Laboratory Diagnosis of Virus Infections MBChB III (Part 1) – 22May19

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Communication (Physician-laboratory)

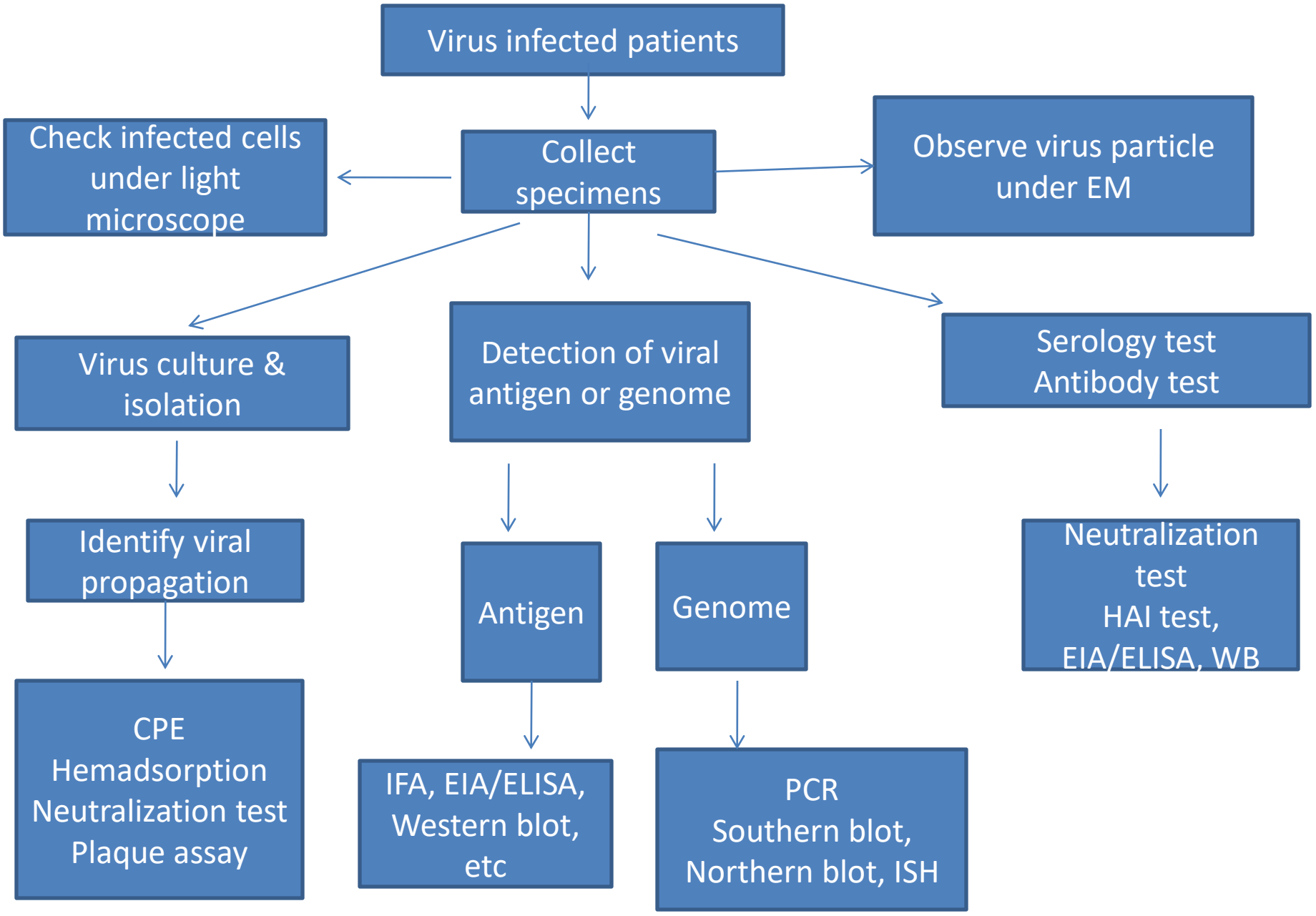
Physician/clinician

- Clinical diagnosis (need further confirmation – lab diagnosis)
- Specific lab requests (based on tentative diagnosis – infection type/infectious agent suspected)
- Proper labeling of specimens
- Physician's name/contact info
- Lab results feedback -> clinician soonest possible
- Treatment (if antivirals available)

Viral Diagnostics in the Clinical Laboratory

1. >70% of all infectious disease cases seen by a physician are due to viral infections.
2. For Lab diagnosis
   - Quality of patient specimens important
   a. Collection
   b. Appropriate tubes/containers
   c. Transportation
   d. Storage
   e. Appropriate test & analysis

Procedures for laboratory viral diagnosis



Three General Approaches for Laboratory Diagnosis of Viral Infections

- **Virus Isolation (Indirect Examination)**
  - CPE and other characters
  - Animal systems (Eggs, mice etc)

- **Direct detection**
  - Microscopy or staining
  - Detection of nucleic acid, antigens

- **Serology**
  - Antibodies (Indirect)
  - Antigen (Direct)

Serology

Serology

Detection of:

1. Rising titres of antibody between acute and convalescent stages of infection, or
2. Detection of IgM in primary infection
   - marker of recent infection
   - short-lived
3. Detection of IgG (systemic immunity)
   - In blood
   - Long-lived
4. Detection of IgA (local immunity)
   - In body secretions (saliva, tears, vaginal fluid etc)
   - Long-lived

Viral Serology

1. Indirect
2. Primary and secondary responses to viral infections
   - IgM (1st exposure)
   - IgG (2nd exposure)

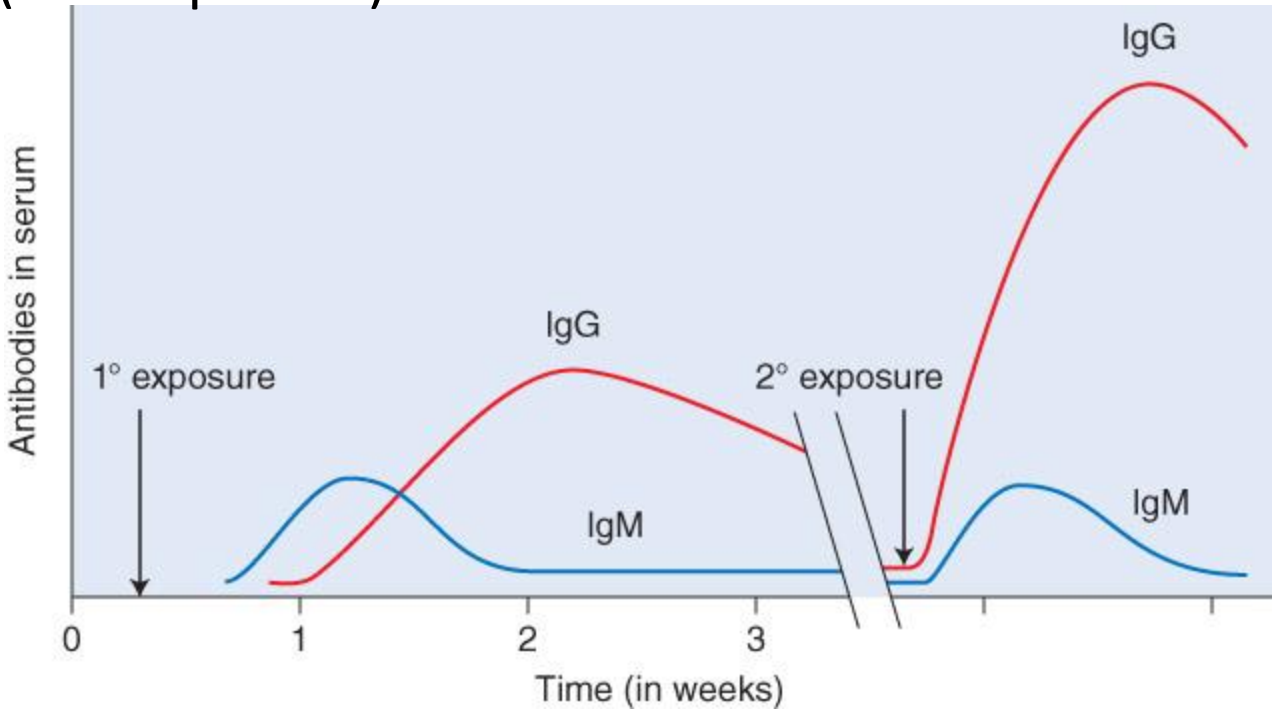


Figure 5.18: Primary (1 degree) and secondary (2 degree) antibody responses toward a viral pathogen.

