

(Foundation Block, Microbiology: 2018)

#### By: Dr.Malak El-Hazmi

Associate professor Consultant Virologist College of Medicine &

## **OBJECTIVES**

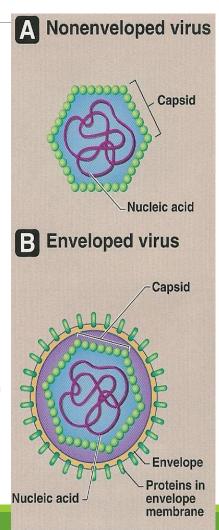
- > Distinguish the viruses from other microorganisms
- General characteristics of viruses.
- > Structure & symmetry of viruses.
- > Classification of viruses.
- > Steps of virus replication .
- > laboratory diagnosis of viral infections.

## **Properties of Microorganisms**

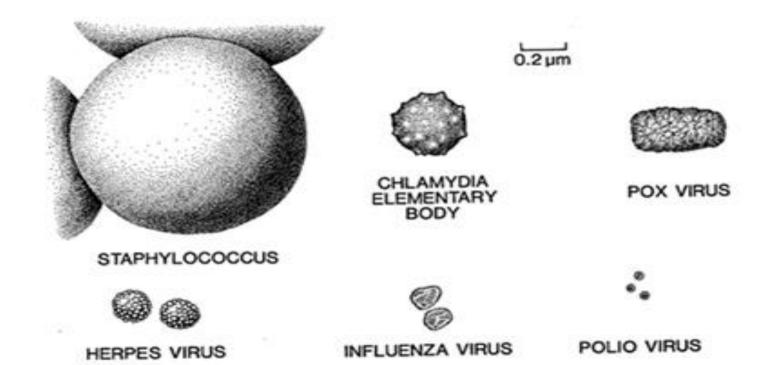
| characteristic  | <b>Parasites</b>     | Fungi                | Bacteria          | Viruses       |
|-----------------|----------------------|----------------------|-------------------|---------------|
| Cell            | Yes                  | Yes                  | Yes               | No            |
| Type of nucleus | Eukaryotic           | Eukaryotic           | Prokaryotic       |               |
| Nucleic acid    | Both<br>DNA<br>& RNA | Both<br>DNA<br>& RNA | Both DNA<br>& RNA | DNA or<br>RNA |
| Ribosomes       | Present              | Present              | Present           | Absent        |
| Mitochondria    | Present              | Present              | Absent            | Absent        |
| Replication     | Mitosis              | Budding or mitosis   | Binary fission    | special       |

#### **Characteristics of viruses**

- Acellular organisms
- ➤ Tiny particles
  - Internal core
  - Protein coat
  - Some Vs have lipoprotein mb
- Obligate intracellular organisms
- Replicate in a manner diff from cells (1V many Vs)



## Size; 20-300 nm

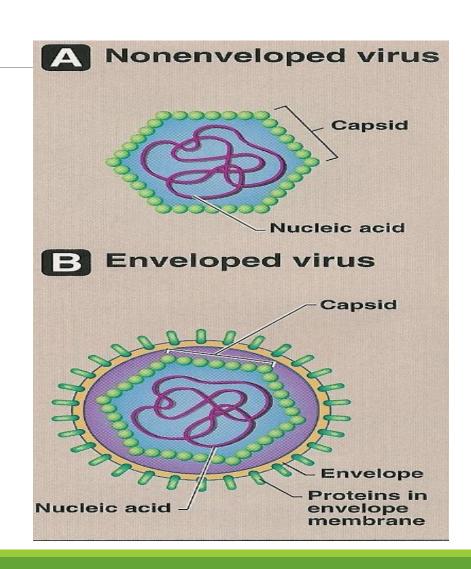


#### Viral Structure

1-Viral genome

2-Capsid

3-Envelope



# Viral Structure

#### 1-Viral genome

#### DNA

(Deoxyribonucleic acid)

- All DNA Vs have ds except Parvoviruses
- Single molecule

## or

#### RNA

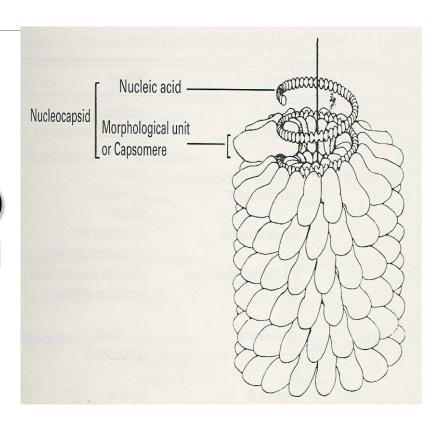
(Ribonucleic acid)

