Chapter 33

Host defenses

Host defenses against viruses may be:

1. **non-specific** (e.g., interferon and natural killer cells)

2. **specific** (humoral and cell mediated immunity).

Non specific defenses

Interferons

-- Interferons are a group of **glycoproteins** produced by human cells after viral infections.

-- They inhibit viral growth by **blocking translation of viral proteins**.

-- There are three types:

alpha (leucocytes), **beta** (fibroblast) and **gamma** (lymphocyte) interferons.

-- First two are induced by viruses, last by antigens.

Induction of alpha and beta interferons

-- The strong inducers are **viruses** and **double stranded RNA**.

-- Induction by viruses is **not specific** but differ in effectiveness.

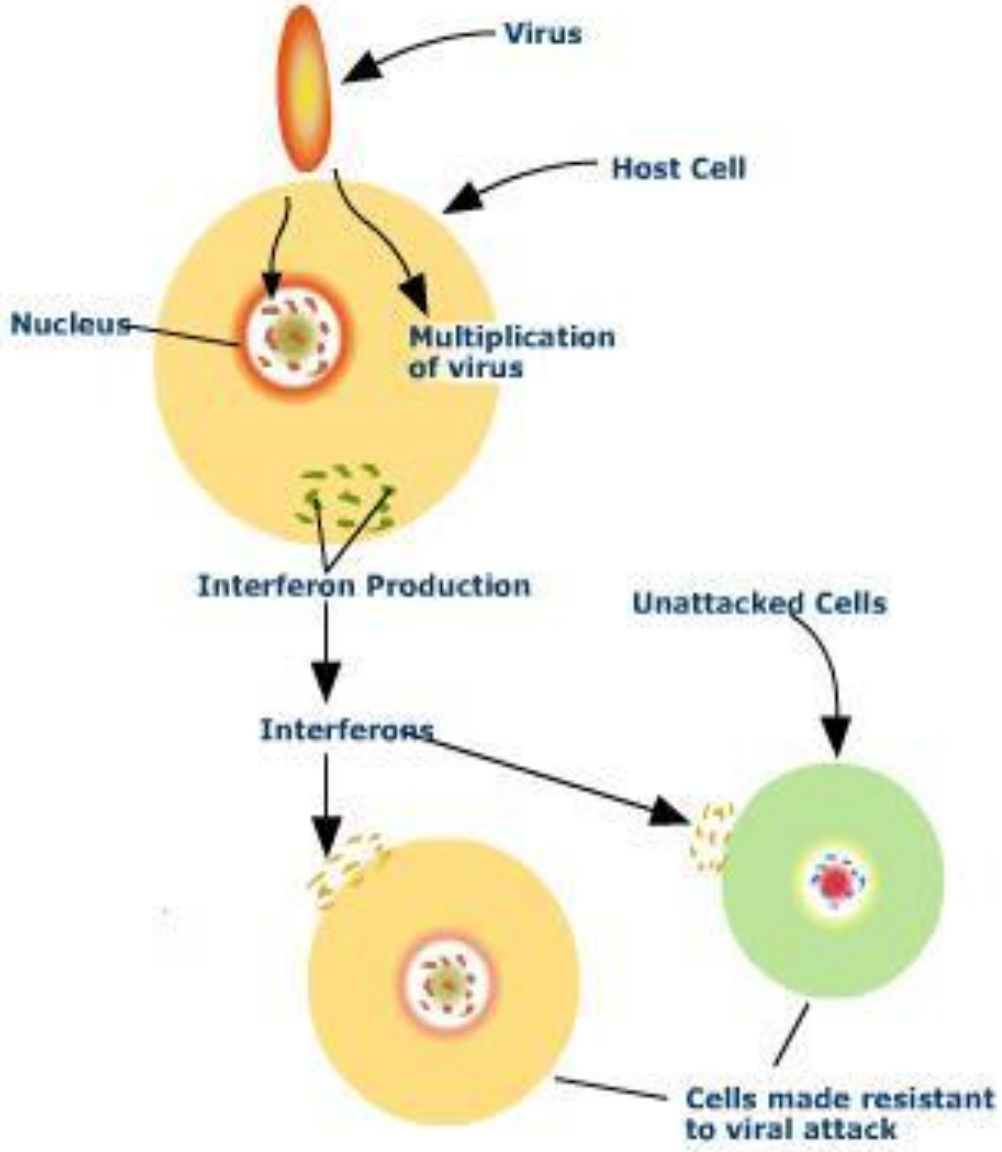
-- Double stranded RNA is a strong inducer and it is observed that **double stranded RNA** is a **part of replicative cycle of all inducing viruses.**

-- **Intracellular bacteria** and **protozoa** can also **induce interferon** production but at a **much weaker level**.

-- Inhibitory action of interferons is not specific for a particular virus but are **typically specific in regard to host species** in which they act.

-- **So interferon produced in other animals is not useful in humans.**

-- **Recombinant interferon** based on human genes is now generally available.


