Lab diagnosis for viral infections

Specimen collection

- Blood samples: PBMC, serum/plasma
- Body fluid: CSF, urine, and saliva
- Swab: nasopharyngeal or skin swab
- Feces
- Tissue

Steps for viral diagnosis

- Direct examination
- Viral isolation and identification
- Ab or Ag detection using serological assay
- Molecular diagnosis

Direct examination

1. Electron microscope

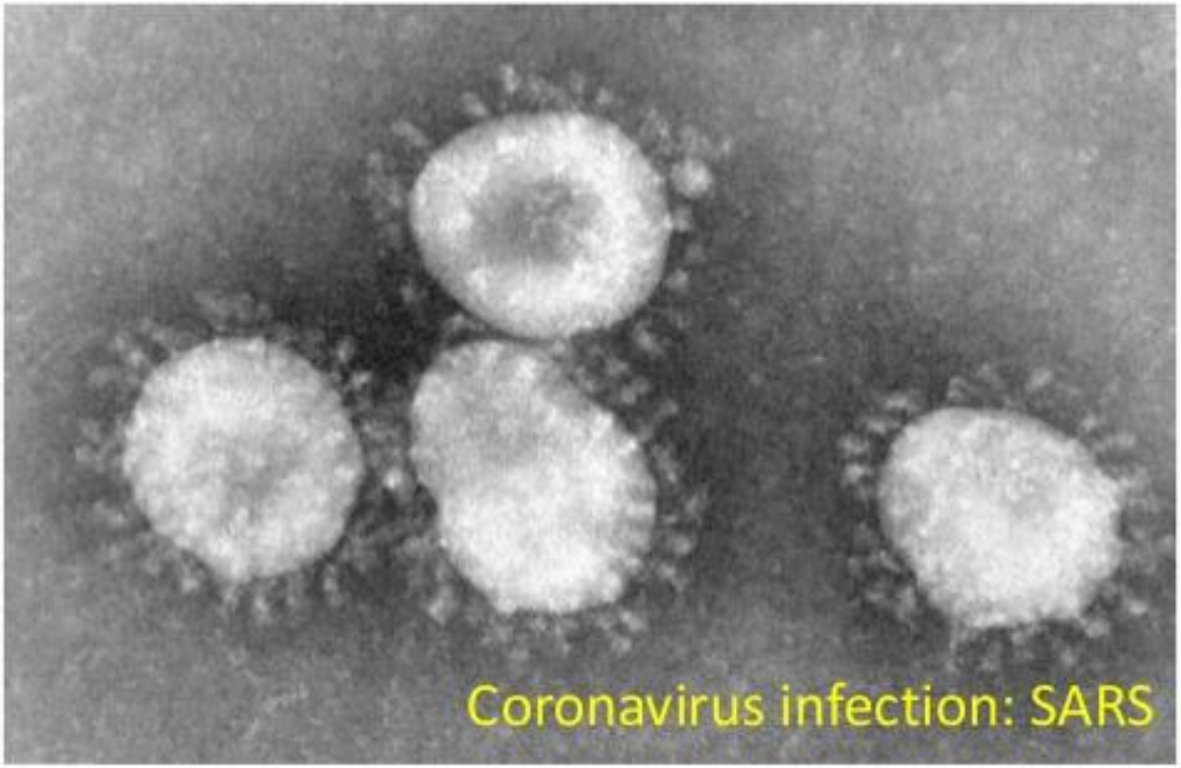
- Transmission electron microscopy (TEM)
  Magnification >500.000X 2D-picture

- Scaning electron microscopy (SEM)
  Magnification 10.000–100.000X 3D-picture

Direct examination

2. Immunostaining assay
3. Cytopathic assay
4. Agglutination assay (latex test)
5. Molecular assay (PCR, RT-PCR, qRT-PCR)

Electron microscope for viral detection and identification



http://www.redorbit.com/education/reference_library/health_1/viruses/2583898/coronavirus/

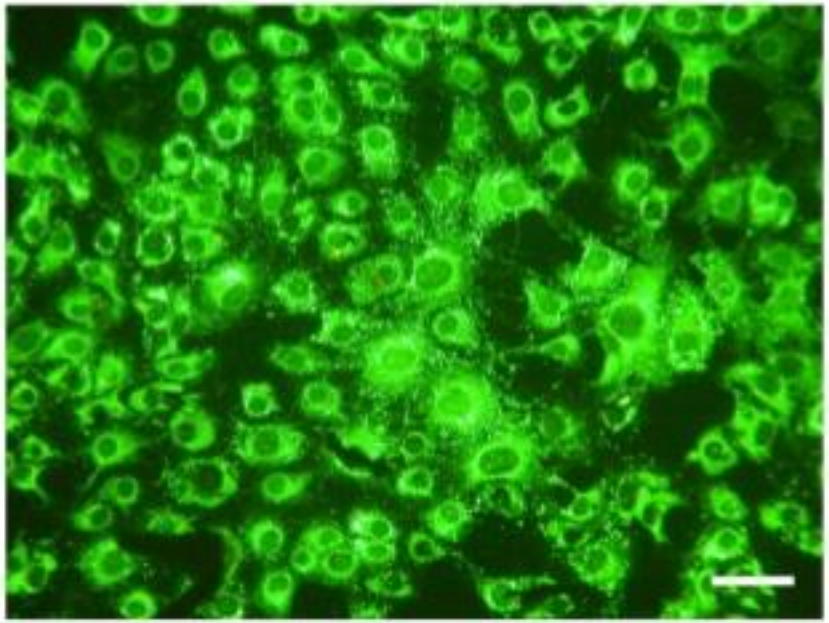
Viral Morphology

- Size and shape of viral particle
- Presence or no presence of viral envelope
- Structure (symmetry) of capsid

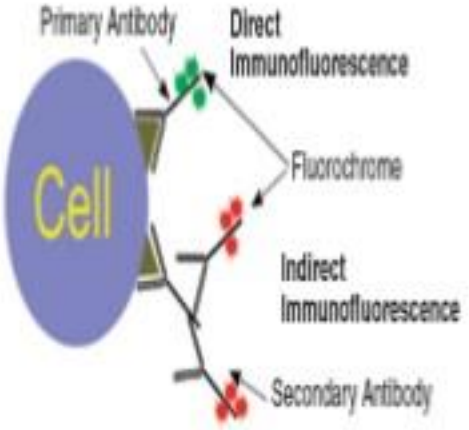
Immunostaining assay

- Using monoclonal antibody to viral antigen in clinical specimen
- Secondary Ab to develop the signal of positive staining
- Secondary Ab tagged with fluorescence dye: immunofluorescence staining assay (IFA)
- Secondary Ab tagged with peroxidase + substrate) immunoperoxidase staining assay (IHC)

