Methods used in Virology

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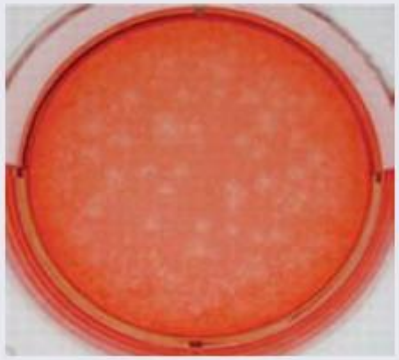
Virus Isolation and Culture



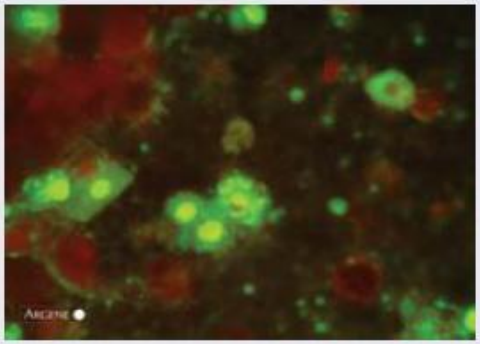
Animal virus plaques
in a cell culture [1]

Phage plaques
in a lawn of
bacterial cells [2]

Density Gradient Centrifugation

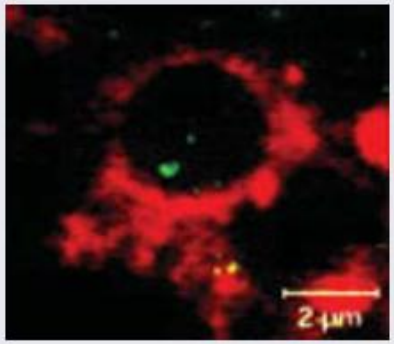
Separation of virus particles
in a density gradient [3]

Fluorescence Microscopy



Virus-infected cells
detected using a
virus-specific antibody
labelled with a
fluorescent dye [4]

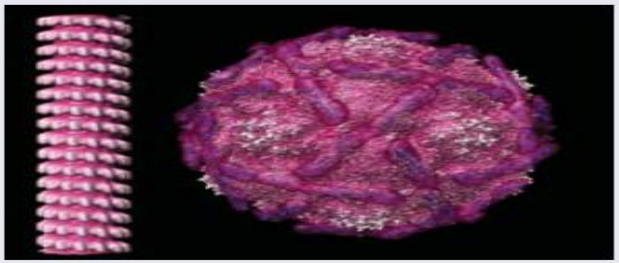
Confocal Microscopy



An endosome (labelled red)
containing virus protein
(labelled green)
in an infected cell [5]

Electron Microscopy

Negatively-stained virus particles [6]



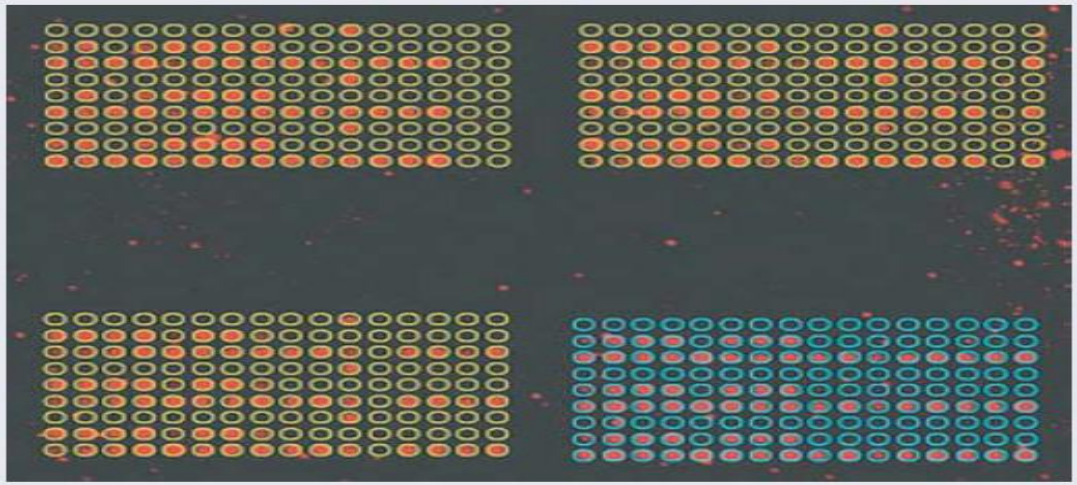
Reconstructed images from cryo-electron microscopy [7]