Virus
Host Cell
Nucleus
Multiplication of virus
Interferon Production
Unattacked Cells
Interferons
Cells made resistant to viral attack

Action of alpha and beta interferon

Interferons generally **do not effect normal cells** because uninfected cells do not possess double stranded RNA.

Inteferons induce synthesis of **three cell encoded proteins** that **inhibit the translation of viral mRNA:**

1. An **oligonucleotide synthetase** that synthesize an **adenine trinucleotide**.
2. A **ribonuclease** that is activated by adenine trinucleotide. It degrades viral mRNAs
3. A **protein kinase** that phosphorylates an initiation factor for protein synthesis (**eIF-2**), thereby inactivating it.

-- Interferons have **no direct effect on extracellular virus particles**.

-- Interferons **do not enter the cell** but act by **binding to a receptor on the cell surface** that signals the cell to synthesize the ribonuclease and other antiviral proteins.

-- Interferons are produced **within a few hours** of the initiation of viral replication, so they act in the early phase of viral diseases to limit spread of virus.

-- In contrast **antibody** appears several days after infection.

-- **Alpha interferon** is used in patients with **condyloma acuminatum** and chronic hepatitis caused by **hepatitis B and C viruses**.

-- **Gamma interferon** reduces **recurrent infections** in patients with **chronic granulomatous disease**.

-- Interferons are also used for patients of **Kaposi's sarcoma** and **hairy cell leukemia.**

Natural killer cells

-- NK cells are important part of the **innate defenses** against virus infected cells.

-- They are called **natural** because they **nonspecifically kill infected cells** and **do not need activation by a virus**.

-- They are **T lymphocytes** but do not have an antigen receptor.

-- They kill by secreting **perforins** and **granzymes** which cause **apoptosis**.

Phagocytosis

-- **Fixed** and **alveolar macrophages** are the important cell types in limiting virus infection.

-- In bacterial infections, **PMN cells** are more important.

A-defensins

-- These are a family of **positively charged peptides** with antiviral activity.

-- They **block entry of HIV** into the cell.

Apolipoprotein B RNA-editing enzyme (APOBEC3G)

-- It is an important member of innate host defenses against **retroviral infections**, esp. **HIV**.

-- It causes a **hypermutation** by **deaminating cytosines** in both mRNA and retroviral DNA, thereby inactivating these molecules and reducing infectivity.

-- **HIV** defends itself by producing **Vif** (virus infectivity protein).

Fever

-- High temperature may **directly inactivate viruses** by effecting **enveloped viruses**.

-- It also **retards the growth** of viruses.

Mucociliary clearance

-- Mucociliary clearance mechanism of the respiratory tract may protect the host.

-- Its damage results in increase frequency of viral infections, e.g., influenza.

Circumcision

-- There is evidence that circumcision prevents infection by three sexually transmitted viruses, **HIV, HPV** and **HSV 2**.

Factors that modify host defenses

-- **Age is significant**. Viral infections are more severe in neonates and in elderly

-- **Increased corticosteroid levels** predispose to more severe infections with some viruses, e.g., **varicella zoster**.

-- **Corticosteroids** cause **lysis of lymphocytes**, **decreased recruitment of monocytes, inhibition of interferon production,** and **stabilization of lysosomes.**

-- **Malnutrition** leads to more severe viral infections.

-- Poor nutrition causes **decreased Ig production and phagocyte activity** as well as reduced skin and mucus membrane integrity.

I am careful not to confuse excellence with perfection.
Excellence, I can reach for;
perfection is God's business.

Michael J. Fox

Chapter 34

Laboratory Diagnosis

There are **five** approaches to the diagnosis of viral diseases by use of clinical samples:

1. Identification of virus in **cell culture**
2. **Microscopic identification** directly in the specimen
3. **Serological procedures** to detect a **rise in antibody titer** or the presence of IgM antibodies
4. **Detection of viral antigens** in blood or body fluids
5. **Detection of nucleic acids** in blood or patient's cells

Identification in cell culture

-- Because the viruses **replicate only within cells**, so cell culture is required.

-- Many viruses are inactivated at room temperature, so it is important to inoculate the specimen as soon as possible.