Immunofluorescence staining



WNV-infected Vero cells



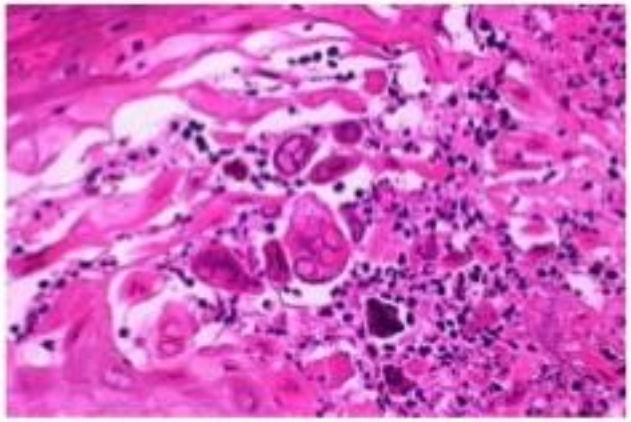
http://www.virologyj.com/content/3/1/71/figure/f3?highres=y
http://www.dako.com/08002_03aug09_ihc_guidebook_5th_edition_chapter_10.pdf

Cytopathic Effect (CPE)

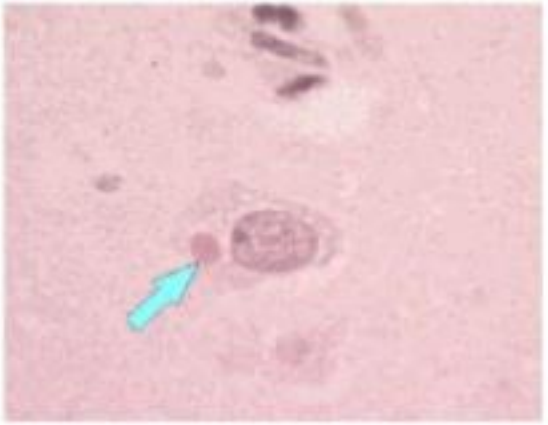
- Uninfected cells are adherent, they normally grow flat and stuck down firmly on the tissue culture flask
- After infection with rhinovirus, the cells change shape, becoming round and more refractile (brighter) under phase contrast microscopy
- Some infected cells detach from the tissue culture flask and float in the medium

Cytopathology assay

- Observation of cell change during viral infection in clinical specimens



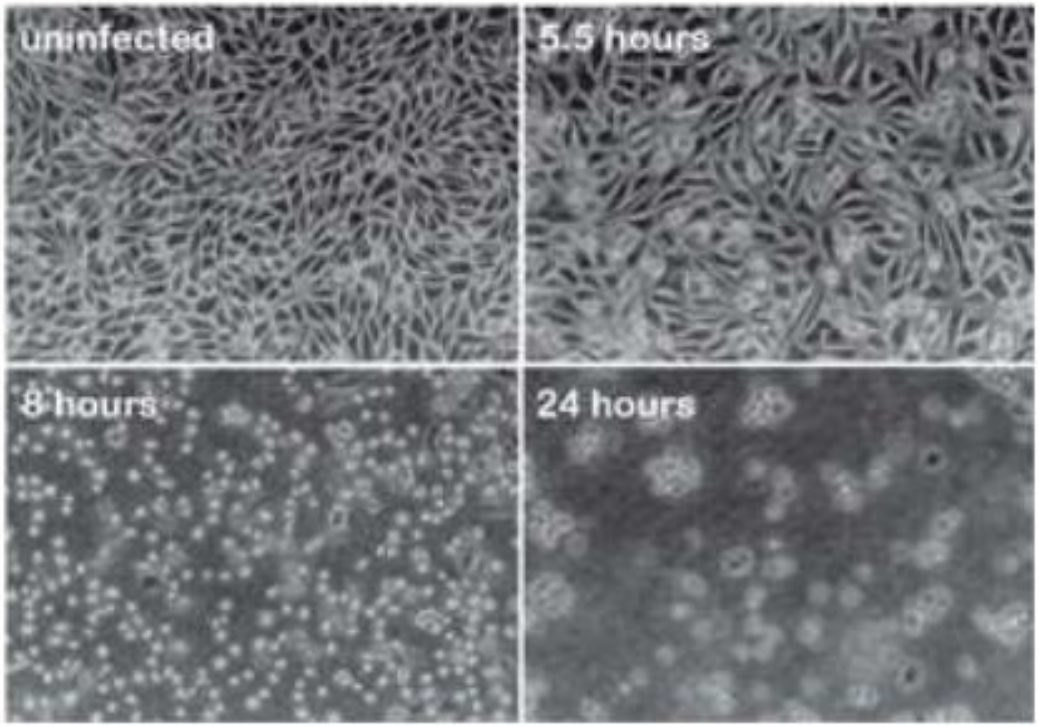
Multinucleated (giant) cell or syncytial cell from Tzanck smear



Negri's body in cytoplasm of neuron cells

http://cai.md.chula.ac.th/chulapatho/chulapatho/lecturenote/infection/Pathology%20of%20infection/inclusion%20bodies/Rabies.htm