Electrophoretic Techniques

Detection of virus proteins by sodium dodecyl sulphate polyacrylamide gel electrophoresis [8]

Analysis of virus nucleic acids by agarose gel electrophoresis [9]

Microarrays



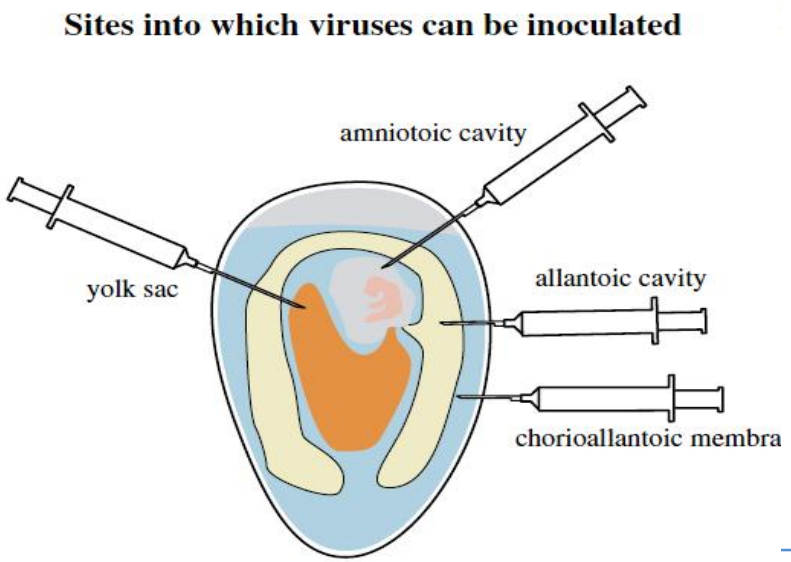
Analysis of virus transcription [10]

Methods used in Virology

- Virus Isolation and Culture
- Microscopic tools: light, fluorescent
- Centrifugation methods
- Electron Microscopy
- Electrophoretic Techniques:
  Detection of virus proteins by sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE), Analysis of virus nucleic acids by agarose gel electrophoresis.
- Microarrays: Analysis of virus transcription