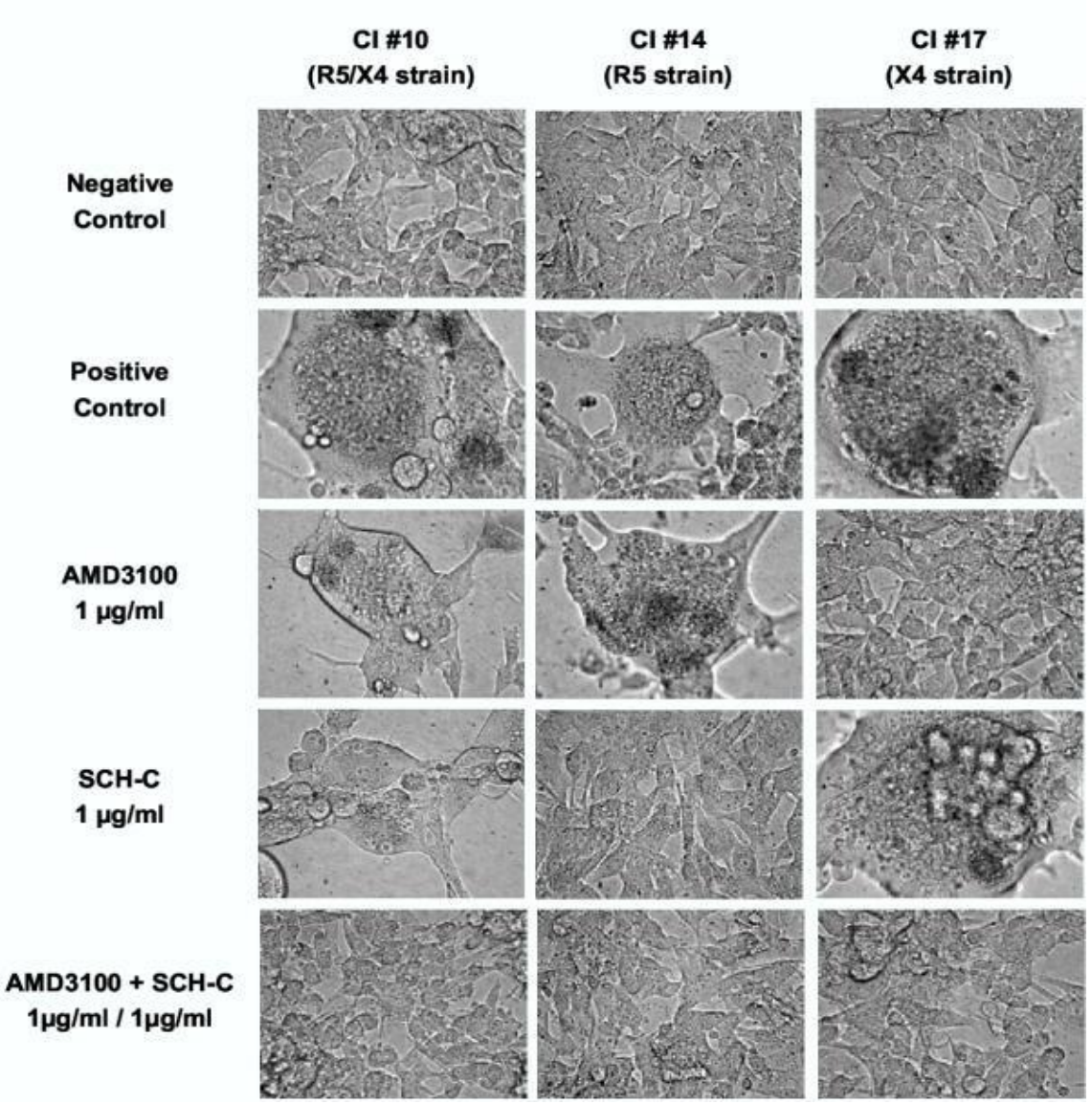
-- Brief transport period or storage at 4°C is acceptable.