Virology Serology

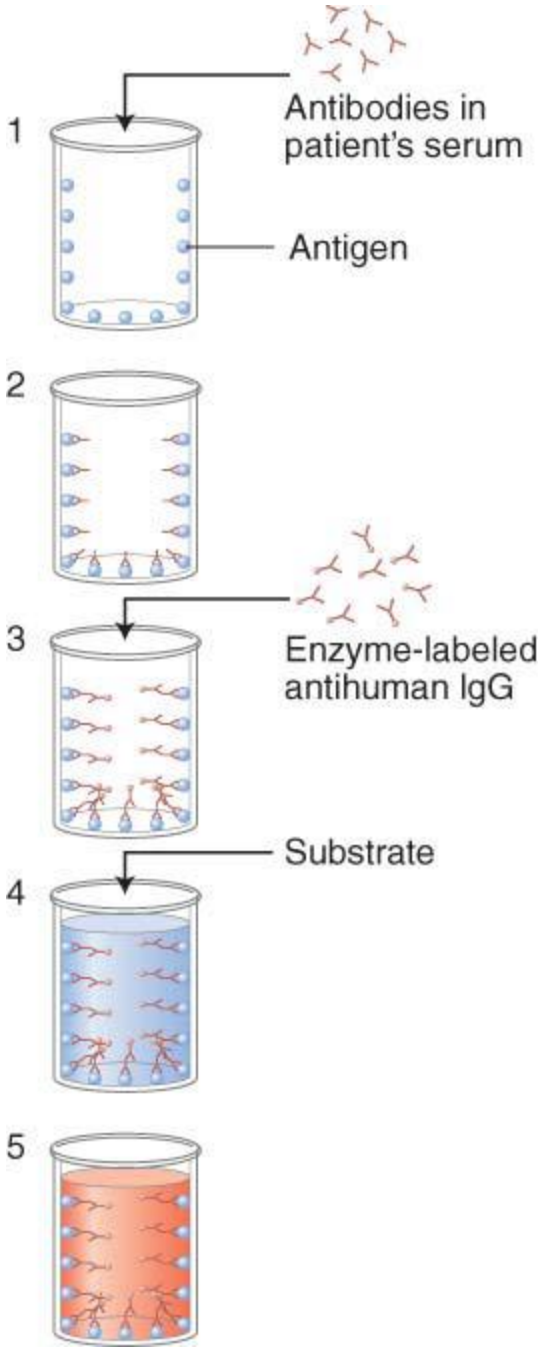
<u>Enzyme-Linked Immunosorbent Assays (ELISAs)</u>

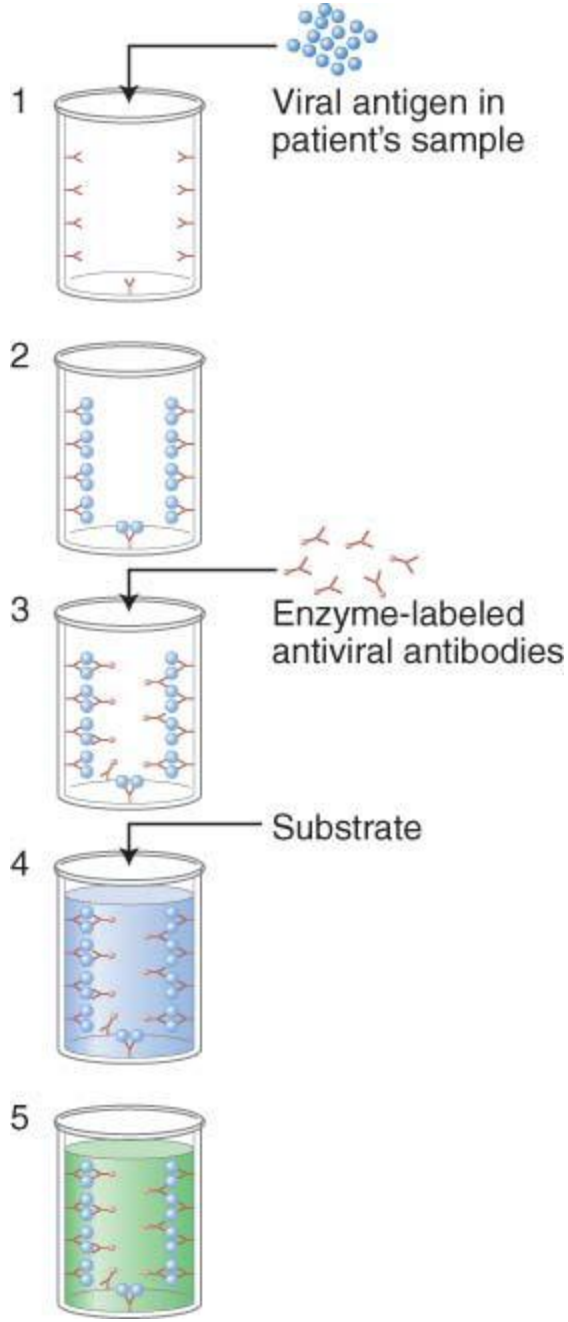
- Enzyme reacts with substrate -> colored product
    - •Very sensitive
    - •HIV test
        - If positive twice, Western Blotting is performed next

-Detection of antigen (Antigen ELISA) – Direct

-Detection of antibodies (Ab ELISA) – Indirect test

ELISA Procedures





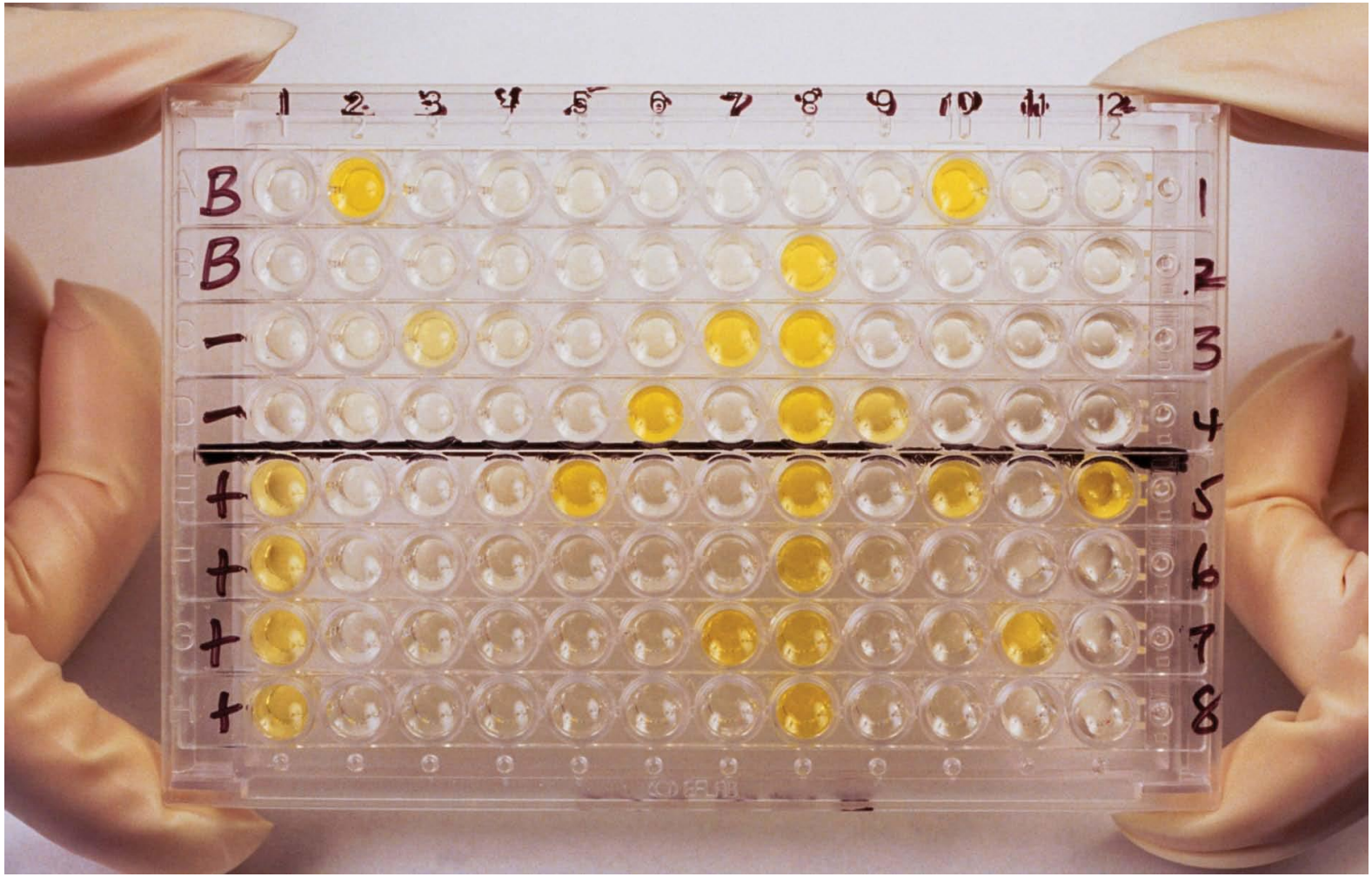


Figure 5.20: HIV ELISA test.