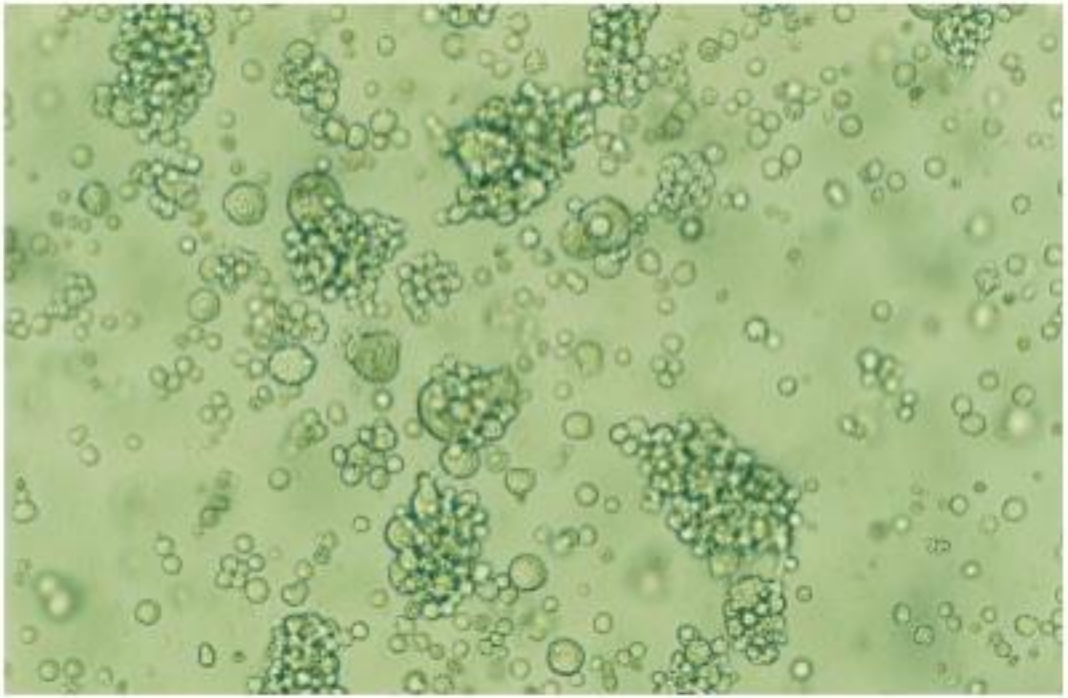
http://cai.md.chula.ac.th/chulapatho/chulapatho/lecturenote/infection/Pathology%20of%20infection/inclusion%20bodies/herpesimplex.html



The upper left panel shows uninfected cells, and the other panels show the cells at the indicated times after infection. As the virus replicates, infected cells round up and detach from the cell culture plate. These visible changes are called *cytopathic effects*.

Cytopathic effect (CPE)

- Cell formation varies in each virus



http://virology-mania.blogspot.com/2009/02/next-up-cytopathic-effect-cpe.html

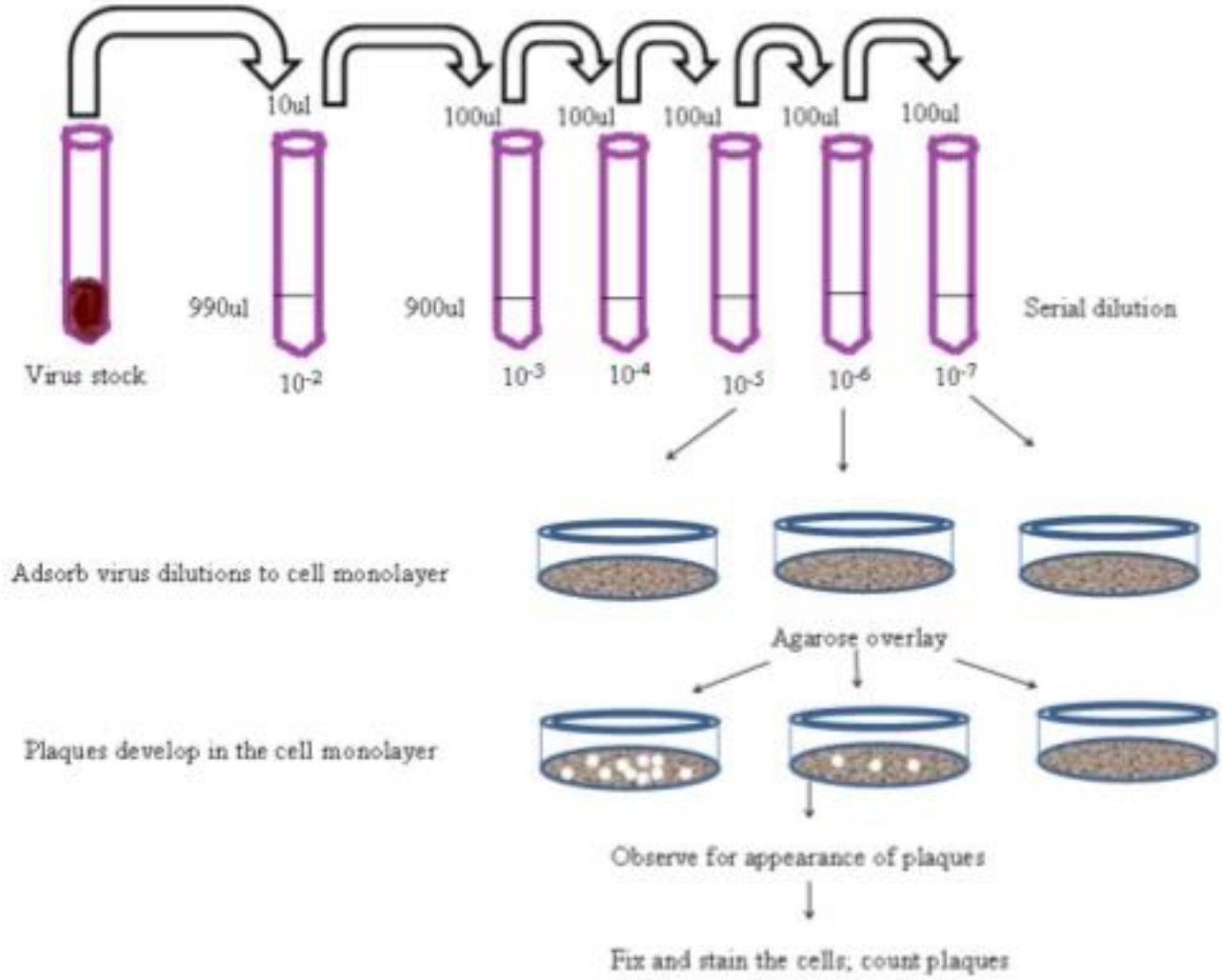
Table 2.1 Some examples of cytopathic effects of viral infection of animal cells