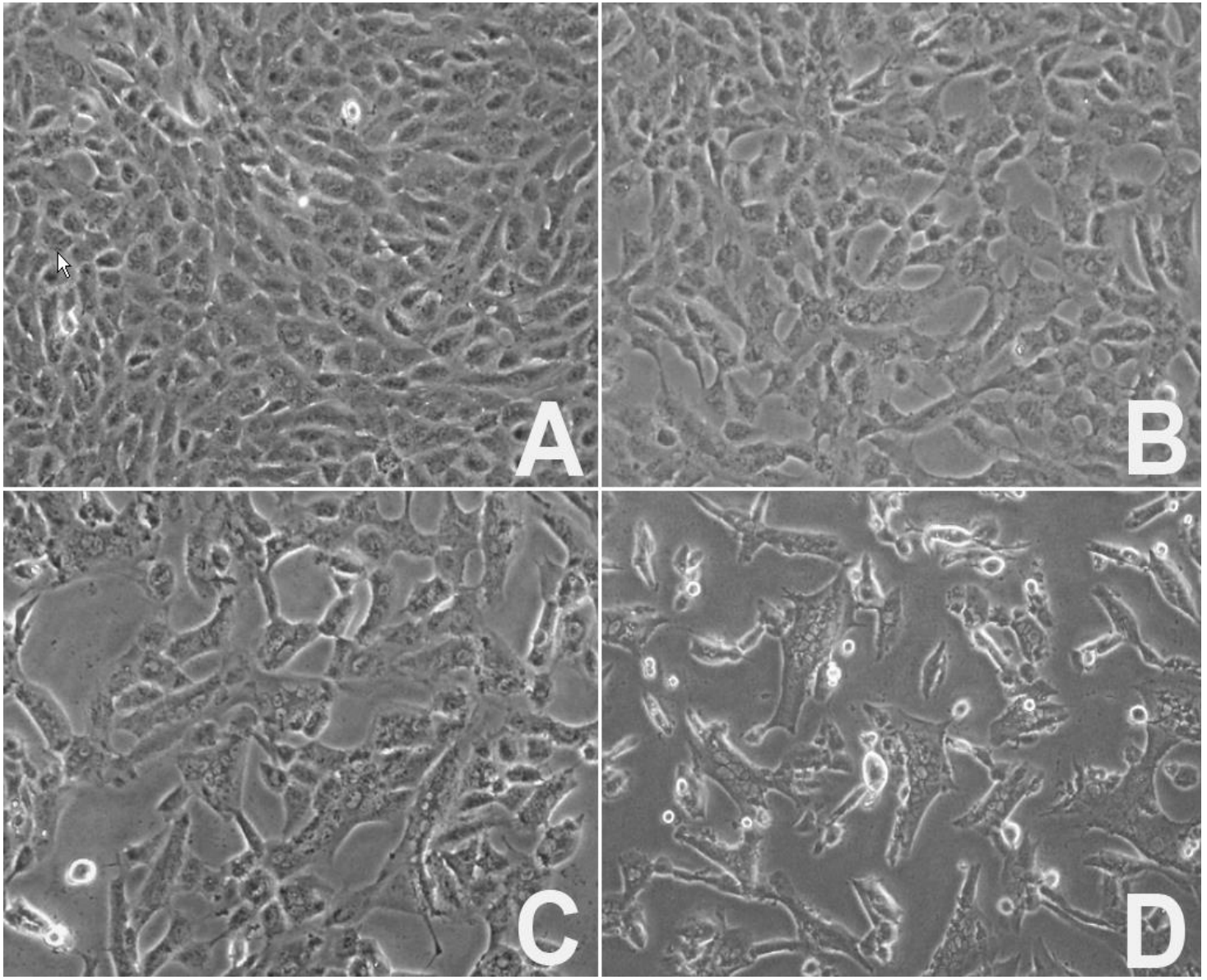
-- CPE can help in **presumptive identification.**

-- CPE can be **change in size**, **shape or formation of multinucleated giant cells (syncytia).**

-- CPE are also seen in **dying or dead cells**.

-- The **time taken** for CPE to appear and **type of cell** in which virus produce CPE are important clues.


CI #10
(R5/X4 strain)
CI #14
(R5 strain)
CI #17
(X4 strain)
Negative Control
Positive Control
AMD3100 1 µg/ml
SCH-C 1 µg/ml
AMD3100 + SCH-C 1µg/ml / 1µg/ml


A
B
C
D

If a virus **does not produce a CPE**, its presence can be detected by several other techniques:

1. **Hemadsorption:** It is attachment of erythrocytes to surface of virus infected cells.

   This technique is limited to viruses with a hemagglutinin protein on their envelope such as **mumps, parainfluenza**, and **influenza viruses**.

2. **Interference with the formation of a CPE by a second virus:**

   For example, **rubella virus,** which does not cause a CPE, can be detected by interference with the formation of a CPE by certain **enteroviruses**, such as **echovirus** and **Coxsackie virus**.

3. A **decrease in acid production** by infected, dying cells.

-- This can be detected by an indicator such as **phenol red** in culture medium.

-- It **remains red** in case of infected cells but **turns to yellow** in the presence of metabolizing normal cells as a result of the acid produced.

-- This technique is useful for detecting some **enteroviruses**.

-- A **definitive identification** of the virus grown in cell culture is made by using **known antibody** in one of several tests.

-- **Complement fixation**, **haemagglutination inhibition**, and **neutralization of CPE** are the most frequently used tests.

-- **Fluorescent antibody**, **radioimmunoassay, ELISA** and **immunoelectron microscopy** are also used in special instances.

Complement fixation

-- If the antigen (viral) and known antibody are **homologous**, **complement will be fixed** (bound) to the antigen-antibody complex.

-- This makes complement **unavailable to lyse** the “indicator” system, which is composed of sensitized red blood cells.

Immunoelectron microscopy

-- If antigen and antibody are **homologous**, **aggregates are seen** in electron microscope.

Haemagglutination inhibition

-- If the virus and antibody are **homologous**, the **virus is blocked** from attaching to the erythrocytes and **no haemagglutination** occurs.

-- Only viruses that agglutinate red blood cells can be identified by this method.