Virology Serology

Western Blotting

1. Viral proteins are separated in SDS-PAGE gel
2. Transferred to a nitrocellulose filter
3. Detected by labeled antibodies

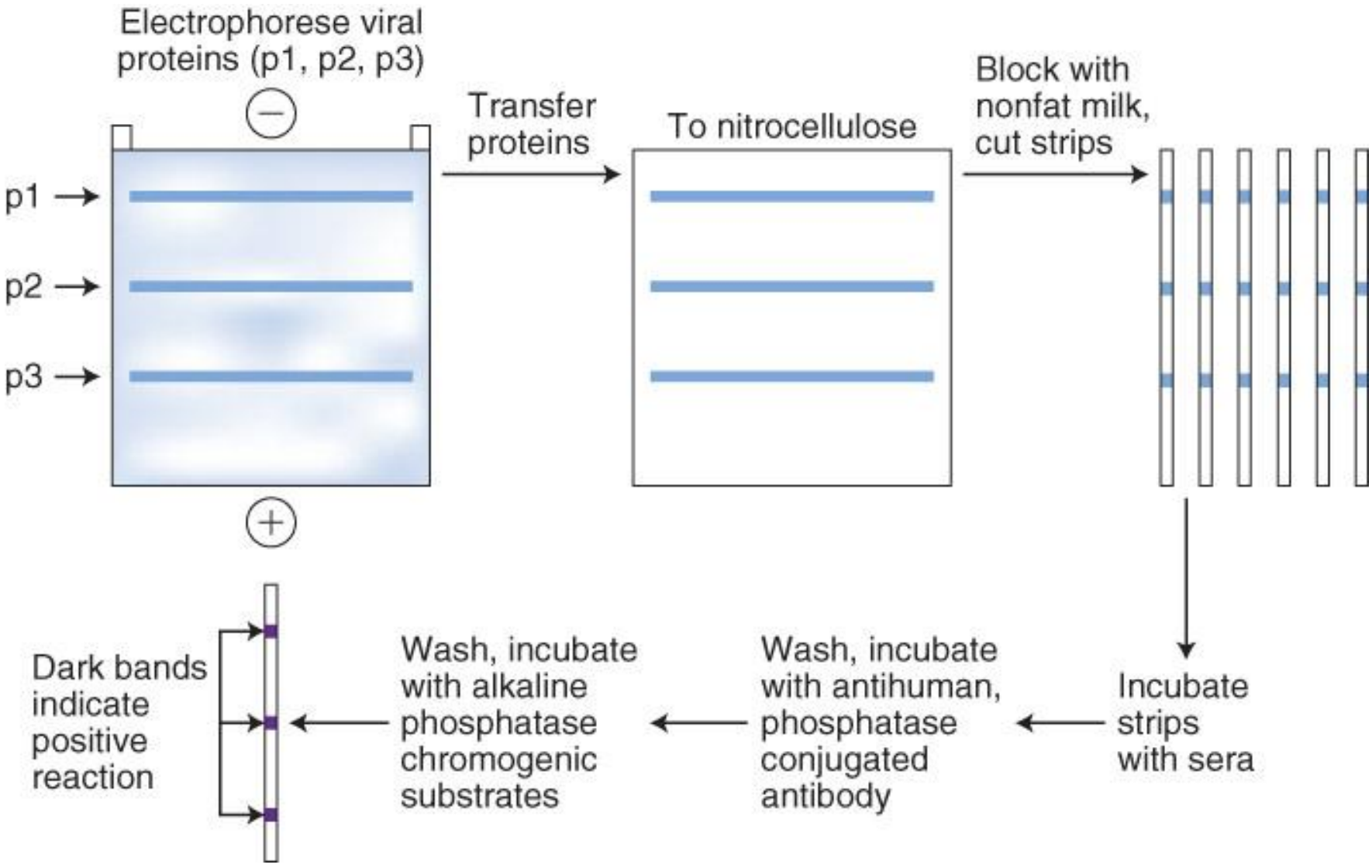
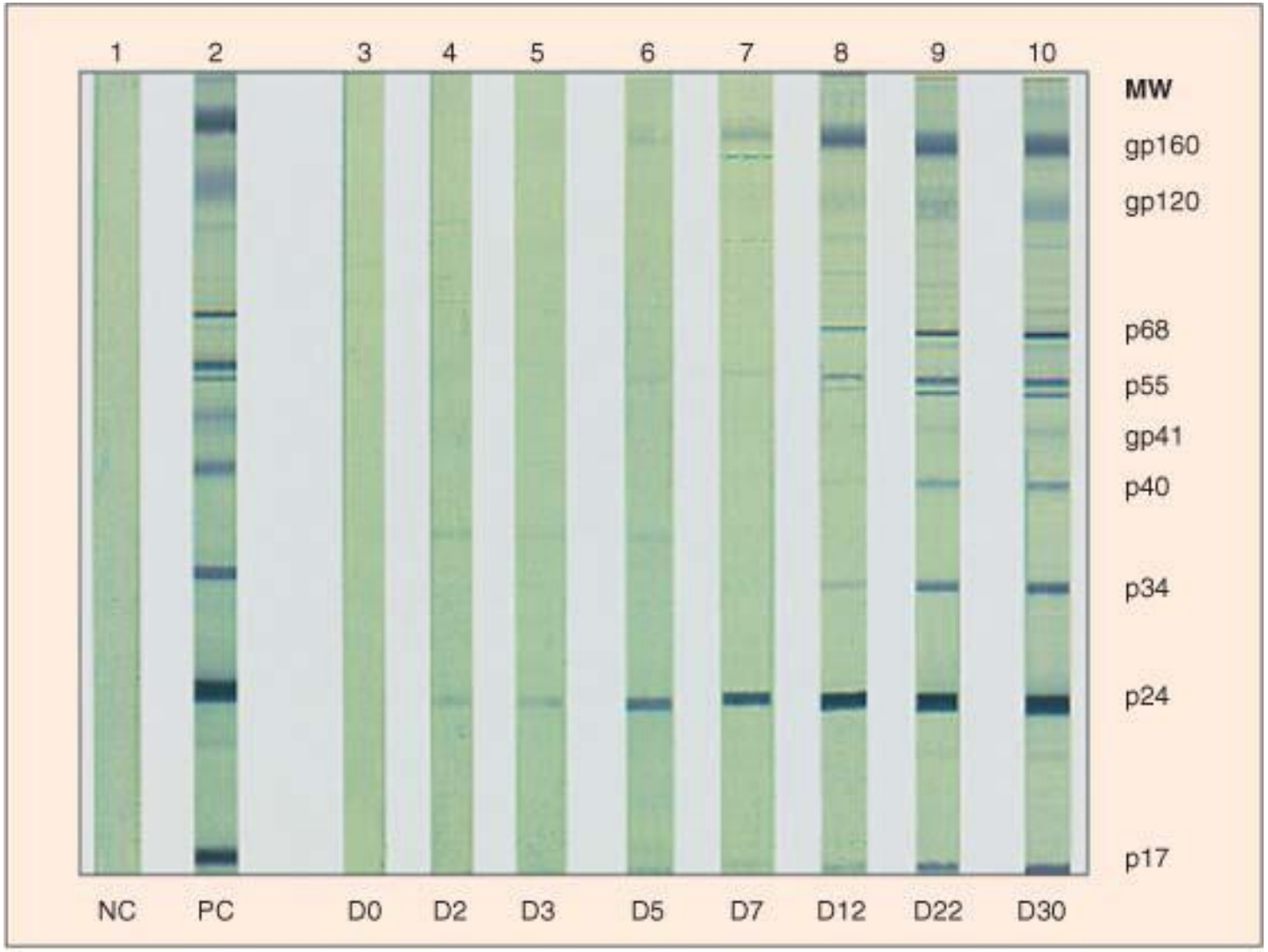


Figure 5.21a: The basic principles behind the Western blotting procedure.

Antibody Detection: Western blot



From Medical Microbiology, 5thed., Murray, Rosenthal & Pfaller, Mosby Inc., 2005, Fig. 51-7.