Virus Culture

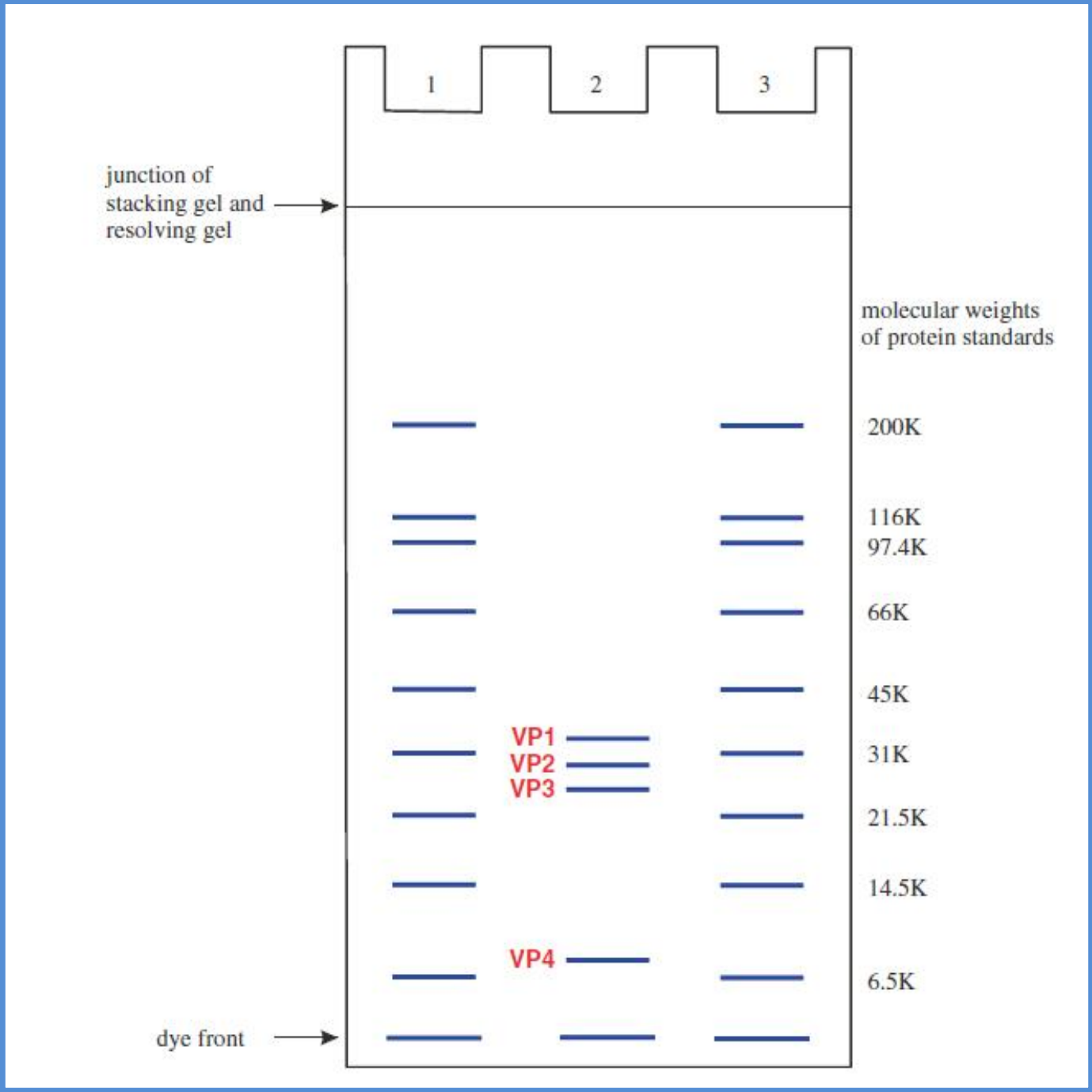
- Need to Specific Hosts: Phages are supplied with bacterial cultures, plant viruses may be supplied with specially cultivated plants or with cultures of protoplasts (plant cells from which the cell wall has been removed), while animal viruses may be supplied with whole organisms, such as mice, eggs containing chick embryos, cell line or insect larvae.



Animal Virus Culture

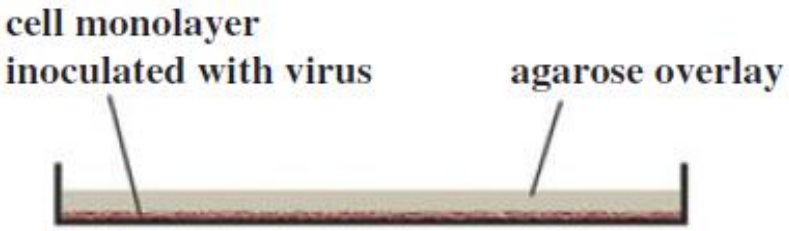
- Cell line such as Hela is a widely used and taken from a cervical carcinoma. Sometimes it is difficult to find a cell line in which a virus can replicate. For many years no suitable cell culture system could be found for hepatitis C virus, but eventually a human hepatoma cell line was found to support replication of an isolate of the virus.
- Cells are cultured in media that provide nutrients. Most media are supplemented with animal serum, which contains substances that promote the growth of many cell lines. Other important roles for the medium are the maintenance of optimum osmotic pressure and pH for the cells. Viruses can be cultivated in cells growing on the surface of a variety of plastic vessels.