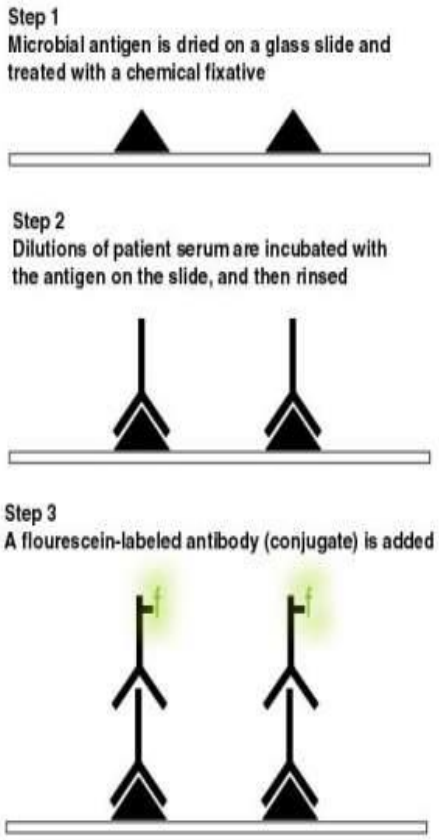
Neutralization

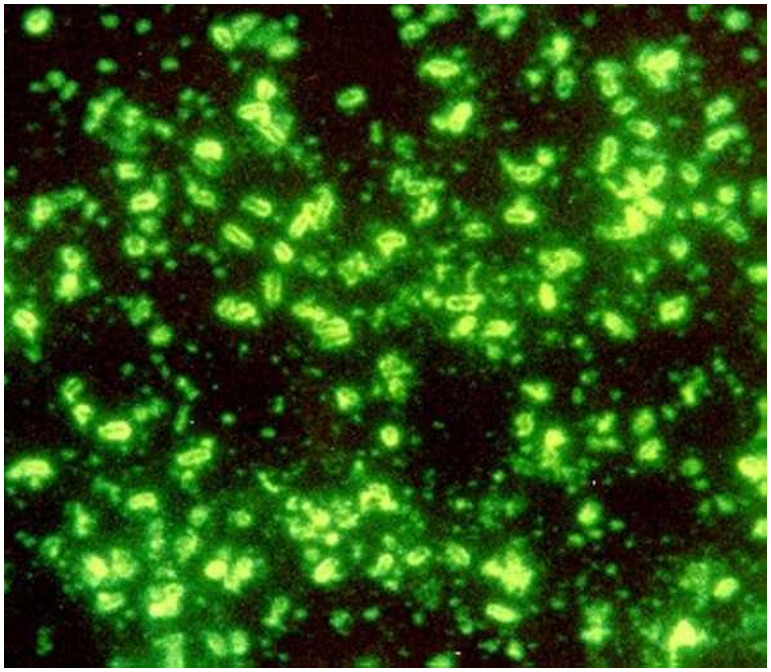
-- If the virus and antibody are **homologous**, the antibody bound to the surface of the virus **blocks its entry into the cell**.

-- This **neutralizes viral infectivity** because it prevents viral replication