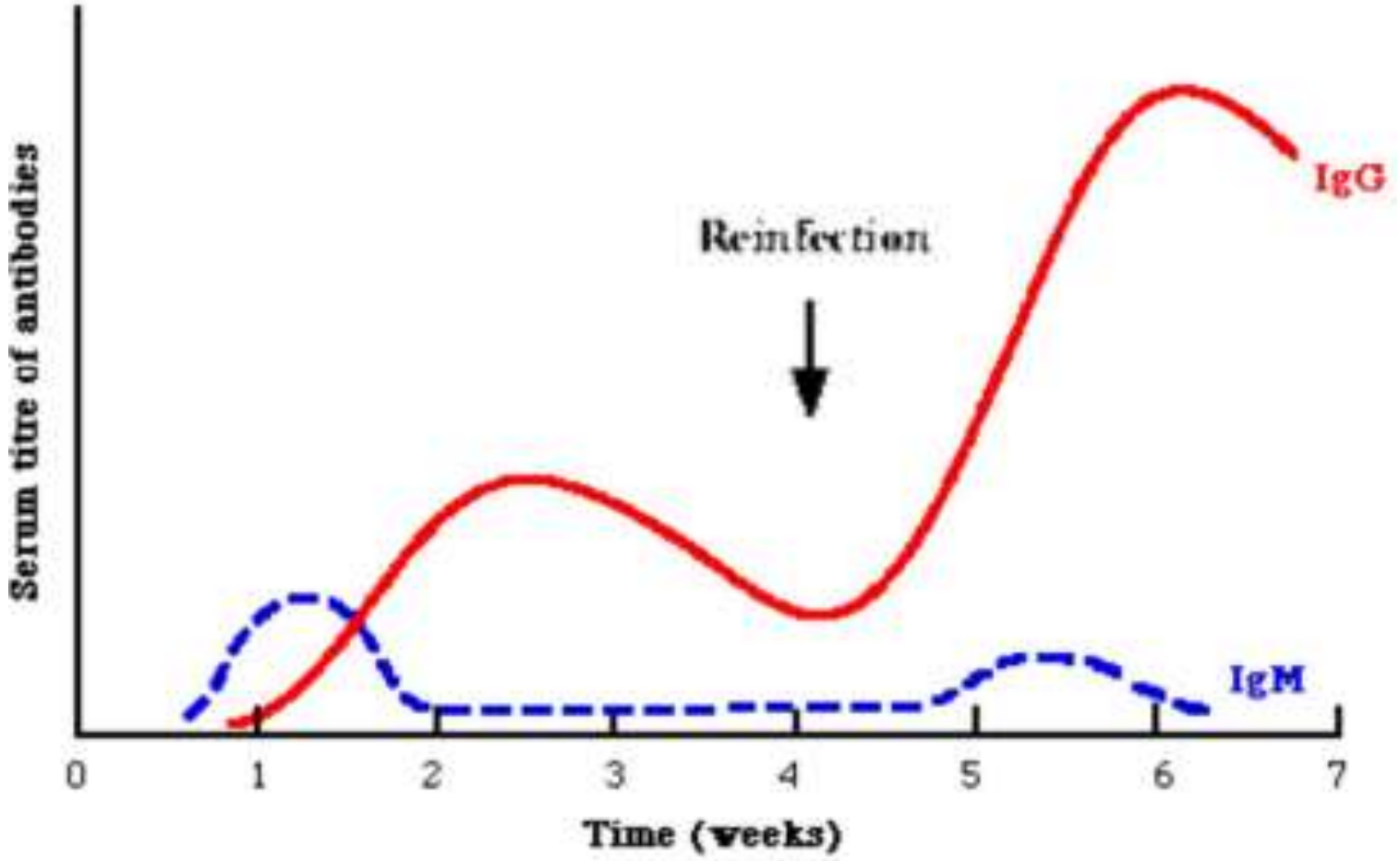
- 4 fold or more increase in titer of IgG or total antibody between acute and convalescent sera
- Presence of IgM
- Seroconversion