Cytopathic effect(s)	Virus(es)
Morphological alterations	
Nuclear shrinking (pyknosis), proliferation of membrane	Picornaviruses
Proliferation of nuclear membrane	Alphaviruses, herpesviruses
Vacuoles in cytoplasm	Polyomaviruses
Syncytia (cell fusion)	Paramyxoviruses, coronaviruses
Margination and breaking of chromosomes	Herpesviruses
Rounding up and detachment of cultured cells	Herpesviruses, rhabdoviruses, adenoviruses, picornaviruses
Inclusion bodies	
Virions in nucleus	Adenovirus
Virions in the cytoplasm (Negri bodies)	Rabies virus
"Factories" in the cytoplasm (Guarnieri bodies)	Poxviruses
Clumps of ribosomes in virions	Arenaviruses
Clumps of chromatin in nucleus	Herpesviruses

Plaque Assay

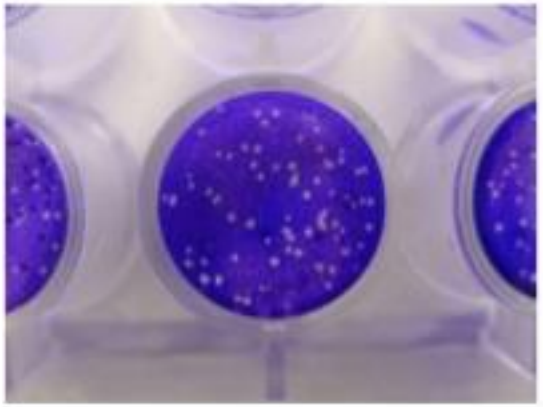
- **Plaque based** assay are standard method used to determine virus concentration in term of infectious dose.
- This assay determines the number of **plaque forming unite** (pfu) in virus sample.
- To measure virus quantity.
- **Plaque** is an area of infection surrounded by uninfected cells,
- It takes 3-14 days

Plaque Assay

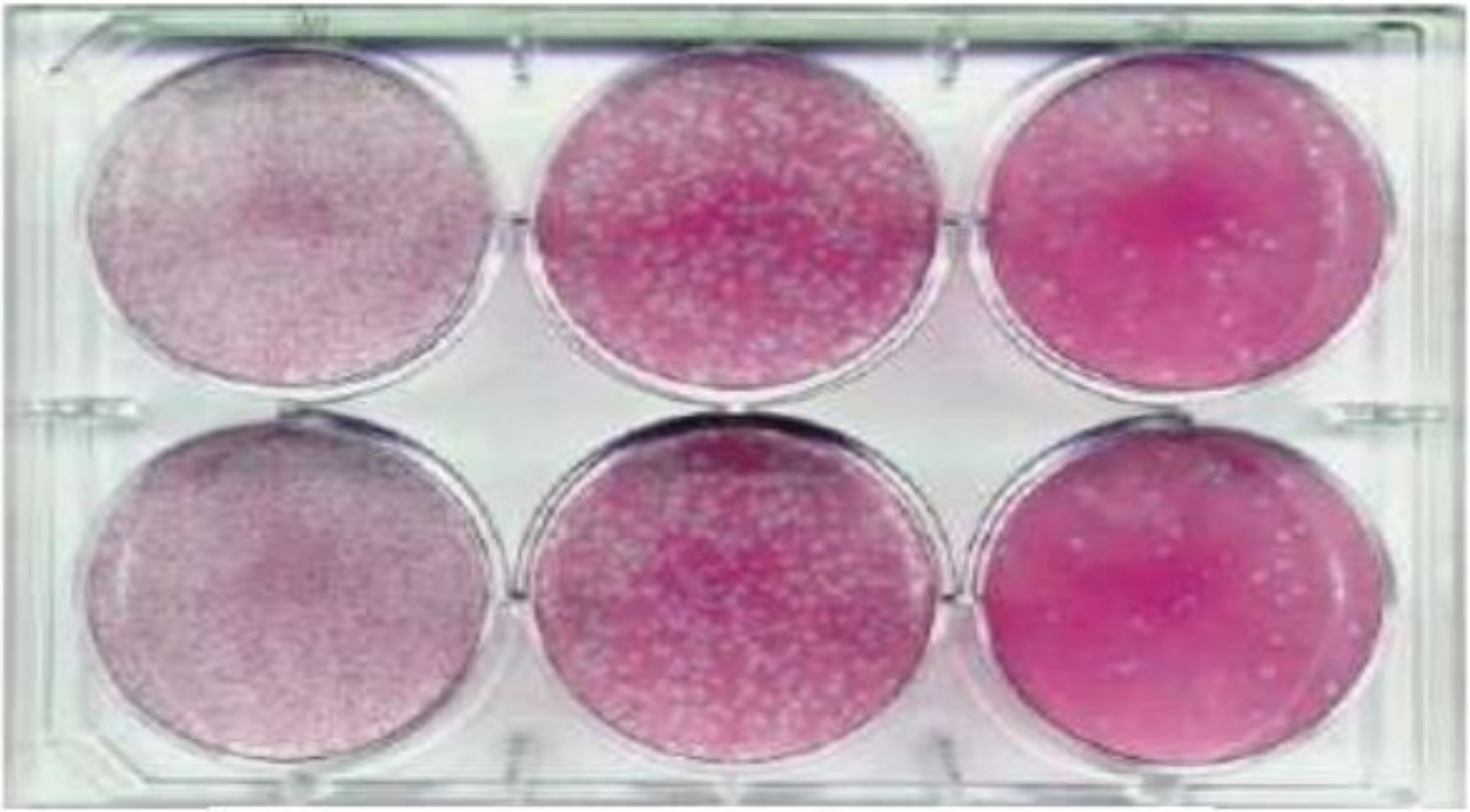


Plaque Assay

- Based on the ability of infectious virus particles to form small areas of cell lysis or foci of infection on the cell monolayer
- This is achieved by first adsorbing the virus onto a confluent cell monolayer and then overlaying the monolayer with agar