and subsequent CPE formation or animal infection.

Fluorescent antibody assay

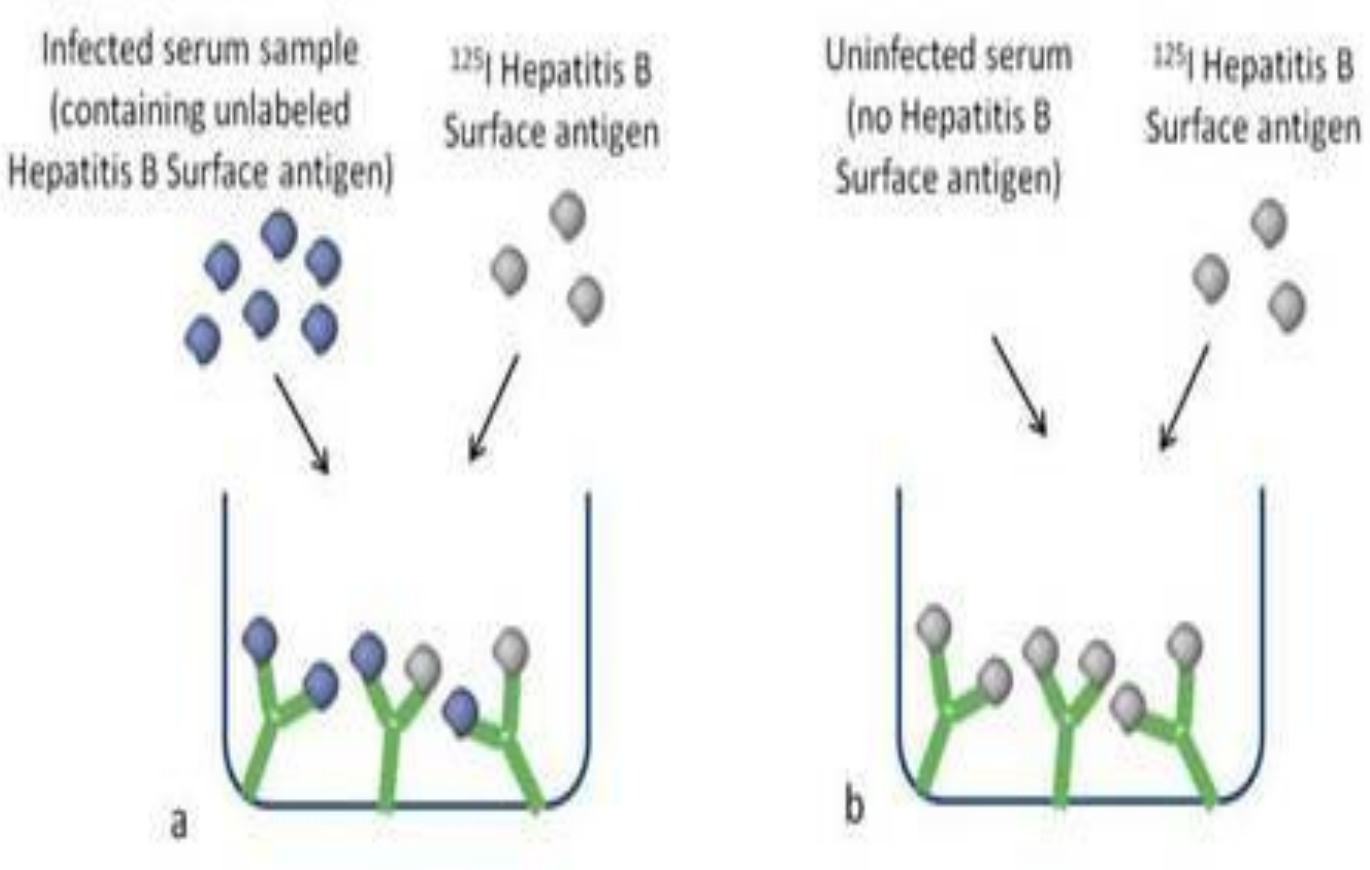
If the virus infected cells and the fluorescein –tagged antibody are **homologous**, the **typical apple-green color of fluorescein** is seen in the cells by **UV microscopy**.