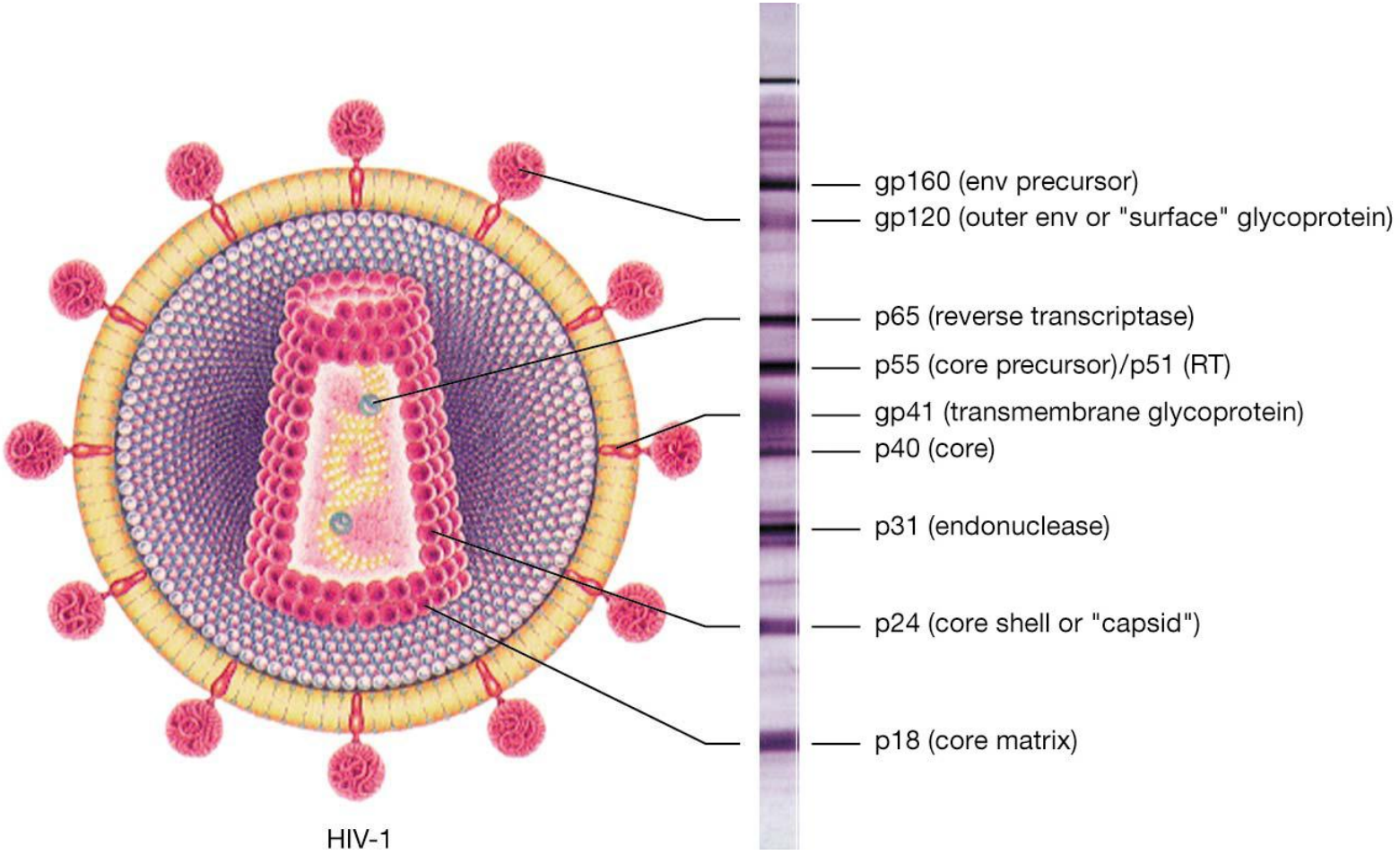


Figure 5.21b: The structure of HIV-1.

Figure 5.21c: The typical results of a Western blot testing patient serum for HIV-1 antibodies.

Stage/Period of Illness	Virus Detectable in Test Materials	Specific Antibody Demonstrable[a]
Incubation	Rarely	No
Prodrome	Occasionally	No
Onset	Frequently	Occasionally
Acute phase	Frequently	Frequently
Recovery	Rarely	Usually
Convalescence	Very rarely	Usually

Direct detection

Direct Detection of Virus

1. Electron Microscopy

- Morphology of virus particles
- Immune electron microscopy

2. Light Microscopy

- Histological appearance
- Inclusion bodies

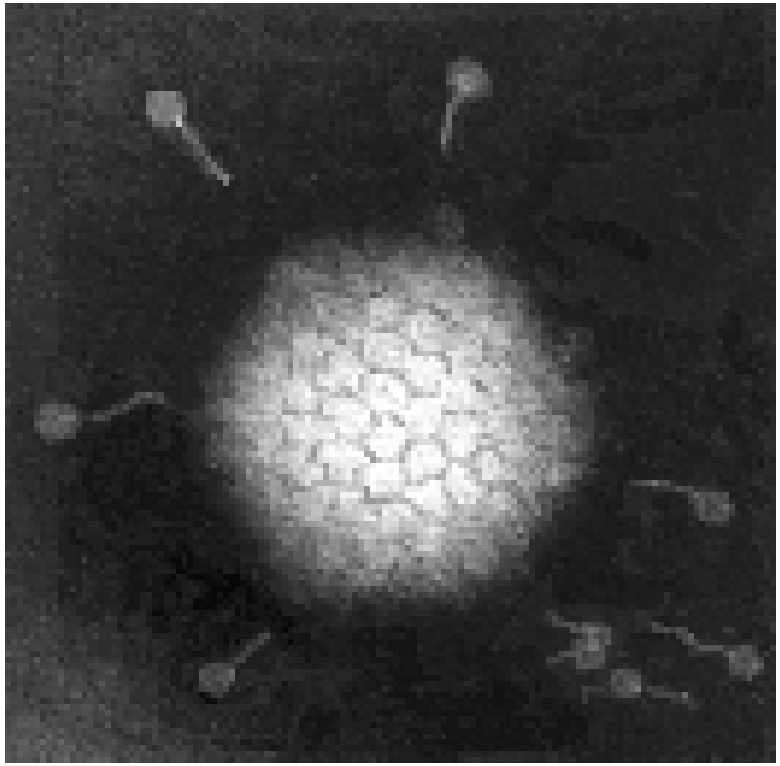
3. Antigen Detection

- Immunofluorescence, ELISA etc.

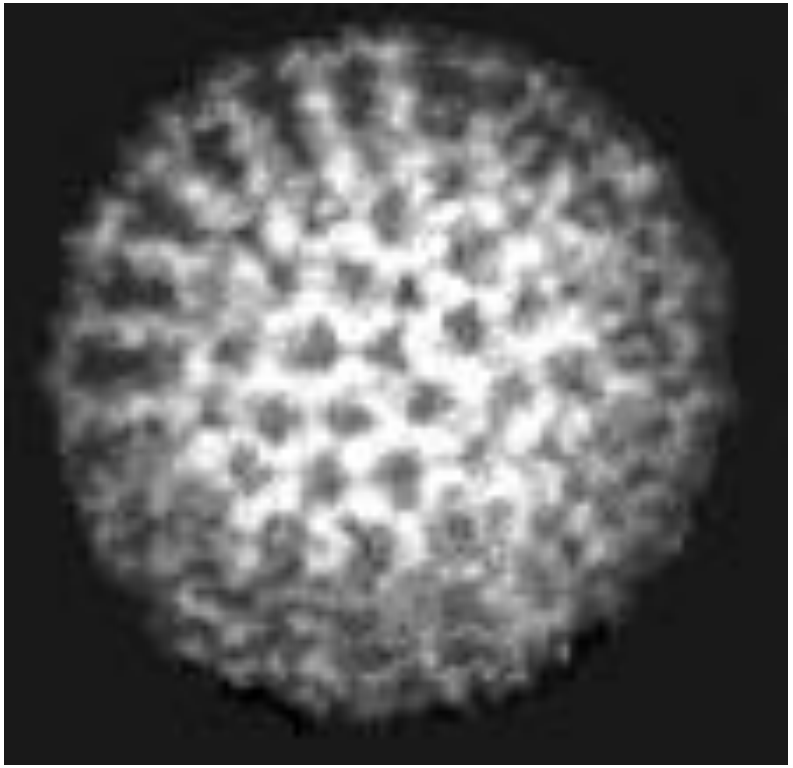
4. Viral Genome Detection

- Hybridization with specific nucleic acid probes
- Polymerase chain reaction (PCR)

Electron Micrographs (EM)