- All RNA Vs have ss except Reoviruses
- single / multiple
- (+) polarity
- (-) polarity

#### Viral structure

## 2-Capsid

- a protein coat
- Subunits (capsomeres)
- Genome (NA) + capsid
  = nucleocapsid
- Function;
  - Protects NA
  - Facilitates its entry into cell



# **Symmetry**

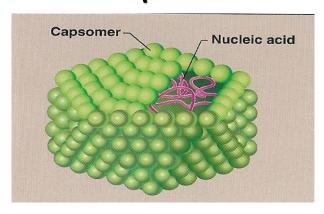
#### based on arrangement of capsomeres

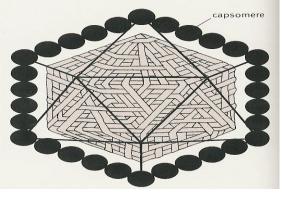
- Cubic symmetry (Icosahederal)
- Helical symmetry
- Complex symmetry

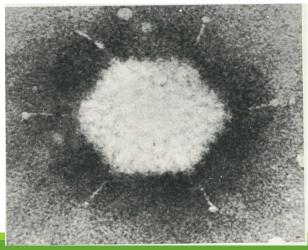
# **Symmetry**

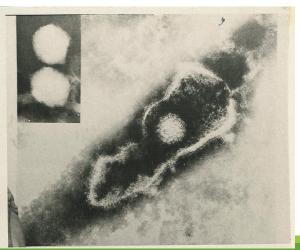
#### based on arrangement of capsomeres

#### 1-Cubic symmetry (Icosahedral)









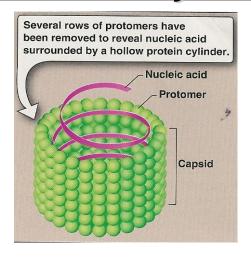
Adenovirus

Herpesvirus

# **Symmetry**

#### based on arrangement of capsomeres

#### > 2- Helical symmetry



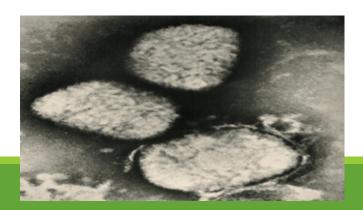




Elongated (filoviruses)

Pleomorphic (influenza v.)

# > 3- Complex symmetry poxviruses



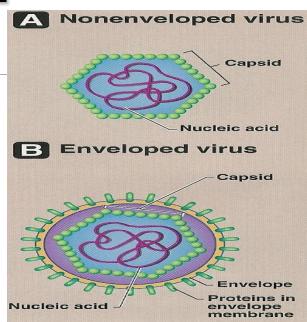
# Viral structure

#### 3-Envelope

Lipoprotein mb (host lipid ,virus specific protein )

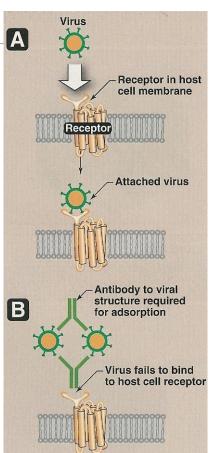
## Budding

- Envelope is derived from cell mb
   except herpesviruses from nuclear mb
- Enveloped Vs are more sensitive to heat ,dry & ether than nonenveloped Vs
- Glycoprotein attaches to host cell receptor