Radioimmunoassay

If the virus and antibody are **homologous**, there is **less antibody remaining to bind** to the known radiolabeled virus.

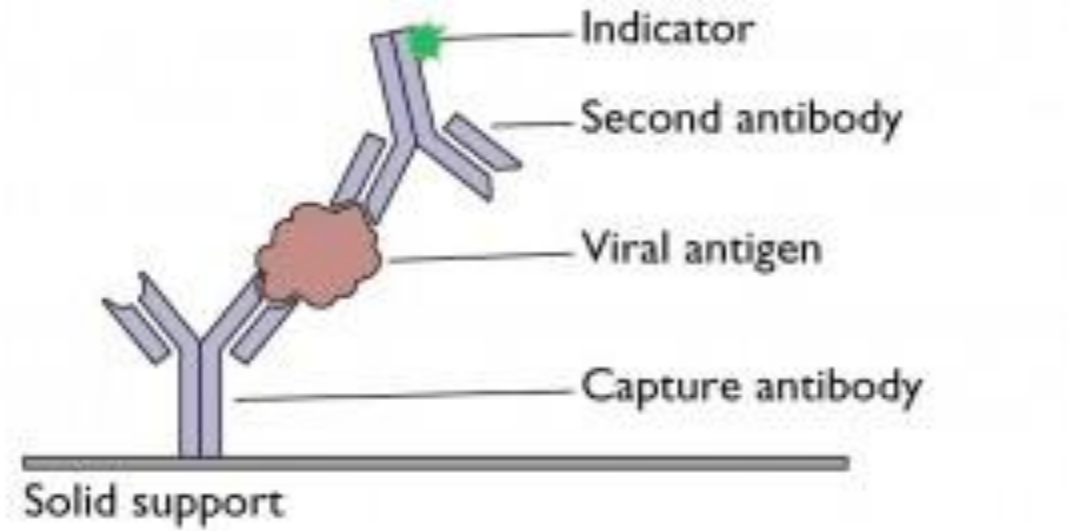


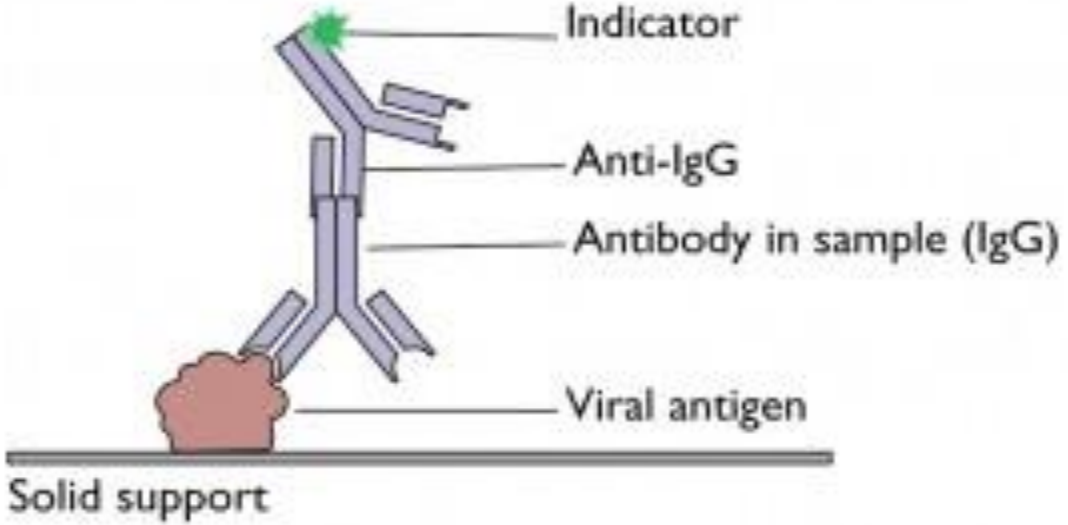
ELISA

-- In the ELISA test to identify a virus, **known antibody is bound to a surface.**

-- **If a virus is present, it will bind to antibody** which is **linked to an enzyme.**

-- If **antigen-antibody complex is made**, the substrate for enzyme which is added later will **change color**.


Indicator
Second antibody
Viral antigen
Capture antibody
Solid support

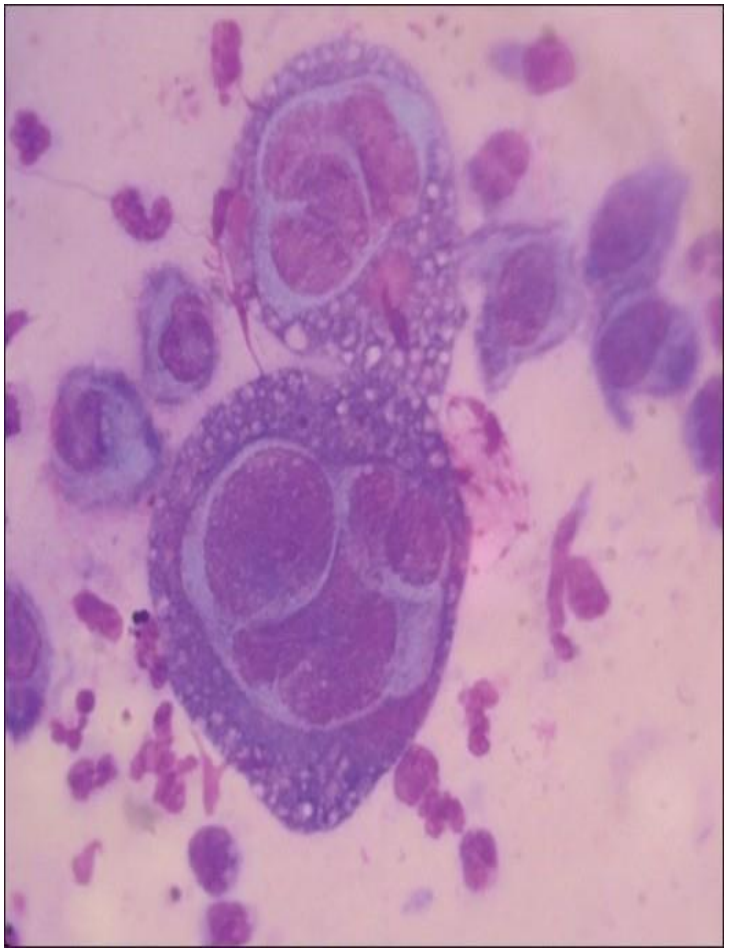

Indicator
Anti-IgG
Antibody in sample (IgG)
Viral antigen
Solid support

Microscopic identification

In some cases, viruses can be detected and identified by **direct microscopy** of **biopsy material** or **skin lesions**.

Three different procedures can be used:

1. **Light microscopy** can reveal characteristic **inclusion bodies** or **multinucleated giant cells**. Tzanck smear which shows herpesvirus induced multinucleated giant cells in vesicular skin lesions is a good example.



2. **UV microscopy** is used for **fluorescent antibody staining of the virus** in infected cells.