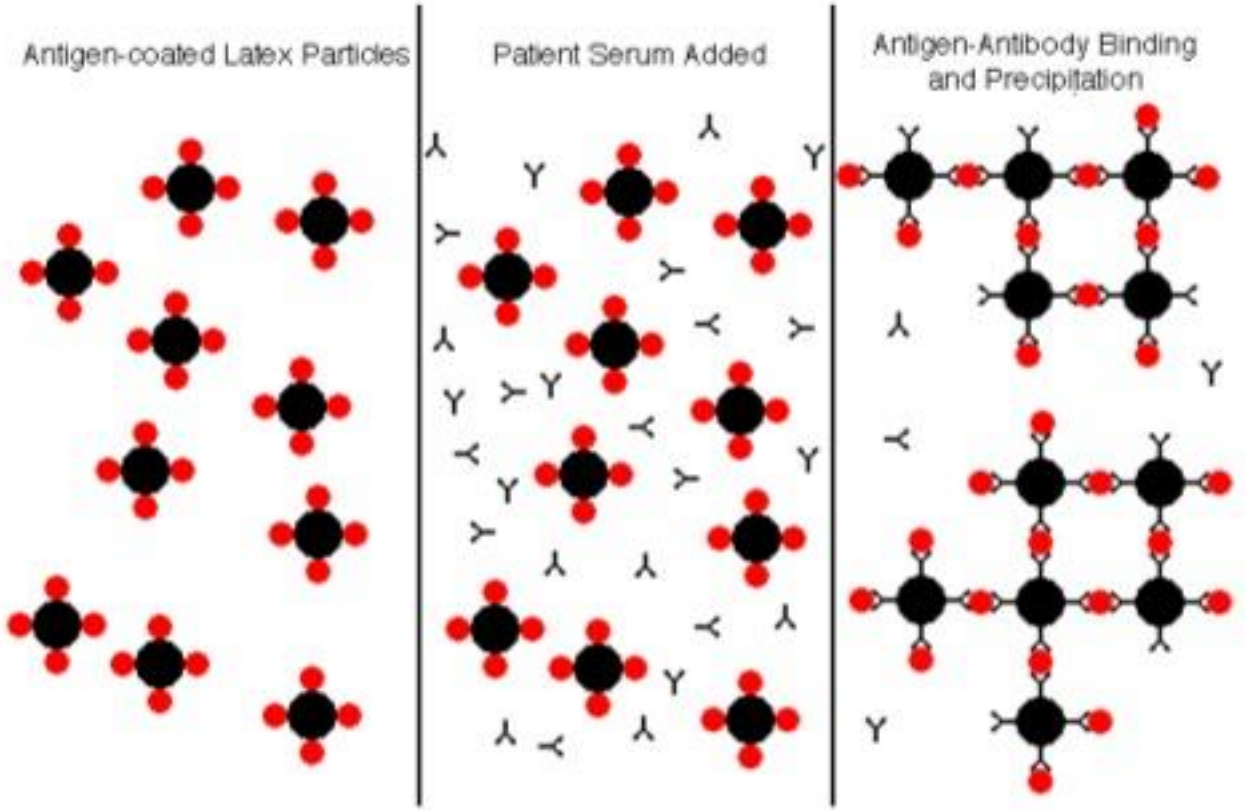
- The overlay medium restricts the spread of secondary infection so that only areas of the cell monolayer adjacent to the initially infected cells will become infected and form plaques or small areas of CPE
- These plaques can then be counted and the viral titer calculated
- Plaque assays can be carried out in 24-well cell cluster plates or cell culture plates



– Each plaque represents 1 PFU (Plaque Forming Unit)

Latex agglutination assay

- Either antibody or antigen detection



http://library.tcmedc.org/webpath/microbio/microbe/microbe05.htm

Molecular Assay

- DNA virus: PCR
- RNA virus: RT-PCR or qRT-PCR
- Virus nucleic acid (hybridisation)
- Detection of viral nucleic acid directly in:

1. Tissues
2. cell smears
3. histopathological preparations

Example: Diagnosis of Human papilloma virus (HPV) infection

PCR method

First step: (DNA or RNA extraction from clinical samples)

Second step: PCR mix PCR Reaction Components

Water Buffer DNA template Primers Nucleotides Mg++ ions DNA Polymerase

Third step:

- Gel electrophoresis then
- Detection and identification of amplified target DNA fragments based on molecular size