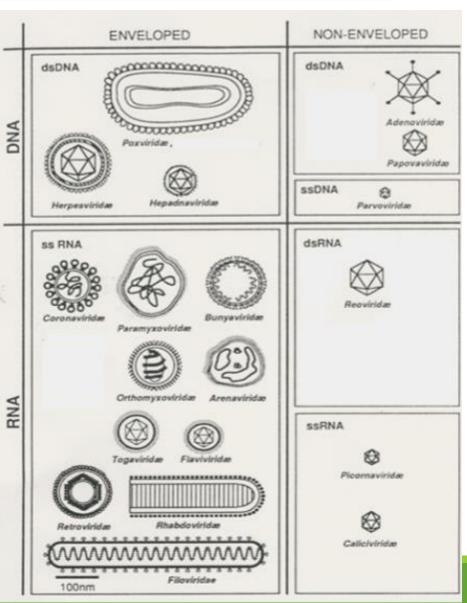
# Viral proteins

- The outer viral ps
- Mediate attachment to specific Rs
- Induce neutralizing Abs
- Target of Abs
- The internal viral ps
- Structural ps ( capsid ps of enveloped Vs )
- Nonstructural ps (enzymes)
  - All ssRNA Vs (-) polarity have transcriptase
     (RNA dependent RNA polymerase) inside virions
  - RetroVs & HBV contain reverse transcriptase

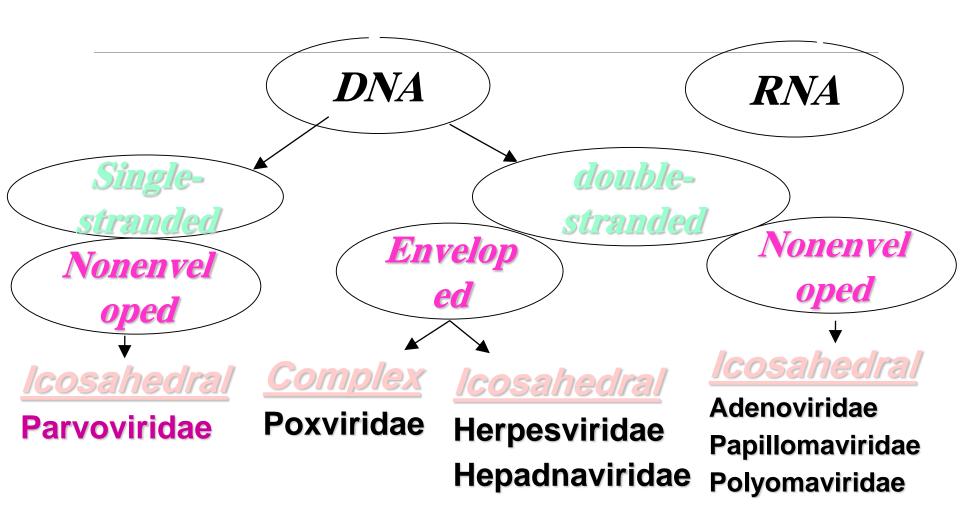


## Classification of viruses

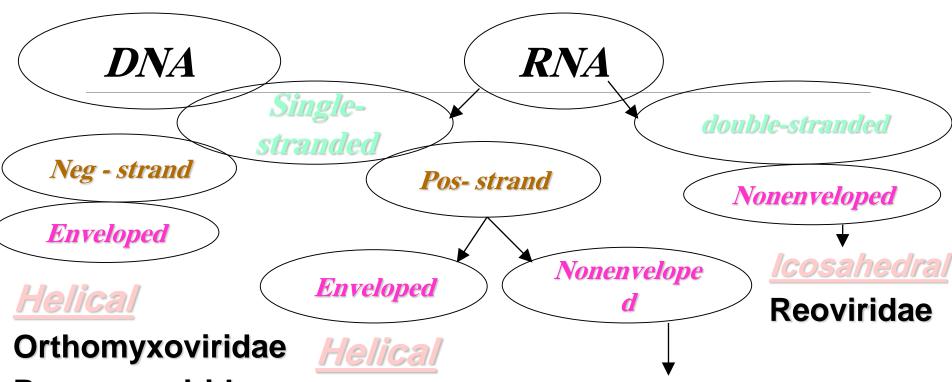
- ➤ Type of NA\*
- ➤ The no. of strand
- The polarity of viral genome
- ➤ The presence or absence of envelor ₹
- Type of symmetry



## Medically Important Viruses



## Medically Important Viruses



Orthomyxoviridae
Paramyxoviridae
Rhabdoviridae
Filoviridae
Bunyaviridae
Arenaviridae