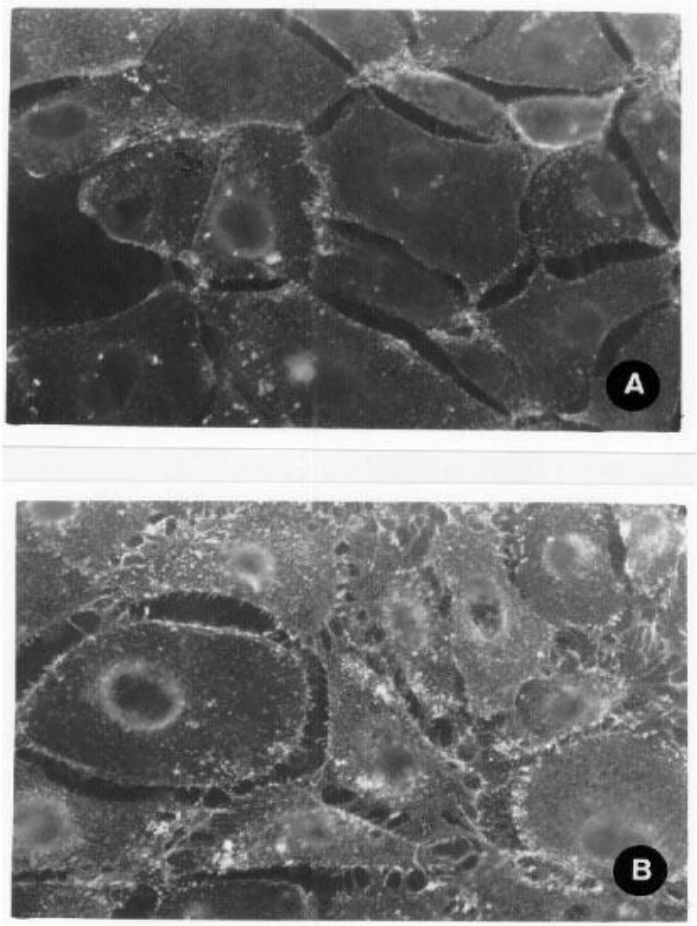
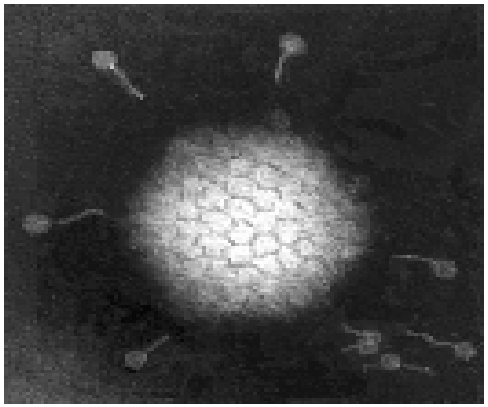
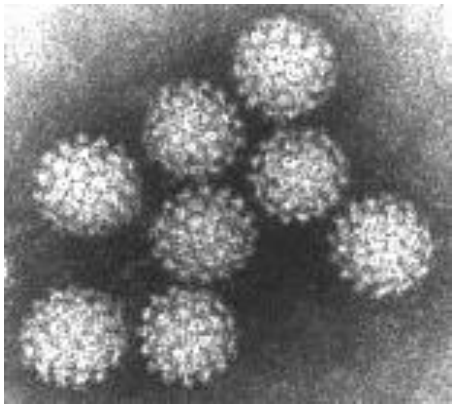


Figure 2 - Fluorescence assay of MA-104 cell cultures incubated with ConA-FITC for 15 min at 4°C (**A**) showing labeling mainly at the cell surface and at 37°C also in the cytoplasm (**B**). 200X.

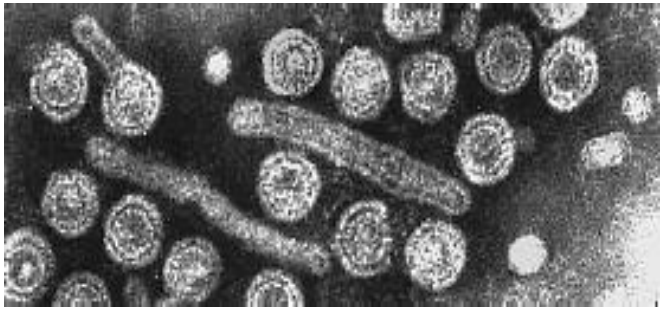
3. **Electron microscopy** detects virus particles which can be characterized by their **size** and **morphology**



Adeno



Papillo



HBV

Serological procedures

-- A **rise in the titer** to the virus can be used to **diagnose current infection**.

-- **Seroconversion** is the term used to describe the finding of antibody to a virus (or any microbe) in a patient's serum when the patient previously had no antibody.

-- A serum sample is obtained in **acute phase** and a second sample is taken in **convalescent phase** after 10- 14 days.

-- If there is **four-fold or higher rise** in titer, patient is considered to be recently infected with that organism.

-- Result of **single sample cannot distinguish** between a previous and current infection.

-- In certain viral infections, the presence of **IgM antibody** is used to diagnose **current infection**.

For example the presence of IgM antibody to core antigen indicates infection by hepatitis B virus.

-- **Heterophile antibody test** (**Monospot**) is a nonspecific test that can be used to detect certain infections, e.g., infectious mononucleosis.

In this, serum is reacted with horse or sheep red blood cells.

If **heterophile antibody** is present (patient is infected with **Epstein Barr virus**), agglutination of RBC occurs.

Detection of viral antigens

-- These are usually detected by **ELISA**.

-- Most common use is to detect **surface antigen of Hepatitis B**.

Detection of viral nucleic acid

-- **Viral nucleic acids** (viral genome or mRNA) can be detected in patient's blood or tissues with **complementary DNA or RNA** (cDNA or cRNA) as a probe.

-- If amount of nucleic acid is very small , **PCR** is used.

-- **Real time PCR** can be used to assay **viral load** during treatment.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Thank You!