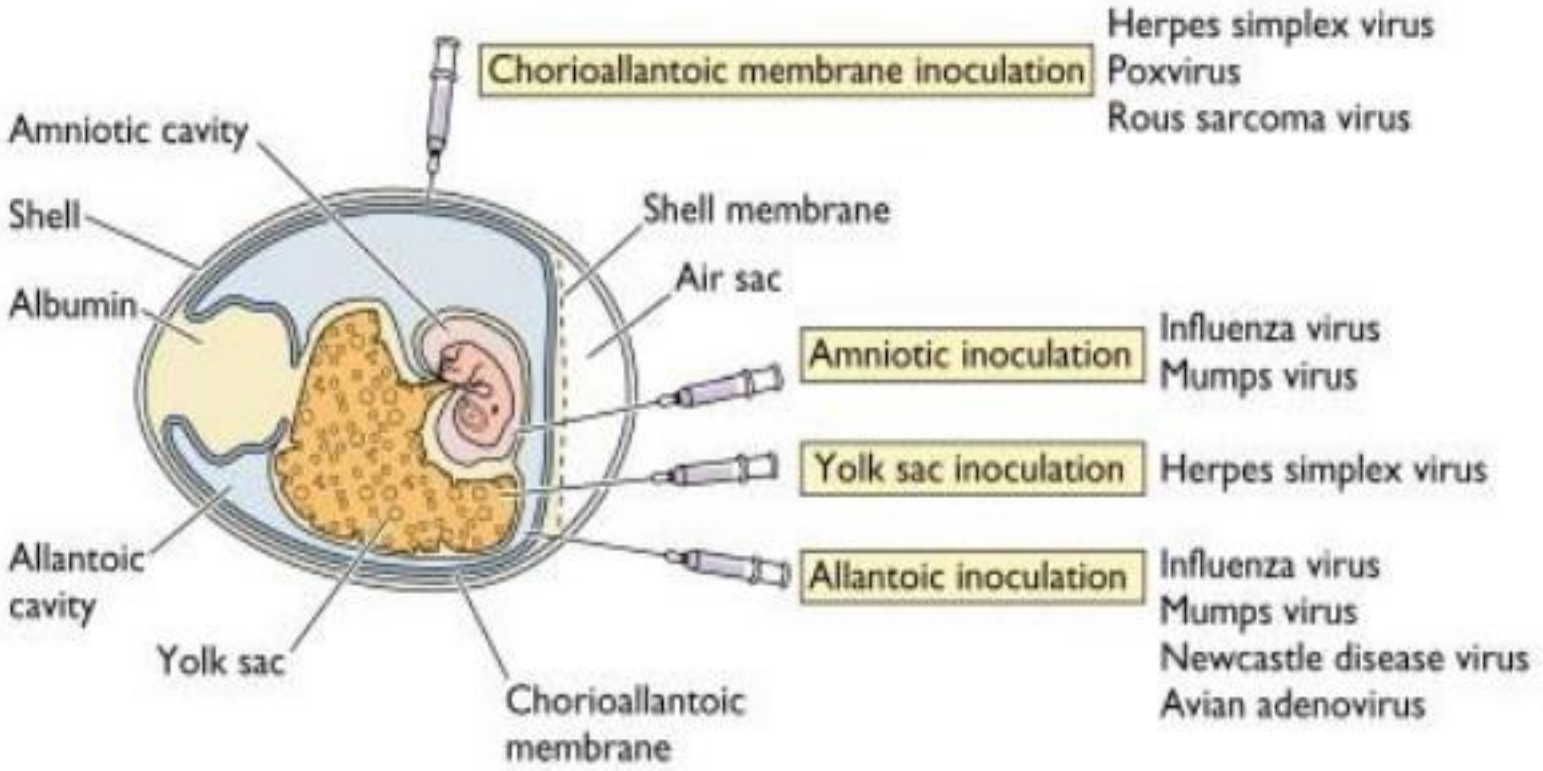
Viral isolation & identification

- Good standard for diagnosis
- Time consuming
- Confirm direct examination and serological assay
- Required facilities for lab

Tools for viral isolation

1. Experimental animal (inoculation)
2. Embryonated egg (Chick embryo)
3. Cell culture (cell line)

Embryonated egg



http://www.virology.ws/2009/12/10/influenza-virus-growth-in-eggs/

Cell culture

1. Primary cell culture (monkey kidney)

- prepared directly from animal or human tissues and can be subcultured only once or twice

2. Semi continuous or diploid cell culture

- derived from human fetal tissue and can be subcultured 20 to 50 times e.g. human diploid fibroblasts such as MRC-5

3. Continuous cell culture

- derived from tumours of human or animal tissue e.g. Vero, Hep2

Cell culture

1. Change of cell formation: CPE, Hyperplasia, and inclusion bodies.

2. Immunostaining assay and molecular assay
3. Cell culture media

Isolation of Viruses in Cell Culture

- However, most of the more common human pathogenic viruses can be cultured relatively easily provided the proper conditions are satisfied
- A wide variety of virus-sensitive cell lines are available either commercially or through one of the national or international cell bank collections such as ATCC & ECACC

Cell line type	Origin
HEL/MRC-5	Human embryonic lung fibroblasts
Hep-2	Human epithelium
E6-VERO	African green monkey
RMK	Rhesus monkey kidney
RD	Human rhabdomyosarcoma
FRK-4r	Fetal rhesus kidney
MDCK	Madin Darby canine kidney

Viral identification

1. **Animal models:** disease or death

- Clinical sign observation
- Specimen collection & sacrifice animal for viral detection, isolation & identification

2. **Embryonated egg**

- Hemeagglutination assay
- Immunostaining assay,
- Embryo observation: died

Serological assay

- Wide used in routine laboratory
- Required paired serum:
- The change of Ab titer (4-fold rising) between Acute and convalescent period (2-3 weeks)
- Very sensitive
- Detection of IgM in primary infection