Coronaviridae

Icosahedral

Togaviridae Flaviviridae

Retroviridae

Icosahedral

Picornaviridae

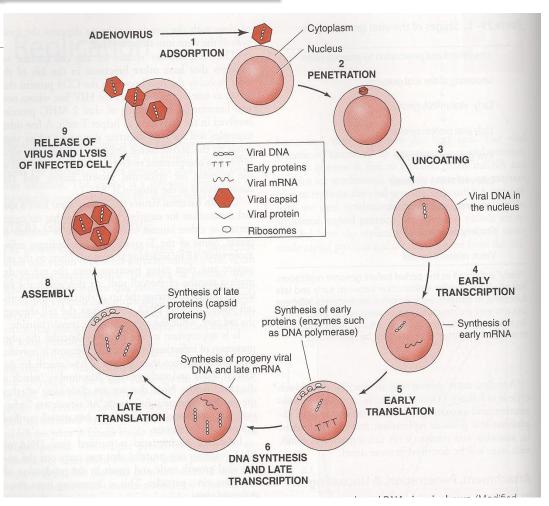
Hepeviridae

Caliciviridae

**Astroviridae** 

# Replication

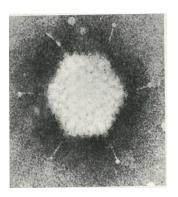
- Adsorption (Attachment)
- Penetration
- Uncoating
- Synthesis of viral components
  - mRNA
  - Viral proteins
  - NA
- Assembly
- Release

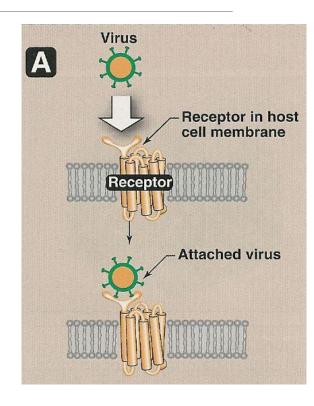


Viral growth cycle

# Adsorption

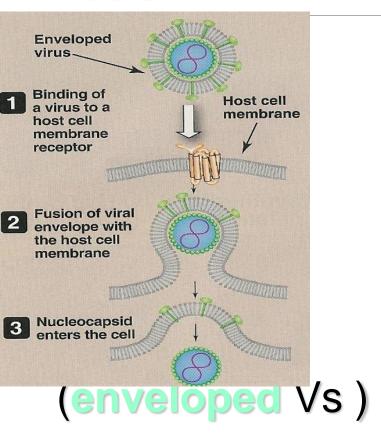
Attachment site; ex- glycoprotein fiber



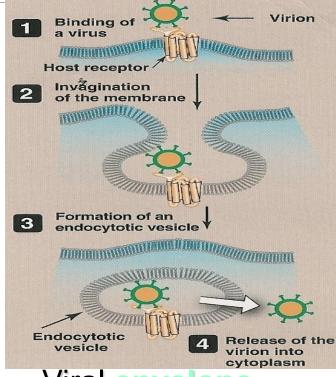


# Penetration

#### 1-Fusion



#### 2-Endocytosis



- Viral envelope fuses with endosome mb
- Nonenveloped \( \subseteq \)
  lysis ,pore

# Replication

- Adsorption (Attachment)
- > Penetration
- Uncoating

Release of viral genome - cytoplasm

- nucleus

## Synthesis of viral components

> mRNA

```
Viral genome transcription mRNA +ssRNA acts directly
```

Viral proteins

mRNA translation viral proteins cell ribosome - enzymes

- structural ps

replication of viral genome

# Replication

- Adsorption (Attachement)
- Penetration
- Uncoating
- Synthesis of viral components
  - mRNA
  - Viral proteins
  - NA

## > Assembly

NA + V. proteins = Virions

➤ Release

# Release

- 1-Budding
   (enveloped Vs)
   -cell mb\*
   -nuclear mb
   (herpesVs)
- Virus-specific glycoproteins are synthesized and transported to the host cell membrane. Host cell membrane Viral protein The cytoplasmic domains of membrane proteins bind nucleocapsids. Nucleocapsid A nucleocapsid is enveloped by the host cell membrane. The host cell membrane provides the viral envelope by a process of "budding" The enveloped virion is released from the host cell.

2- Cell lysisor rupture(nonenveloped)

## <u>laboratory diagnosis of viral</u> <u>infections</u>

- > Microscopic examination.
- > Cell culture.
- > Serological tests .
- > Detection of viral Ag.
- > Molecular method .