Figure 2.3 *Cell culture flasks, dishes and plates.* Photographs of TPP cell culture products courtesy of MIDSCI.


1
2
3
junction of
stacking gel and
resolving gel
molecular weights
of protein standards
200K
116K
97.4K
66K
45K
VP1
VP2
VP3
31K
21.5K
14.5K
VP4
6.5K
dye front

Isolation of Viruses

- Many viruses can be isolated as a result of their ability to form discrete visible zones (plaques) in layers of host cells.
- Plaques can be formed by many animal viruses in monolayers if the cells are overlaid with agarose gel to maintain the progeny virus in a discrete zone. Plaques can also be formed by phages in lawns of bacterial growth



A cell monolayer is inoculated with virus and overlaid with agarose.

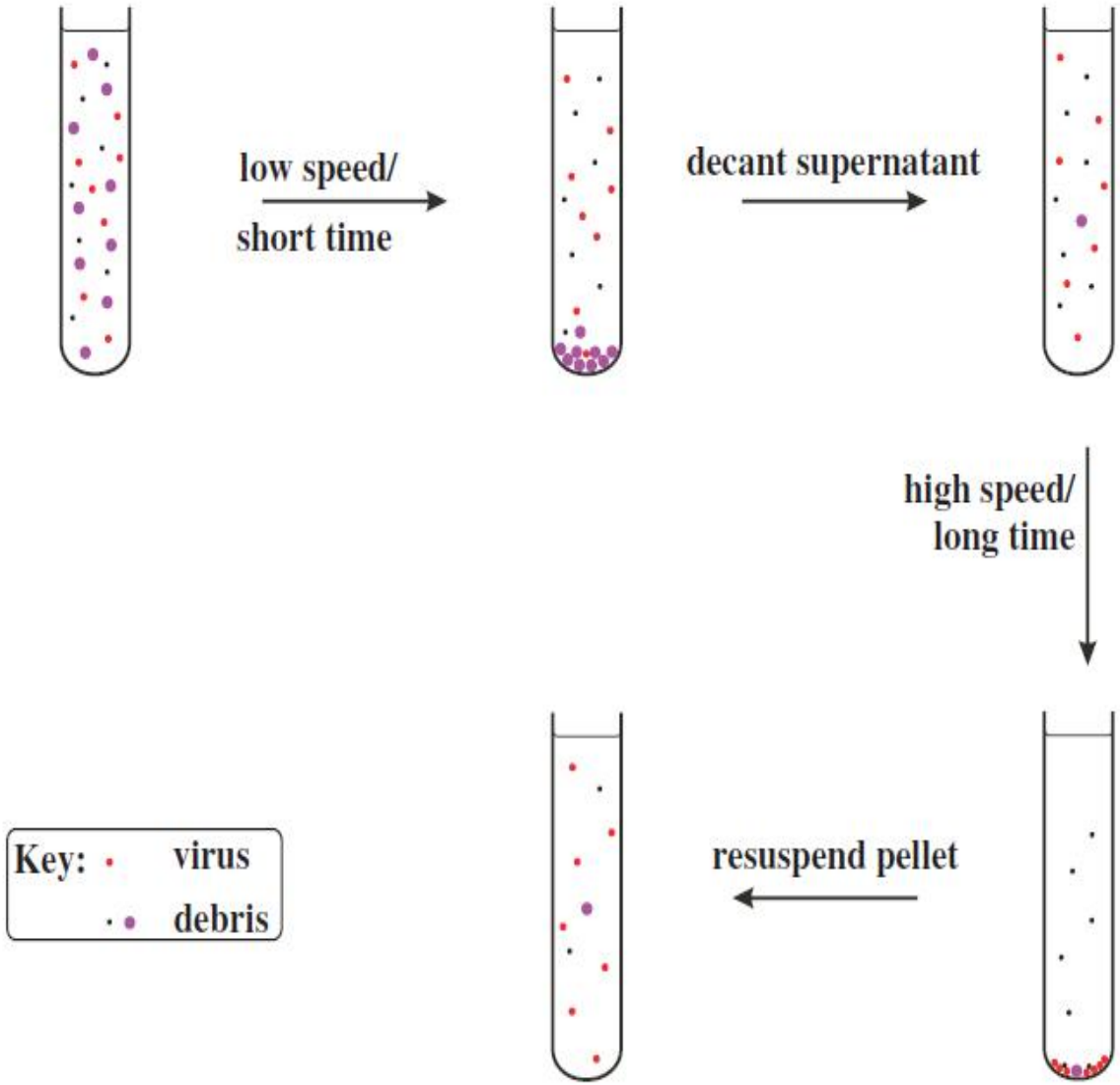
Isolation of Viruses

Plaques formed by West Nile virus in a cell monolayer

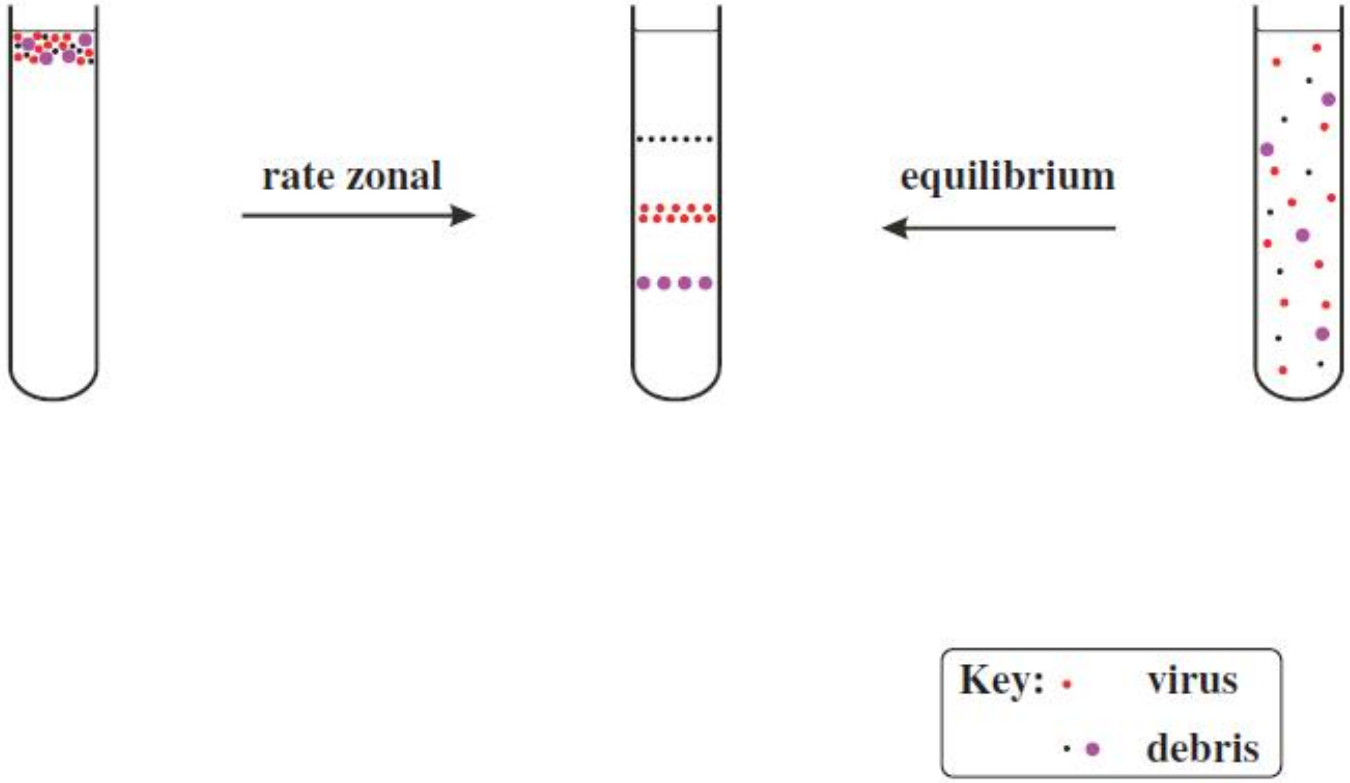
- A plaque is the result of the infection of a cell by a single virion. If this is the case then all virus produced from virus in the plaque should be a clone (virus strain).
- Plaque purified= a plaque might be derived from two or more virions so, to increase the probability that a genetically pure strain of virus has been obtained, material from a plaque can be inoculated onto further monolayers and virus can be derived from an individual plaque.