HIV test

If positive twice, Western Blotting is performed next

Could detect viral antigens or antibodies

ELISA Procedures

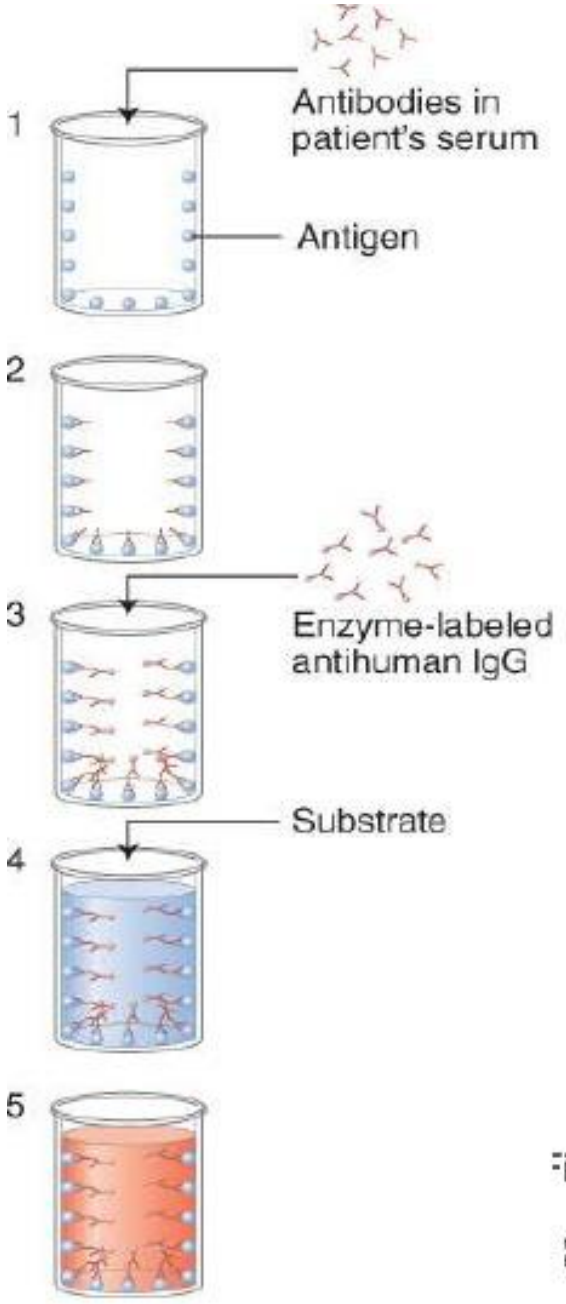


Figure 5-19a

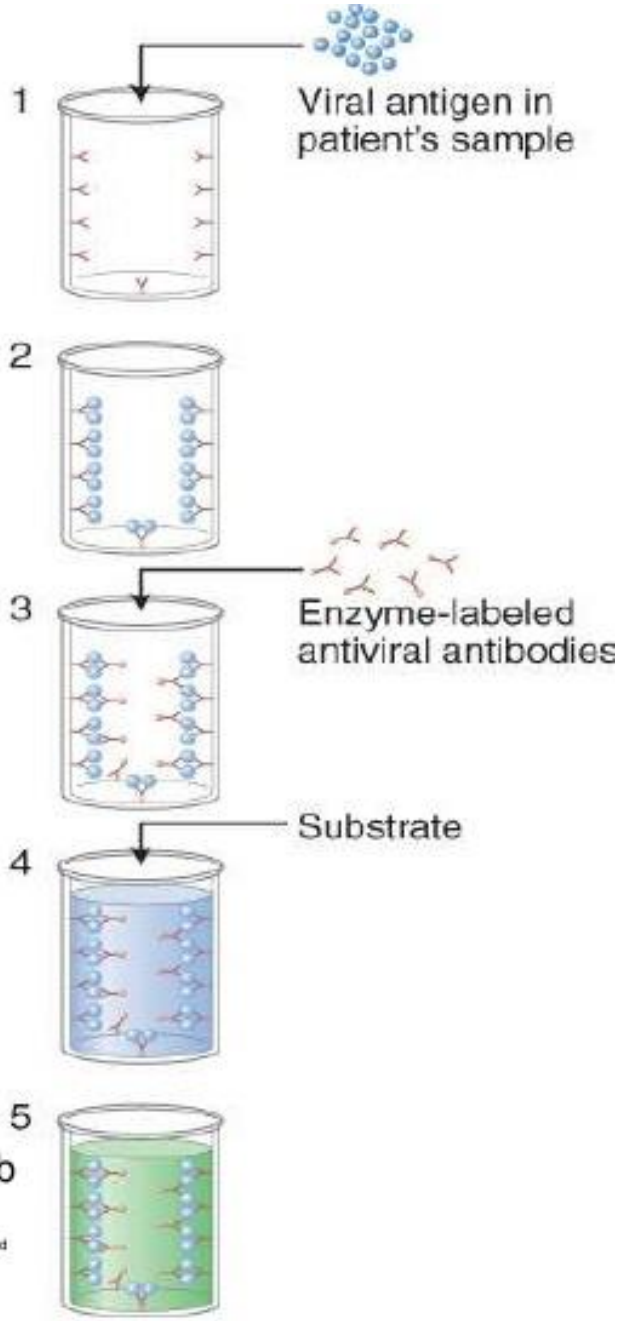


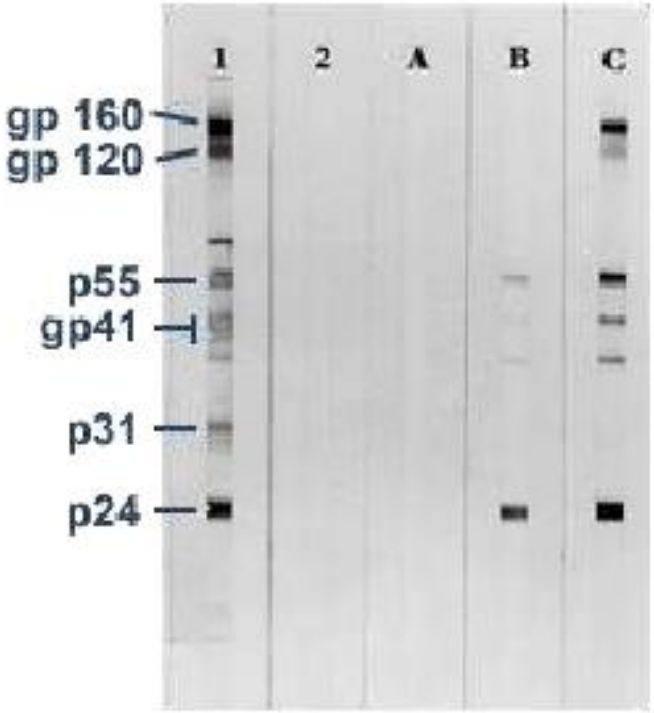
Figure 5-19b

Modified from Specter, S. C., R. L. Hodinka and S. A. Young. Clinical Virology Manual, Third Edition . ASM Press, 2000.

Western Blot

HIV-1 Western Blot

- Lane1: Positive Control
- Lane 2: Negative Control
- Sample A: Negative
- Sample B: Indeterminate
- Sample C: Positive