## Microscopic examination

#### > Light microscopy,

Histological appearance Ex. Inclusion bodies



#### Electron microscopy;

- Morphology& size of virions
- Ex.
  - Dx of skin lesion caused by herpesv, poxv.
- It is replaced by Ag detection & molecular tests

#### Electron micrographs

## 

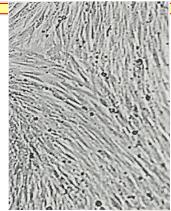


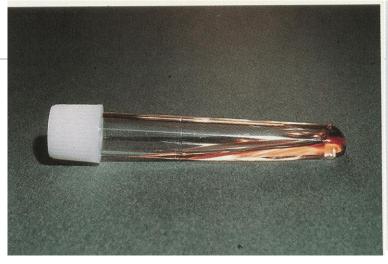
## **Poxvirus**



# Virus cultivation

- Laboratory animal
- > Embryonated egg
- > Cell culture







## *Cell culture* c/c)

- 1-Primary C/C
- 2-Diploid C/C

[semi continuous]

3-Continuous cell line

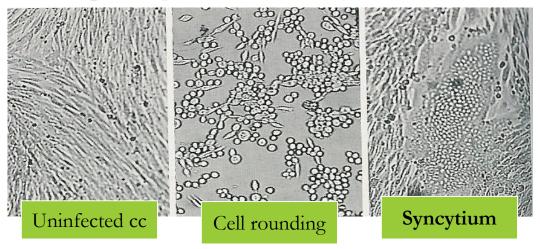
## Variation in Sensitivity of cell cultures to infection by viruses commonly isolated in clinical virology laboratories

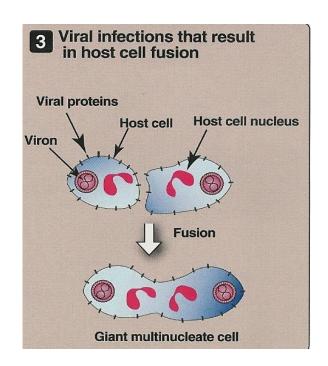
| Virus           | Cell culture <sup>a</sup> |     |       |
|-----------------|---------------------------|-----|-------|
| VII us          | PMK                       | HDF | HEp-2 |
| RNA virus       |                           |     |       |
| Enterovirus     | +++                       | ++  | +/-   |
| Rhinovirus      | +                         | +++ | +     |
| Influenza virus | +++                       | +   | -     |
| RSV             | ++                        | +   | +++   |
| DNA virus       |                           |     |       |
| Adenovirus      | +                         | ++  | +++   |
| HSV             | +                         | ++  | ++    |
| VZV             | +                         | +++ | -     |
| CMV             | _                         | +++ | -     |

PMK, primary MK. Degree of sensitivity: +++, highly sensitive;++, moderately sensitive; +, low sensitivity; +/-, variable; -, not sensitive

## Detection of viral growth

## Cytopathic effects





#### ➤ Others

#### Problems with cell culture;

- Long incubation
- Sensitivity is variable
- Susceptible to bacterial contamination
- ➤ Some Vs do not grow in c/c ex. HCV

# Rapid culture technique

- ➤ Shell Vial Assay
- ➤ Detect viral antigens
- ≥1-3 days

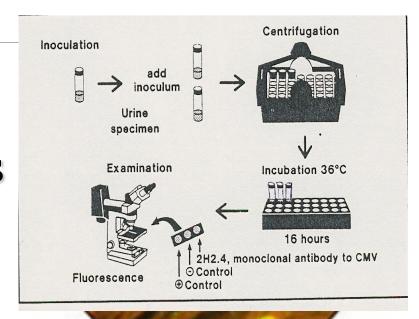


Fig. 2, CMV centrifugation culture fixed and stained 16 hrs after inoculation showing viral proteins in nuclei of infected human fibroblast cells

# Serological test; Antigen detection;

<u>sample</u>

virus

test

>Skin scrapings

**HSV** 

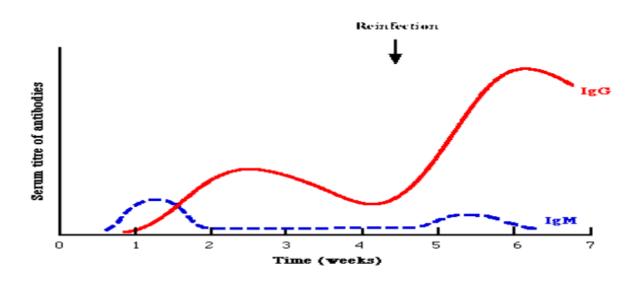
IF

➤ Blood

HBV(HBsAg)