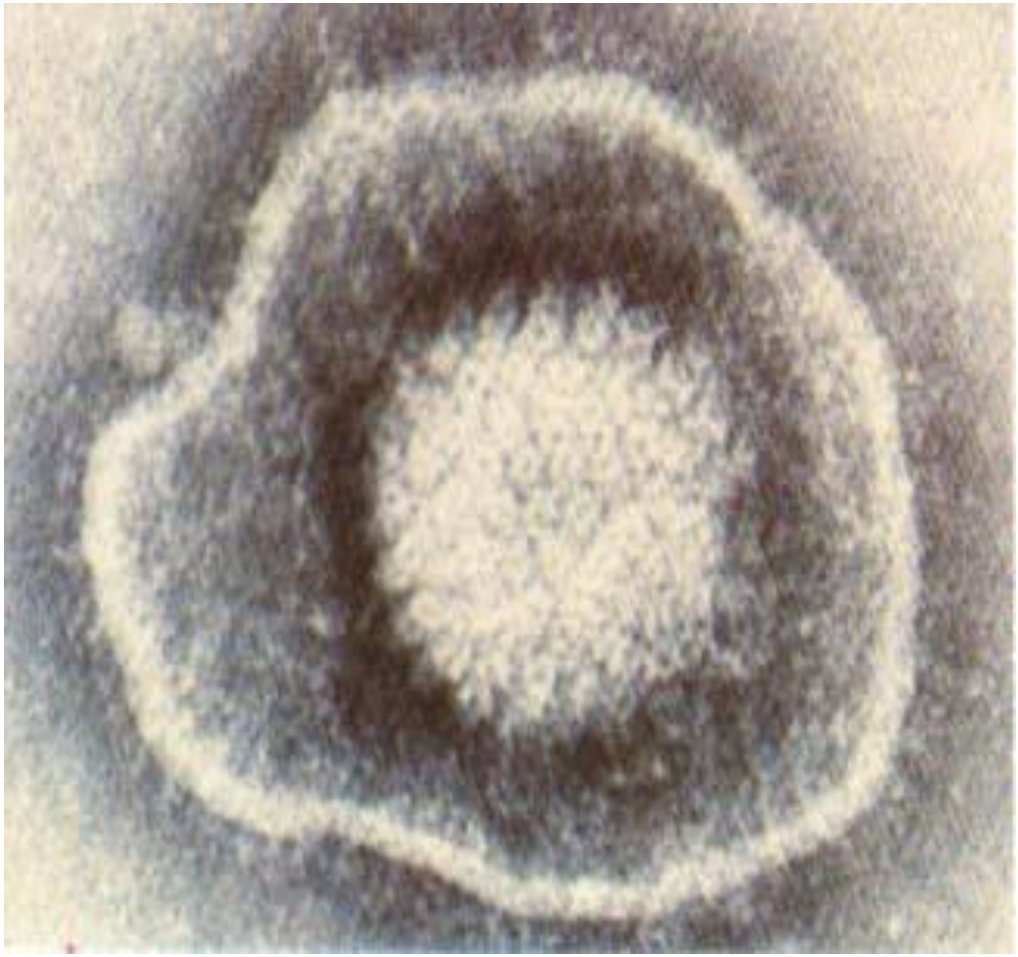
Adenovirus



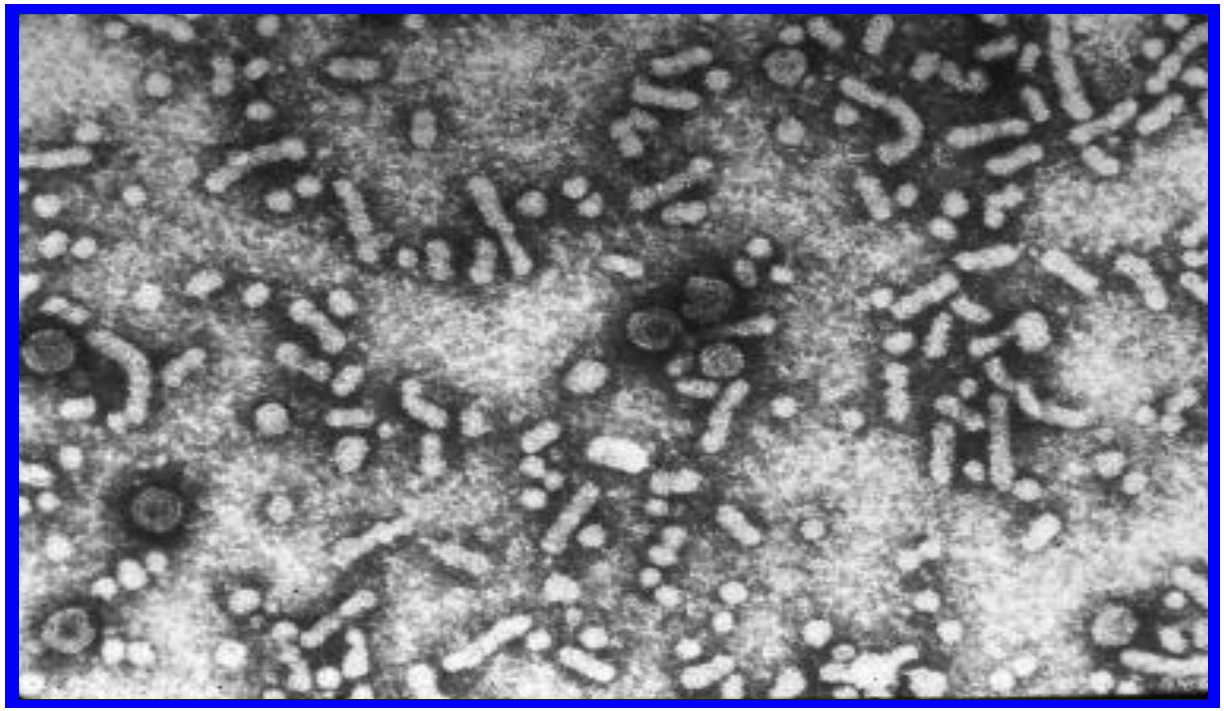
Rotavirus

(courtesy of Linda Stannard, University of Cape Town, S.A.)

Electron micrograph (EM) of a virus particle (HSV)



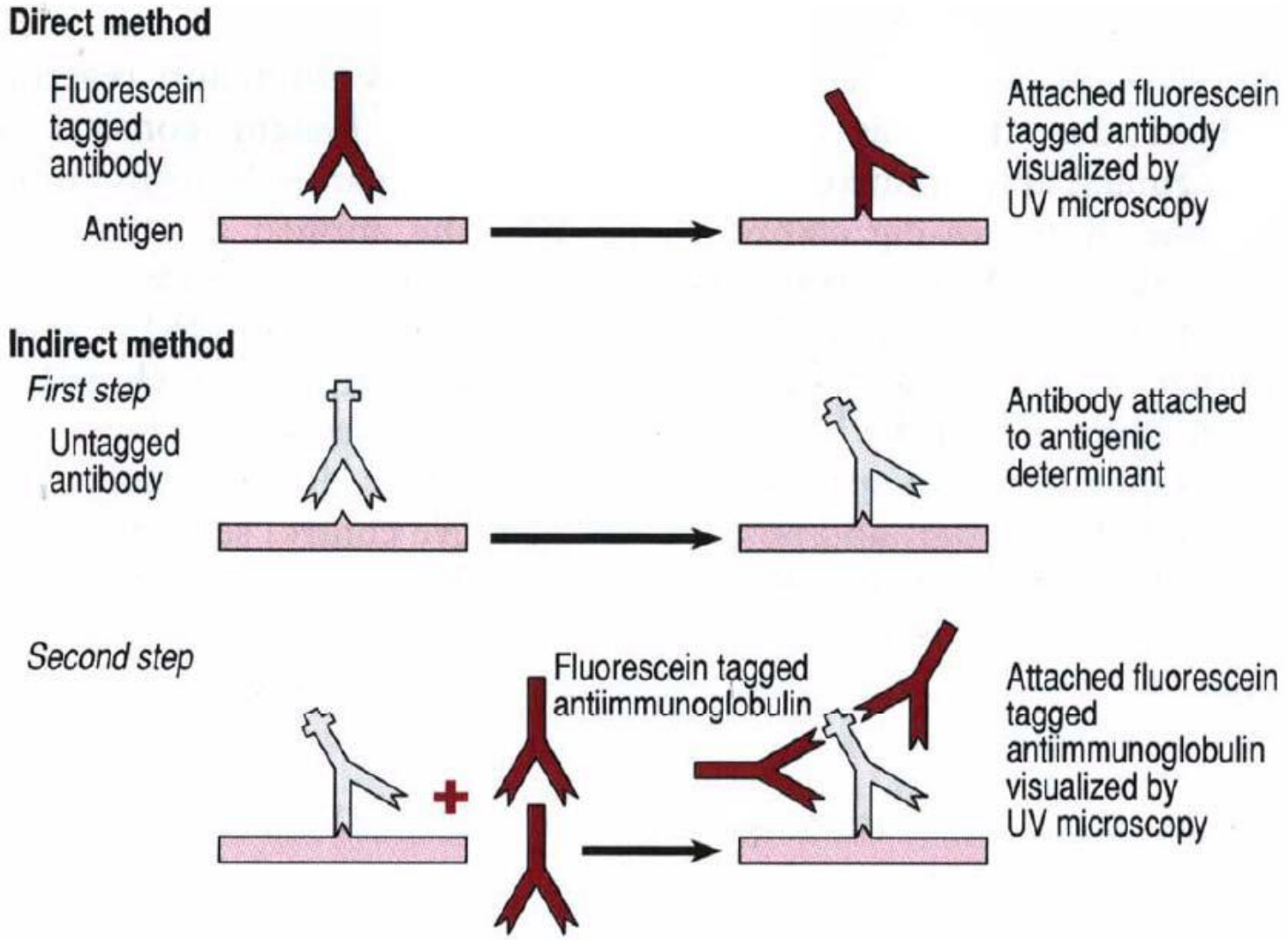
Hepatitis B virus/Dane particles



Immunofluorescence Assay (IFA)