Virus Purification

- Use centrifugation for removing host cell debris and other contaminants.
- The pure virus particles can be used for laboratory studies, for incorporation into a vaccine, or for some other purpose.
- Centrifugation Types:
  density gradient centrifugation
  differential centrifugation



Partial purification of virions by differential centrifugation. A crude preparation of



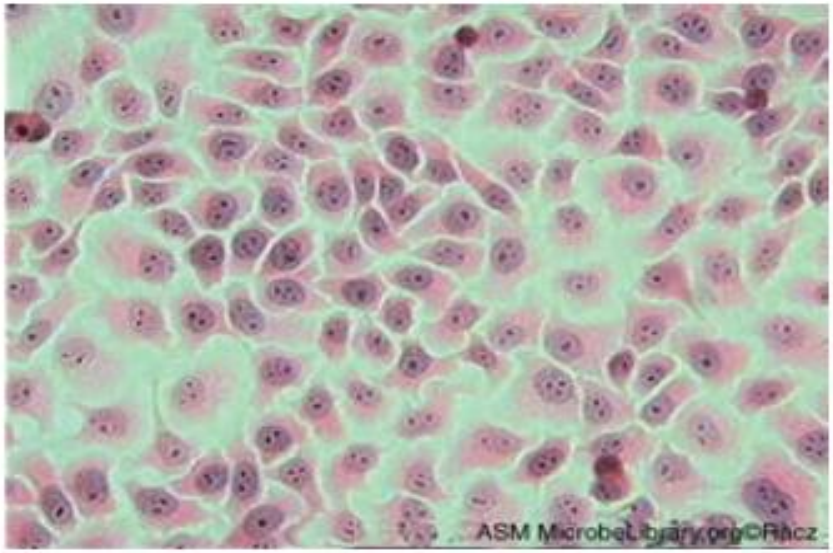
Purification of virions by density gradient centrifugation. A partly purified preparation

Microscopic Techniques

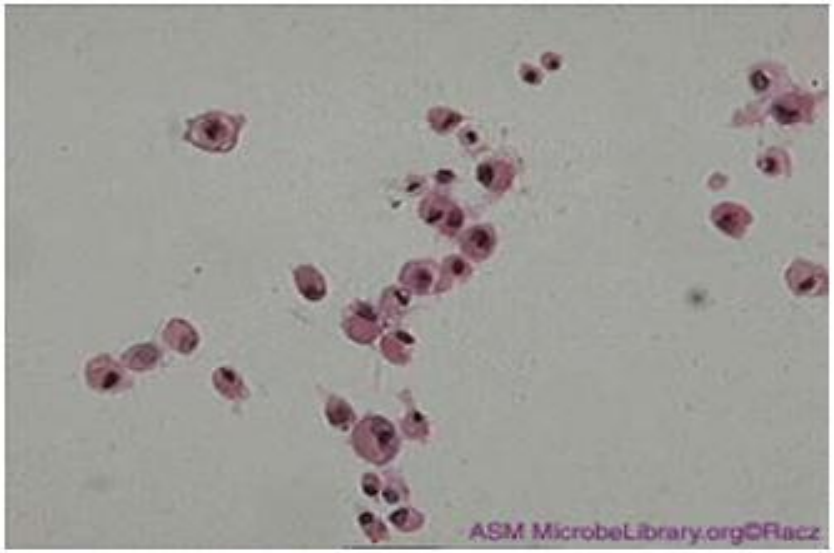
- The light microscopy has useful applications in detecting virus-infected cells, for example by observing cytopathic effects (CPE) or by detecting a fluorescent dye linked to antibody molecules that have bound to a virus antigen (fluorescence microscopy).
- Many investigations of the structure of virions or of virus-infected cells involve electron microscopy.
- X-ray crystallography is another technique that is revealing detailed information about the three dimensional structures of virions (and DNA, proteins and DNA–protein complexes).