**ELISA** 

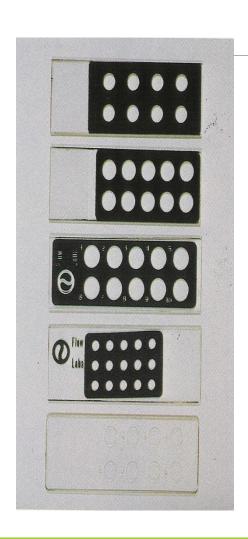
# Serological test; Antibody detection;

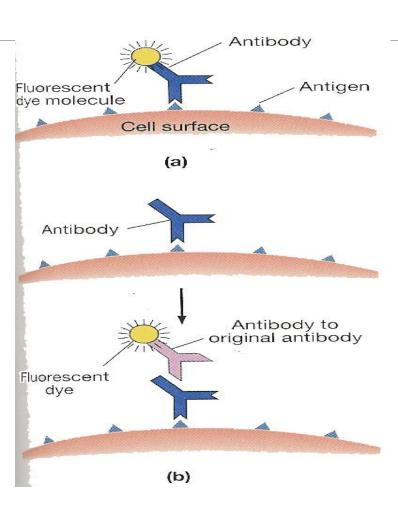


#### ➤ Ex of techniques

- Immunofluorescence (IF)
- Enzyme- linked immunosorbent assay (ELISA)

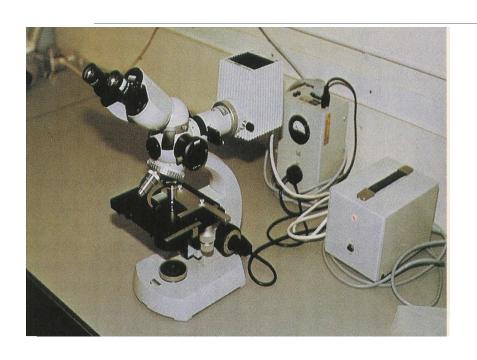
## Immunofluorescence ; IF





A- DirectAg detection;Sample (Ag)

B- IndirectAb detection;Sample (Ab)



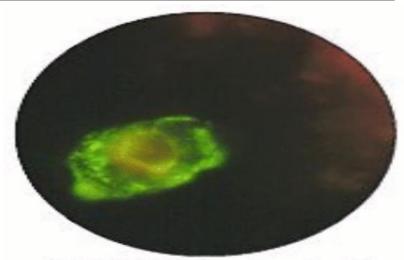
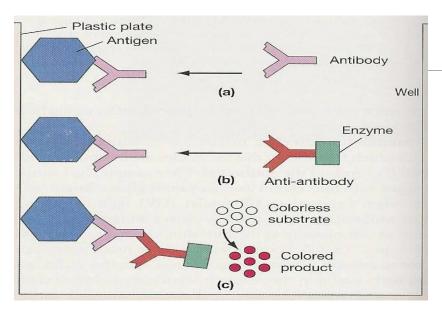


Fig. 3, HSV-infected epithelial cell from skin lesion (DFA)

#### **ELISA**

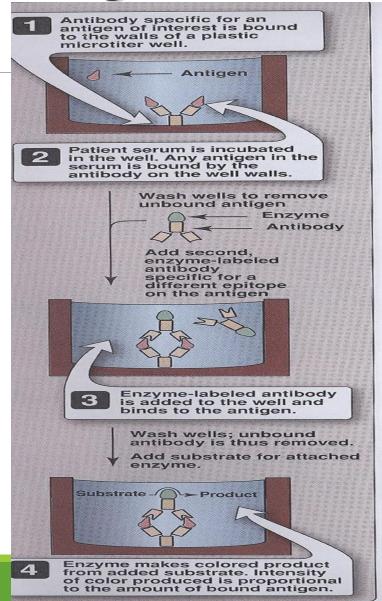
#### Ab detection





Indirect ELISA for Ab detection; coloured wells indicate reactivity

#### Ag detection



## Molecular test;

- Polymerase chain reaction (PCR)
  - Amplification tech.
  - Viral genome
- ➤ Uses;
  - Dx
  - Monitoring response to Rx



#### Reference books