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

Diagnostics of viral diseases

Serology

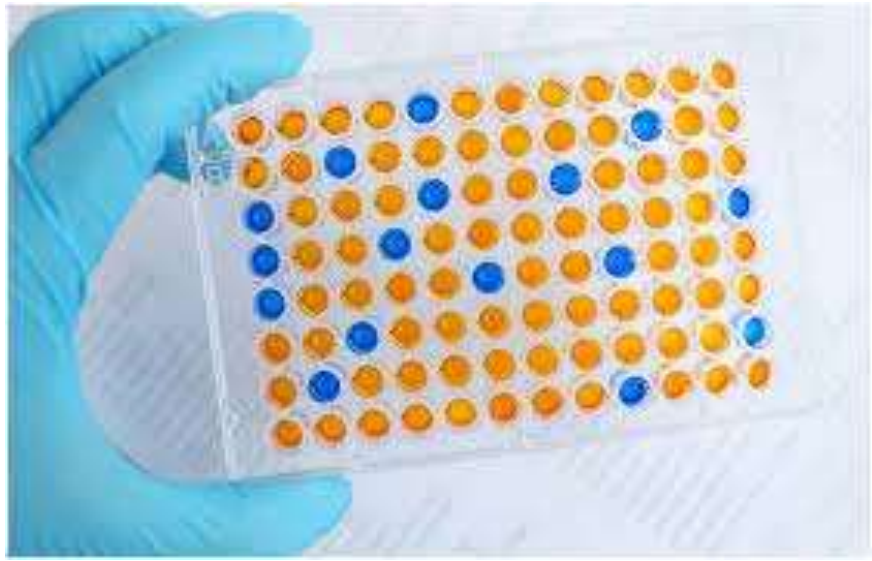


Note that during reinfection, IgM may be absent or present at a low level transiently