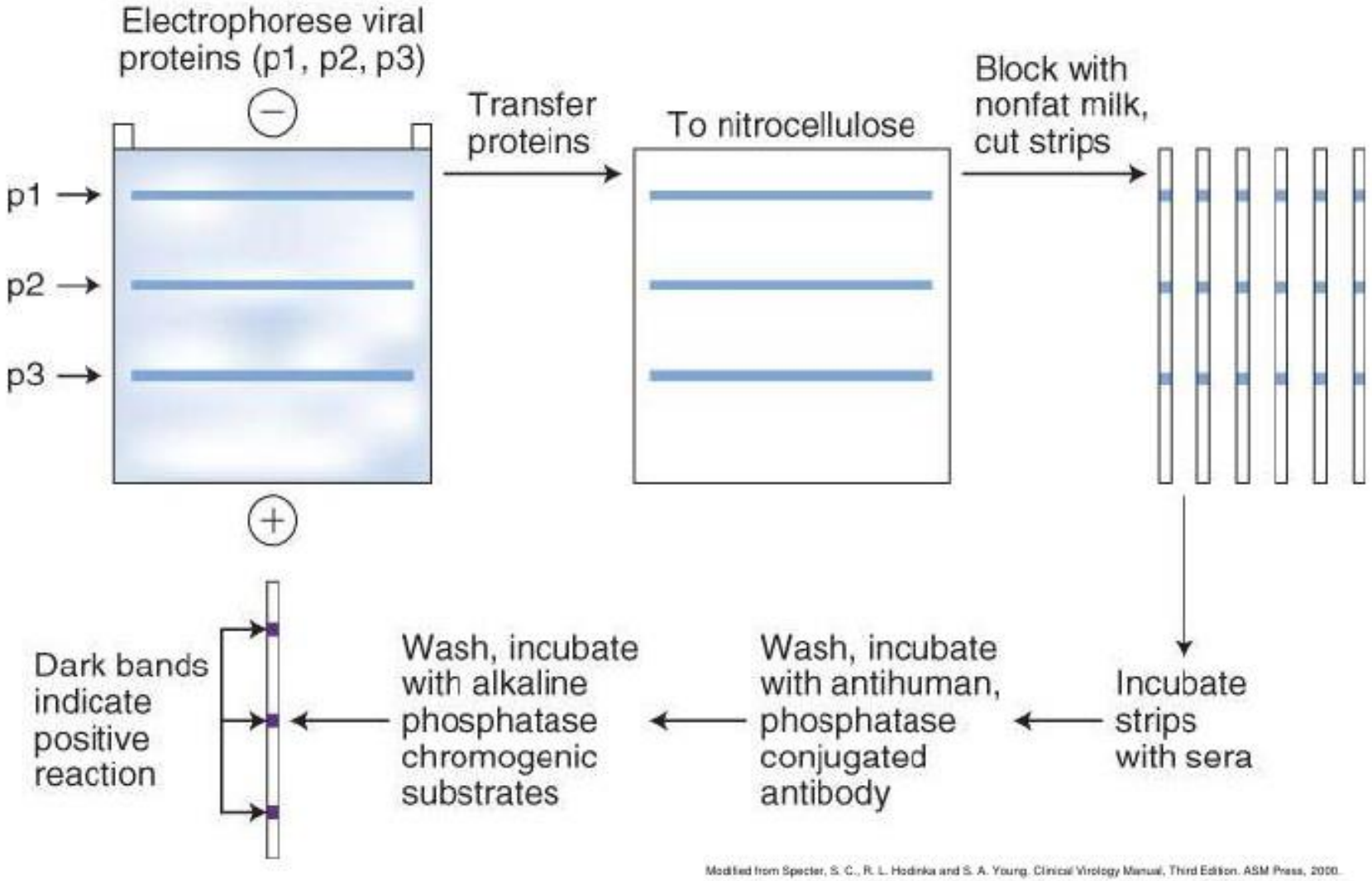


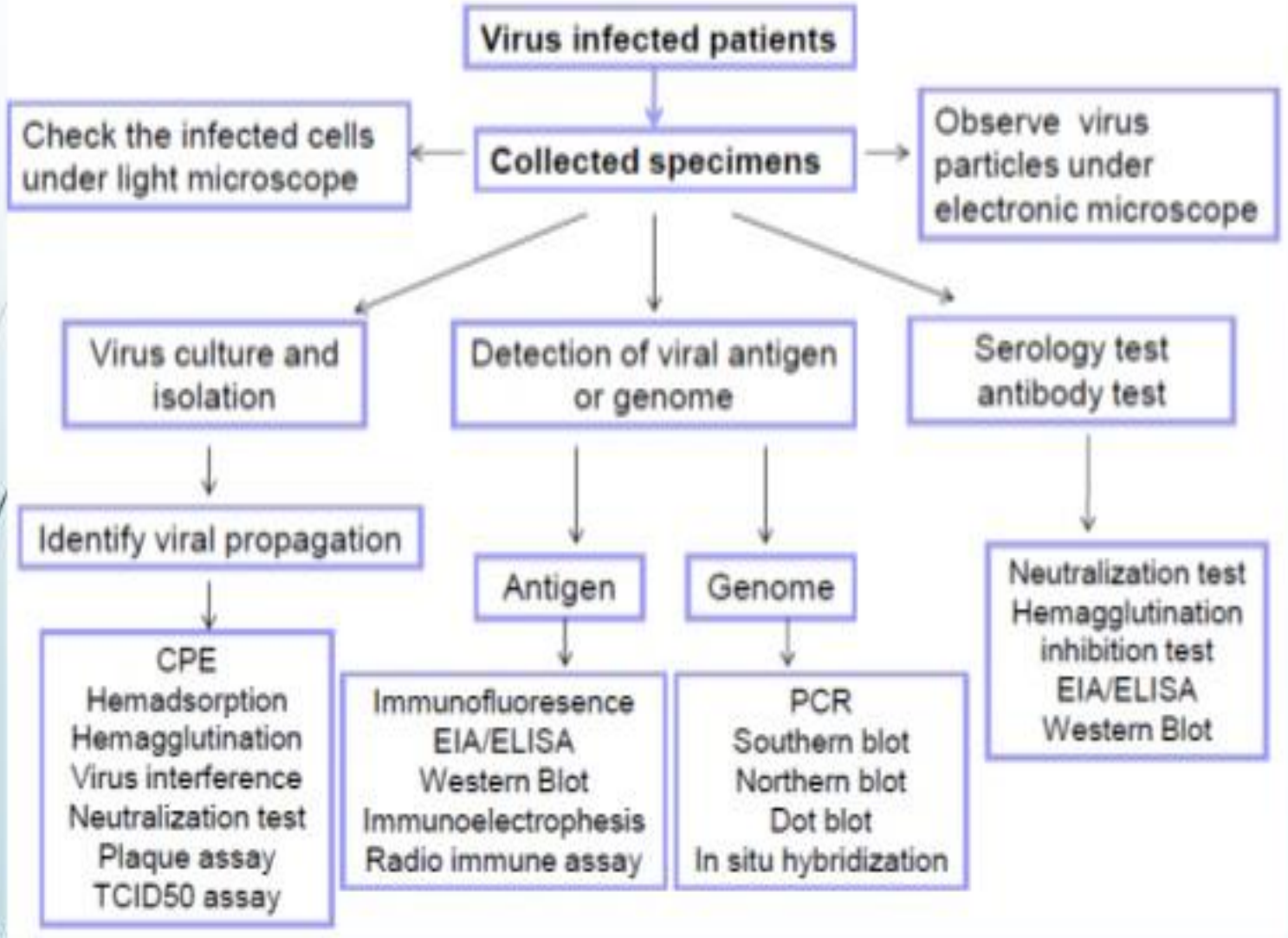
Figure 5.21a: The basic principles behind the Western blotting procedure.

Tools for Ab Detection

- Neutralising test
- Complement fixation test
- ELISA test
- Hemagglutination inhibition test
- WB test
- Rapid test (Strip test) for Ab or Ag detection

DIAGNOSIS OF VIRAL DISEASE:

The diagnosis of viral diseases are showed by flow chart…..



Thank you