- Use monoclonal antibodies (MoAbs) labelled with a fluorescent dye
- MoAbs bind to specific epitope on viral protein
- Visualize infected cells using fluorescent microscopy
- Only virus-infected cells will fluoresce

Immunofluorescence Assay (IFA)



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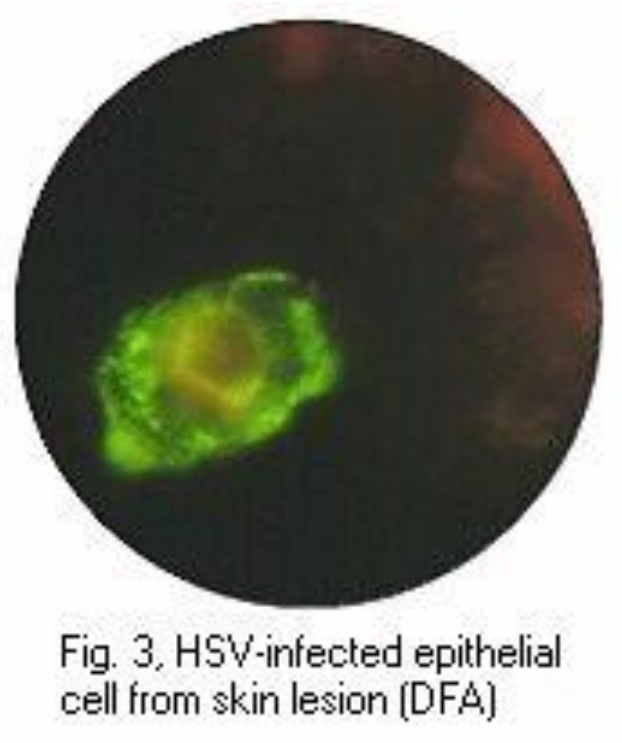


Fig. 3, HSV-infected epithelial cell from skin lesion (DFA)

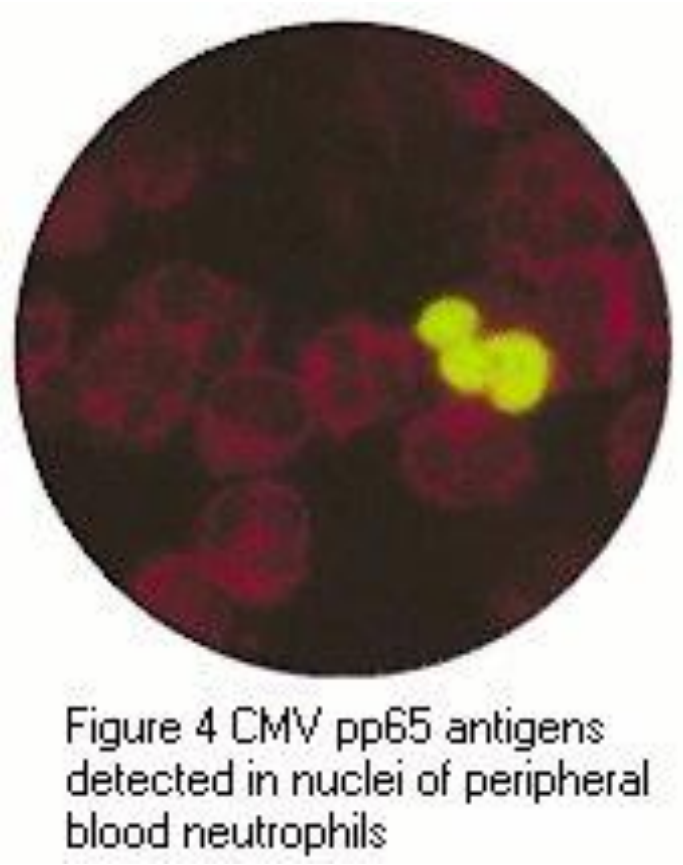


Figure 4 CMV pp65 antigens detected in nuclei of peripheral blood neutrophils

(Virology Laboratory, Yale-New Haven Hospital)

IFA test for CMV

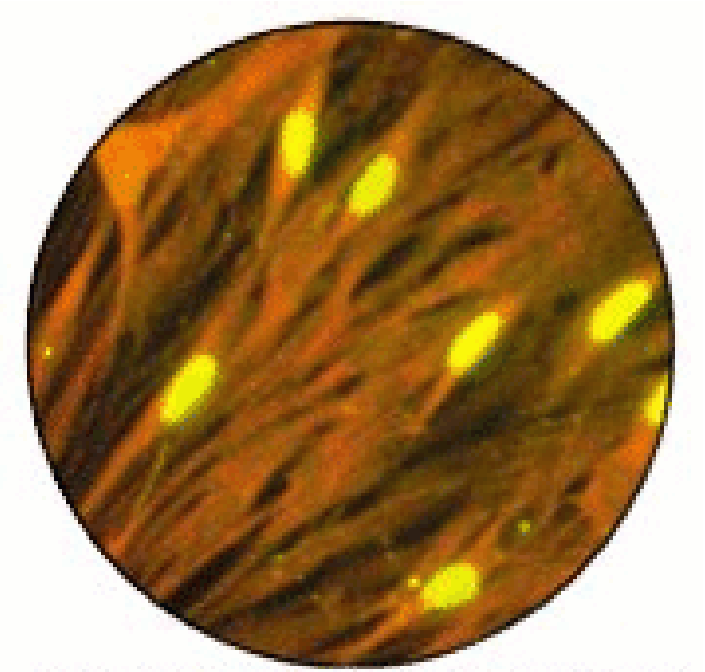


Fig. 2, CMV centrifugation culture fixed and stained 16 hrs after inoculation showing viral proteins in nuclei of infected human fibroblast cells

(Virology Laboratory, Yale-New Haven Hospital)

Advantages & Disadvantages of IFA

- Advantages
  - Result available quickly, usually within a few hours.

- Disadvantages
  - Low sensitivity (compared to cell culture)
  - Poor specificity
  - Requires good specimens.
  - Tedious/time consuming procedure
  - Expensive (lab time & equipment)