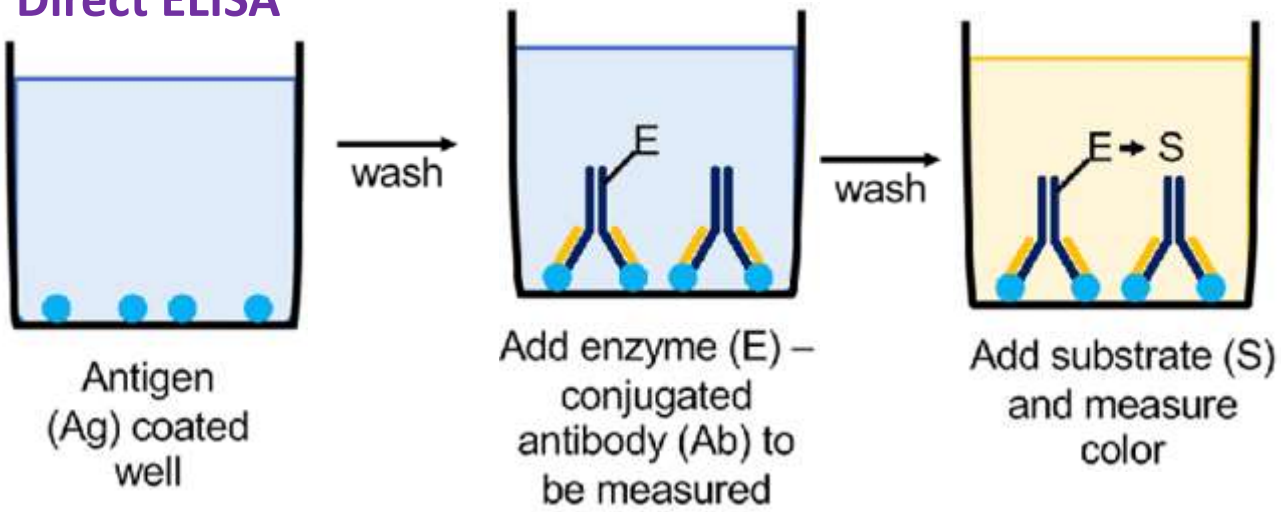
Diagnostics of viral diseases

Indirect methods

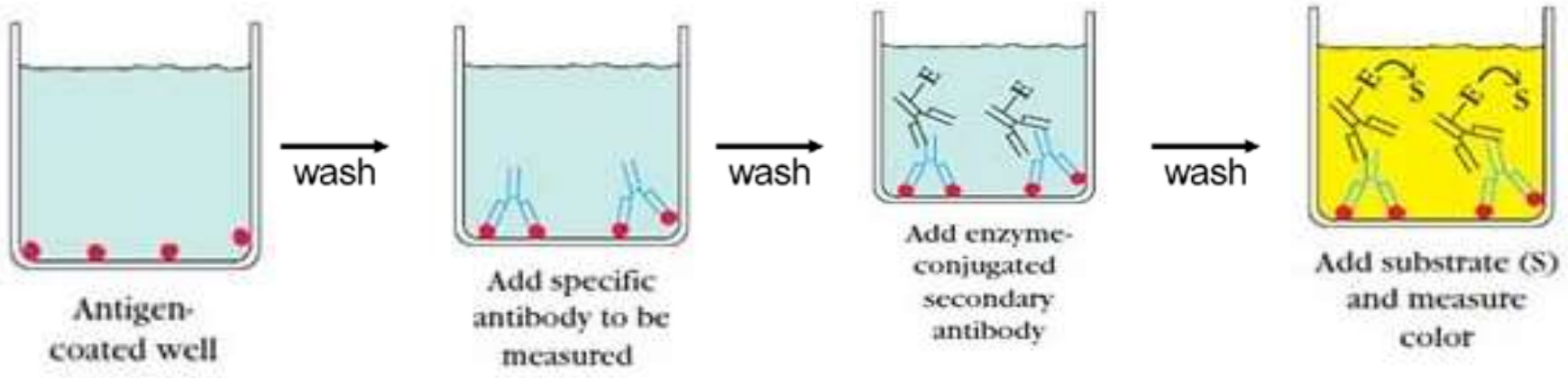
Serology



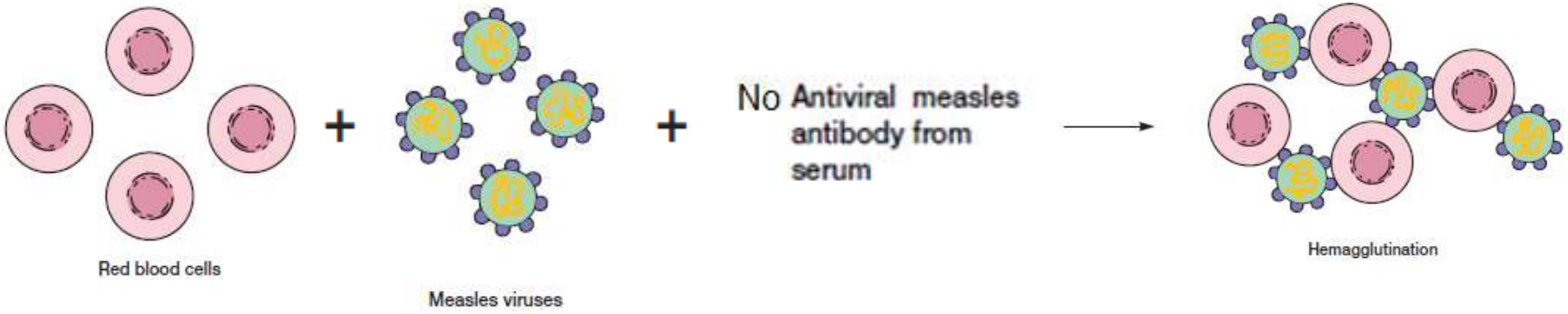
Direct ELISA

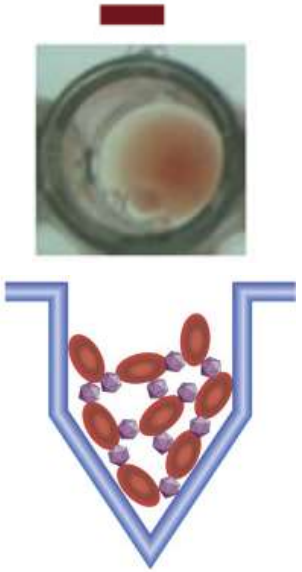


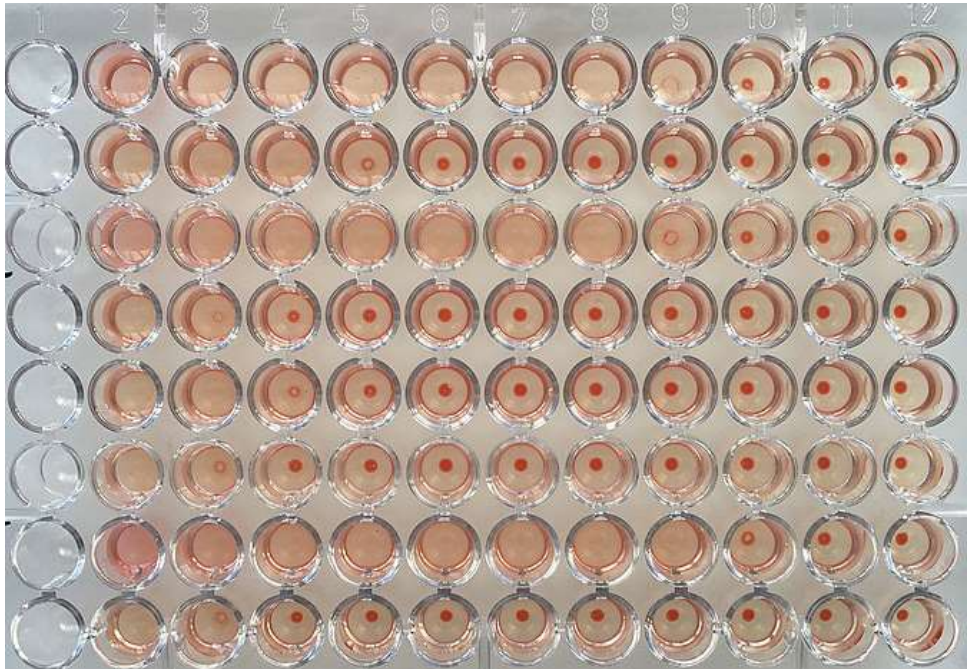
Indirect ELISA



Haemagglutination inhibition test







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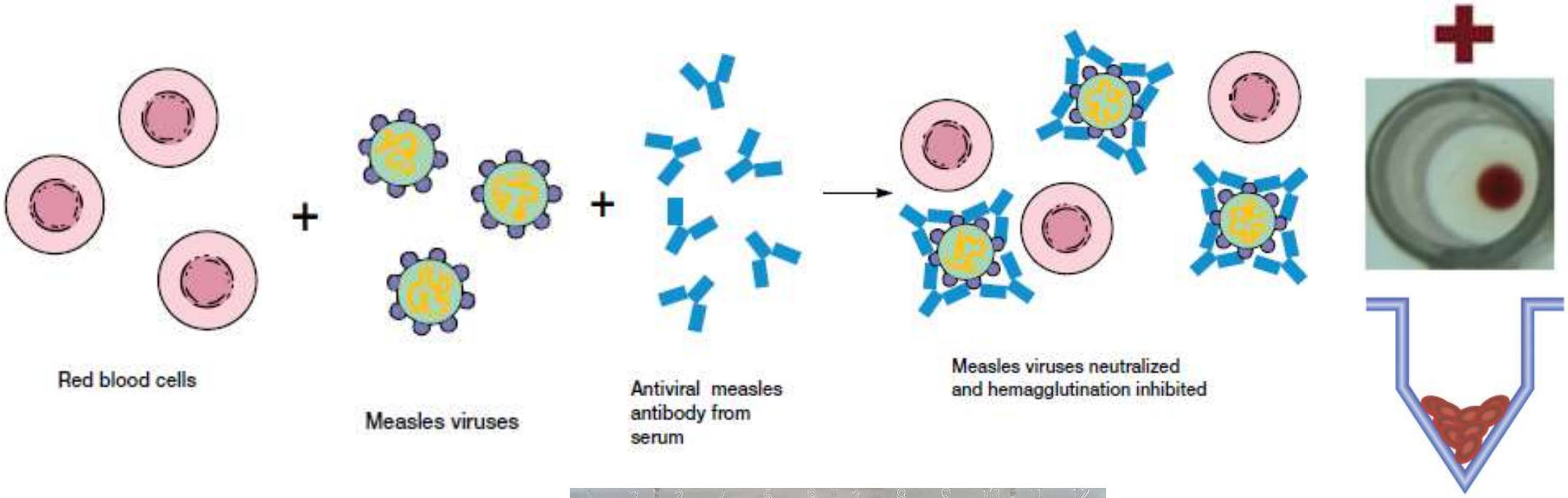

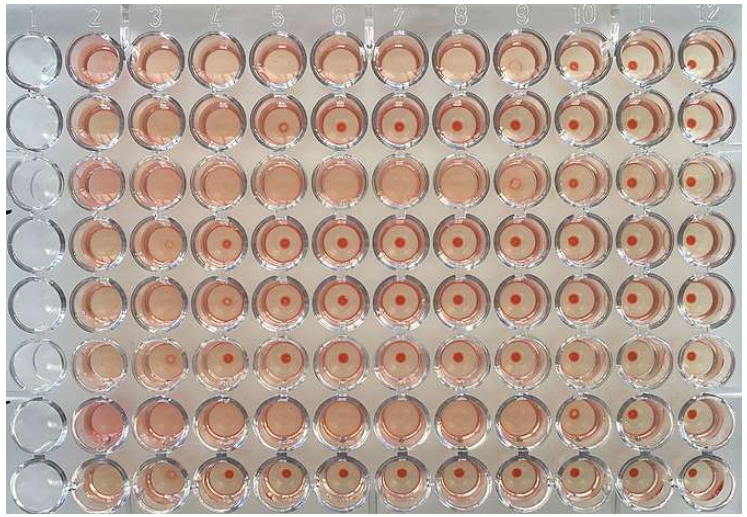

Diagnostics of viral diseases

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