Other Detection Tests

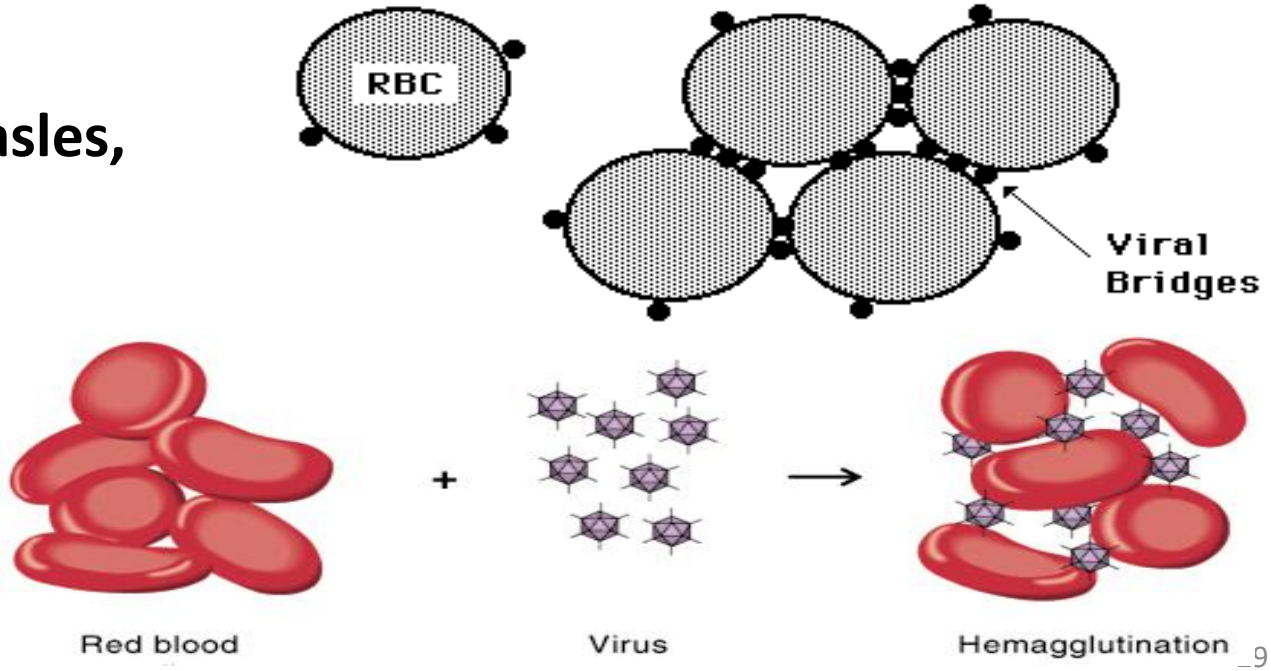
Detection of Viral Proteins

- Hemagglutination (HA) and HA Inhibition (HAI) assays
- Plaque Assays

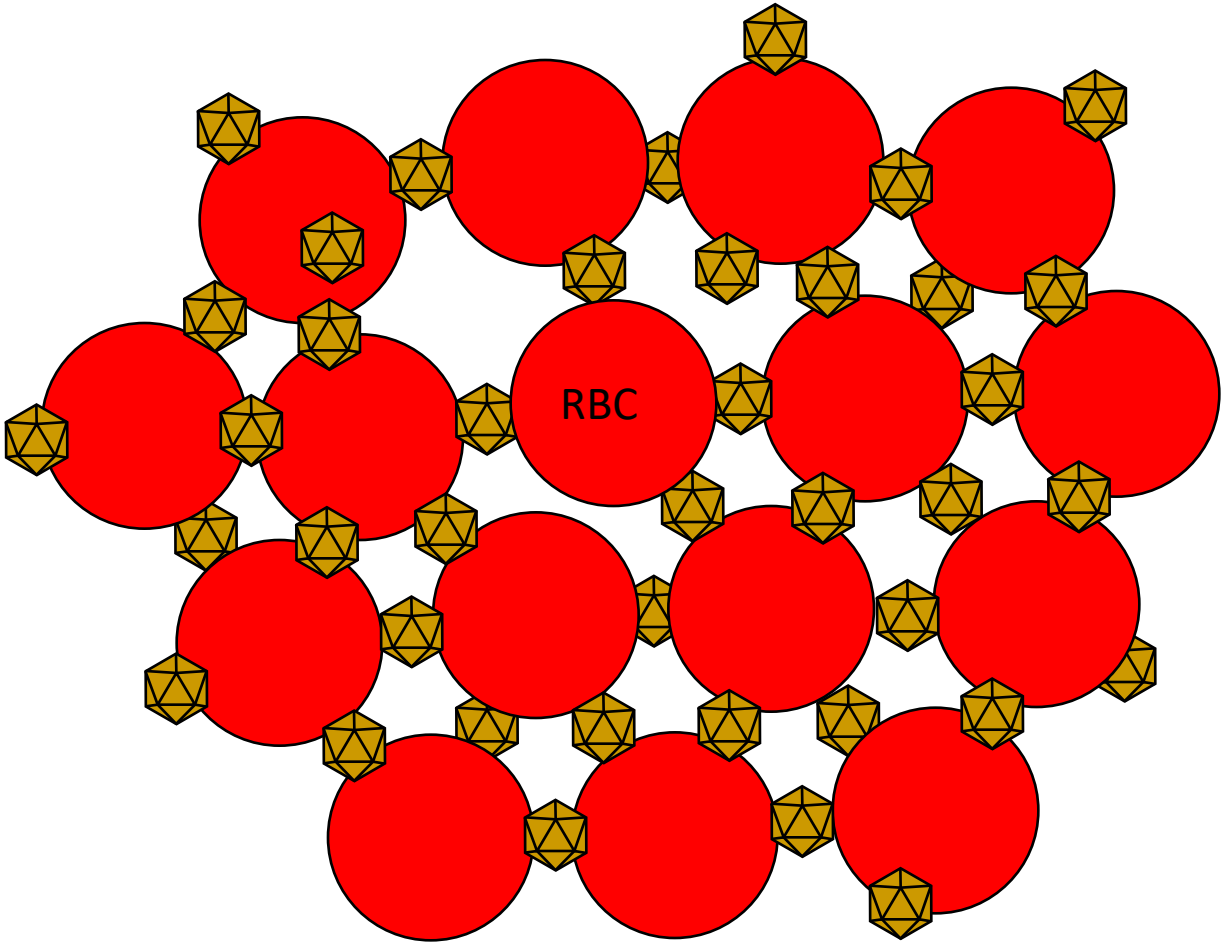
Viral Haemagglutination

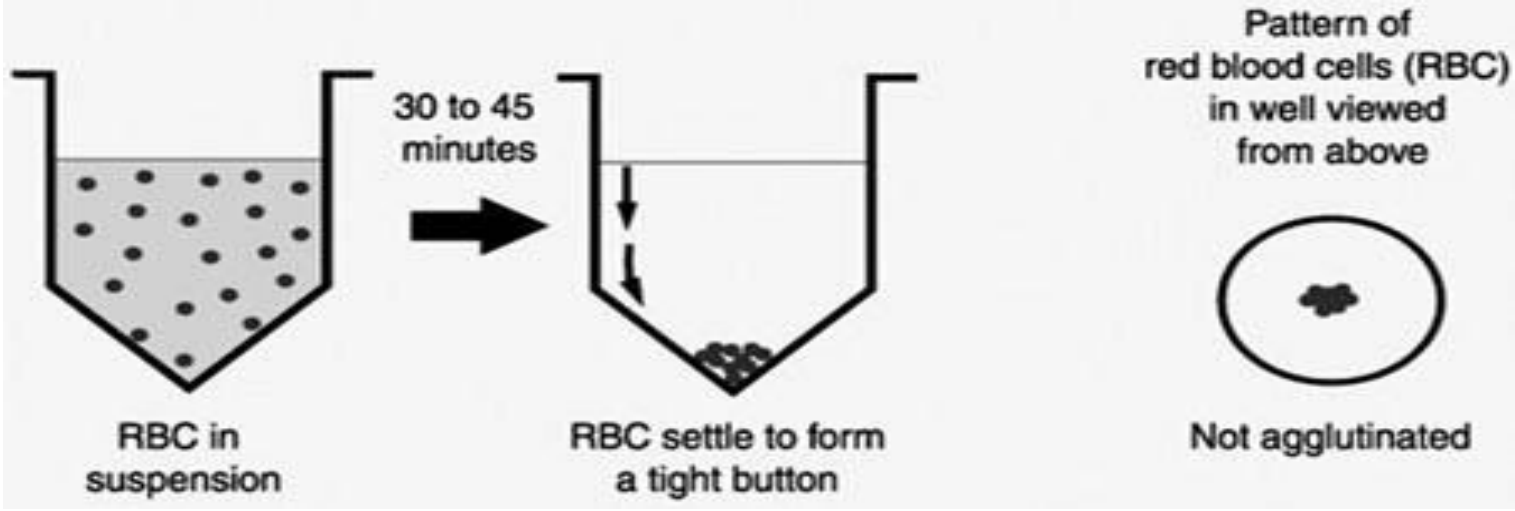
- Some viruses and microbes contain proteins which bind to erythrocytes (red blood cells) causing them to clump together

- **Paramyxoviruses**
  e.g. mumps, measles, Parainfluenza
- **Influenza virus**
- **Adenovirus**
- **Etc**

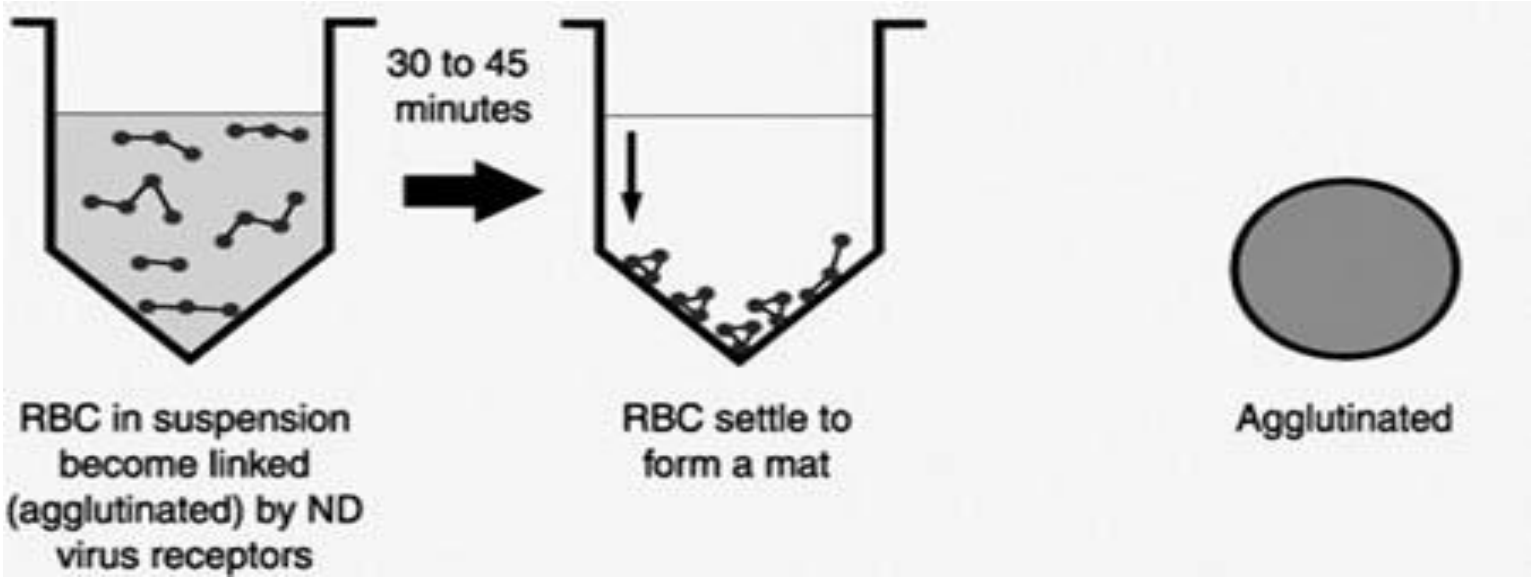


Haemagglutination

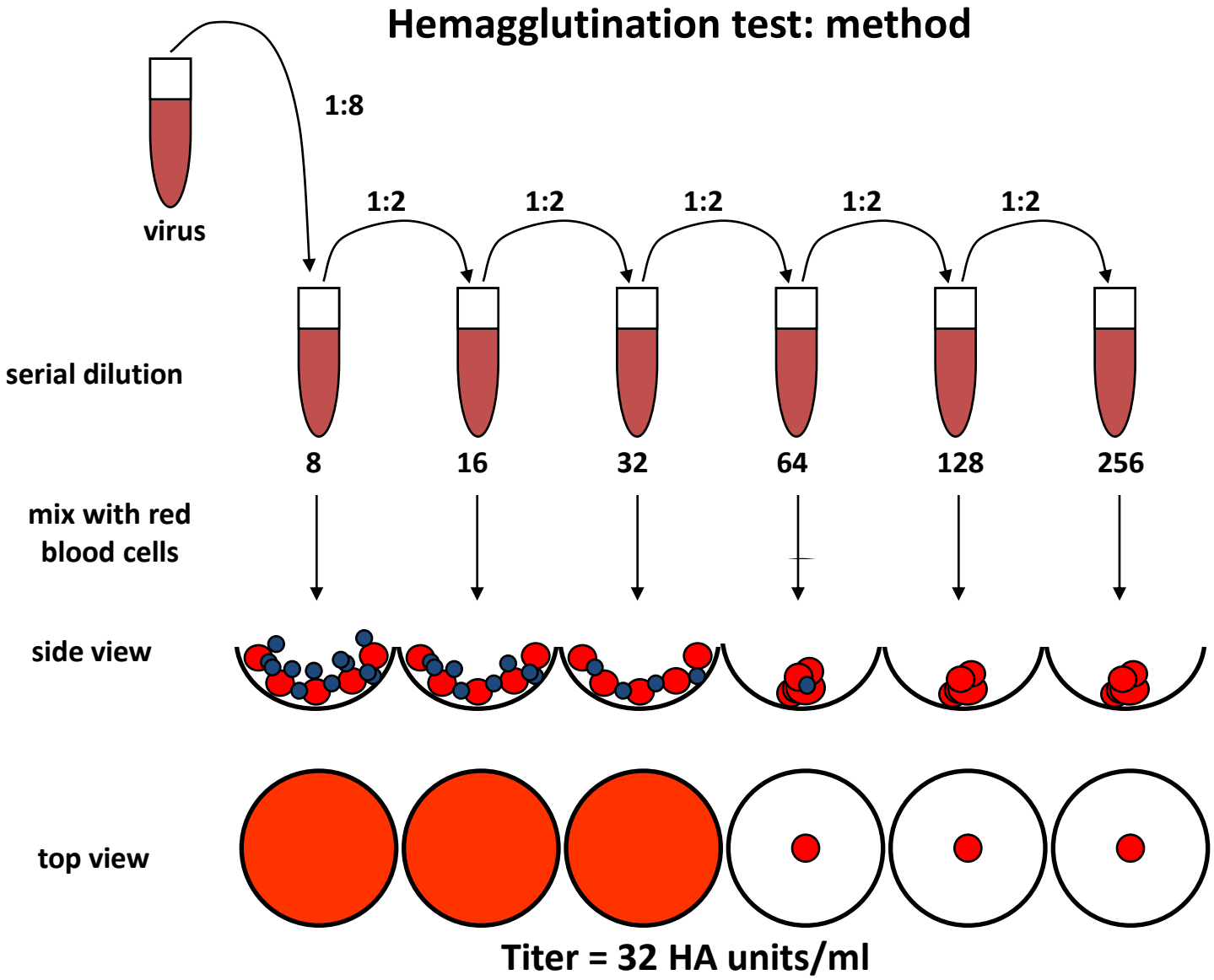




Negative control well (only RBCs+ buffer) (no haemagglutinin)



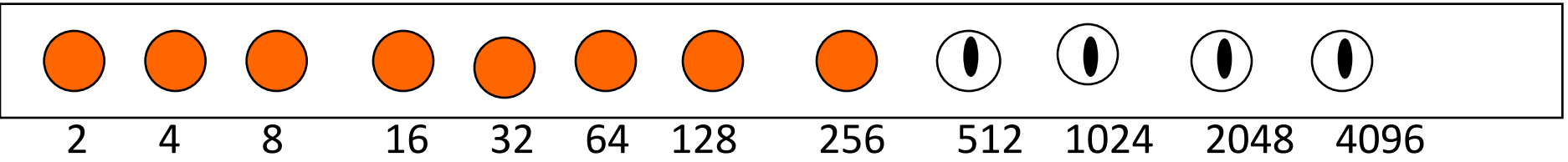
Positive control well (contains haemagglutinin)



One HA unit :minimum amount of virus that causes complete agglutination of RBCs

Readings The results

- **Titer:** The maximum dilution that gives visible agglutination.
- **The end point:** is the well with the lowest concentration of the virus where there is haemagglutination



The HA titer of this virus in this row is 256 or 2^8

(1:256 dilution contains (**1 HA unit**) (one haemagglutinating unit)

Example of readings

Patient	1/2	1/4	1/8	1/16	1/32	1/64	1/128	1/256	1/512	1/1024	Pos.	Neg.	Titer
1	●	●	●	●	●	●	◎	◎	◎	◎	●	◎	64
2	●	●	●	◎	◎	◎	◎	◎	◎	◎	●	◎	8
3	●	●	●	●	●	●	●	●	●	◎	●	◎	512
4	◎	◎	◎	◎	◎	◎	◎	◎	◎	◎	●	◎	<2
5	●	●	●	●	●	◎	◎	◎	◎	◎	●	◎	32
6	◎	◎	●	●	●	●	●	◎	◎	◎	●	◎	128
7	●	●	●	●	●	◎	◎	◎	◎	◎	●	◎	32
8	●	●	◎	◎	◎	◎	◎	◎	◎	◎	●	◎	4

Respiratory Diseases

Syndrome & Virus	Specimen	Detection System
Influenza viruses	Nasopharyngeal washings, swabs, sputum, invasively obtained specimens	Cell culture, embryonated eggs, direct FA, EIA, PCR
Parainfluenza viruses	Nasopharyngeal washings, swabs, sputum	Cell culture, direct FA, PCR
Respiratory Syncytial virus (RSV)	Nasopharyngeal washings	Cell culture, direct FA, PCR
Adenovirus	Nasopharyngeal washings, swab, feces, conjuctivial swab	Cell culture, direct FA, PCR, EIA (for enteric Ad 40/41)
Rhinovirus	Nasopharyngeal washings	Cell culture, direct FA, PCR

Encephalitis & Meningitis

Syndrome & Virus	Specimen	Detection System
Arboviruses	Serum CSF Nasopharyngeal swab	Cell culture Suckling mice
Enteroviruses	Feces Throat swab CSF	Cell culture PCR
Rabies virus	Saliva Brain biopsy	Direct FA Suckling mice
Herpesvirus	CSF	PCR
Mumps	CSF Nasopharyngeal swab Urine	Cell culture

Febrile Diseases

Syndrome & Virus	Specimen	Detection System
Dengue , other arboviruses	Serum, CSF, autopsy specimens, vector (mosquitoes & ticks)	Cell culture Suckling mice