Vero (monkey kidney) cell line

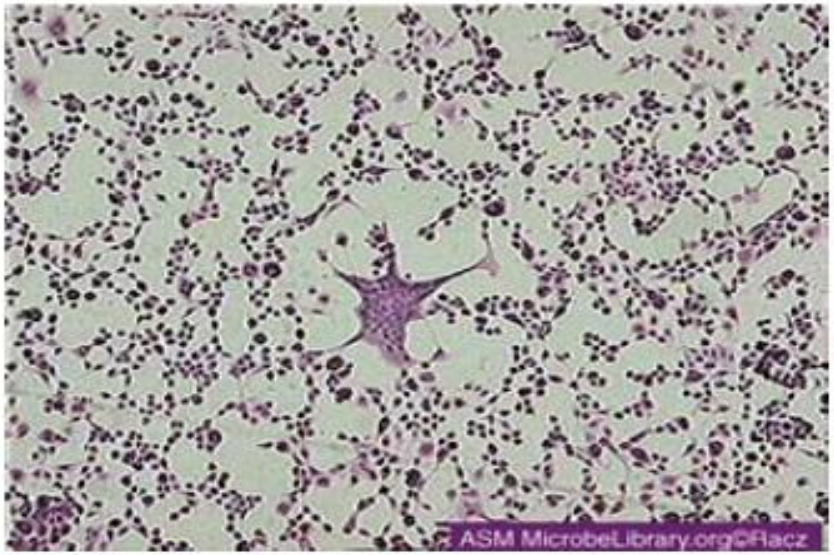
(a) Normal Cell



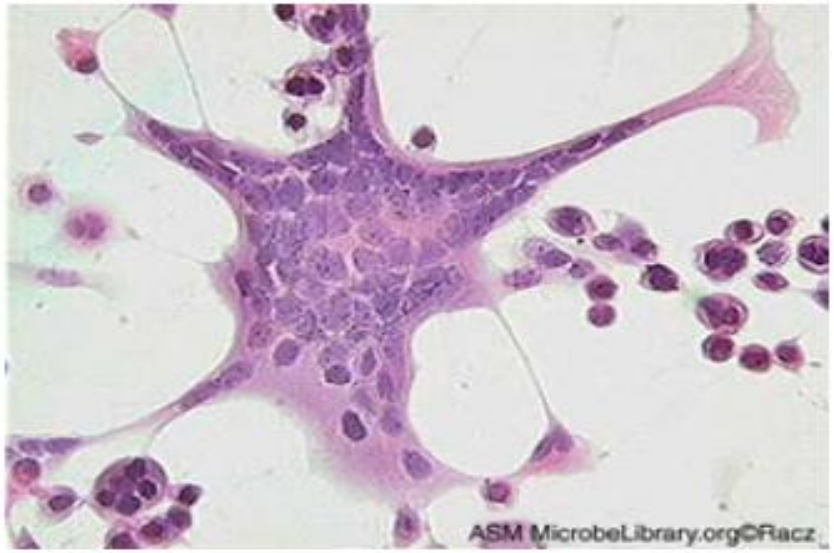
(b) Polio Virus



(c) HSV



(d)

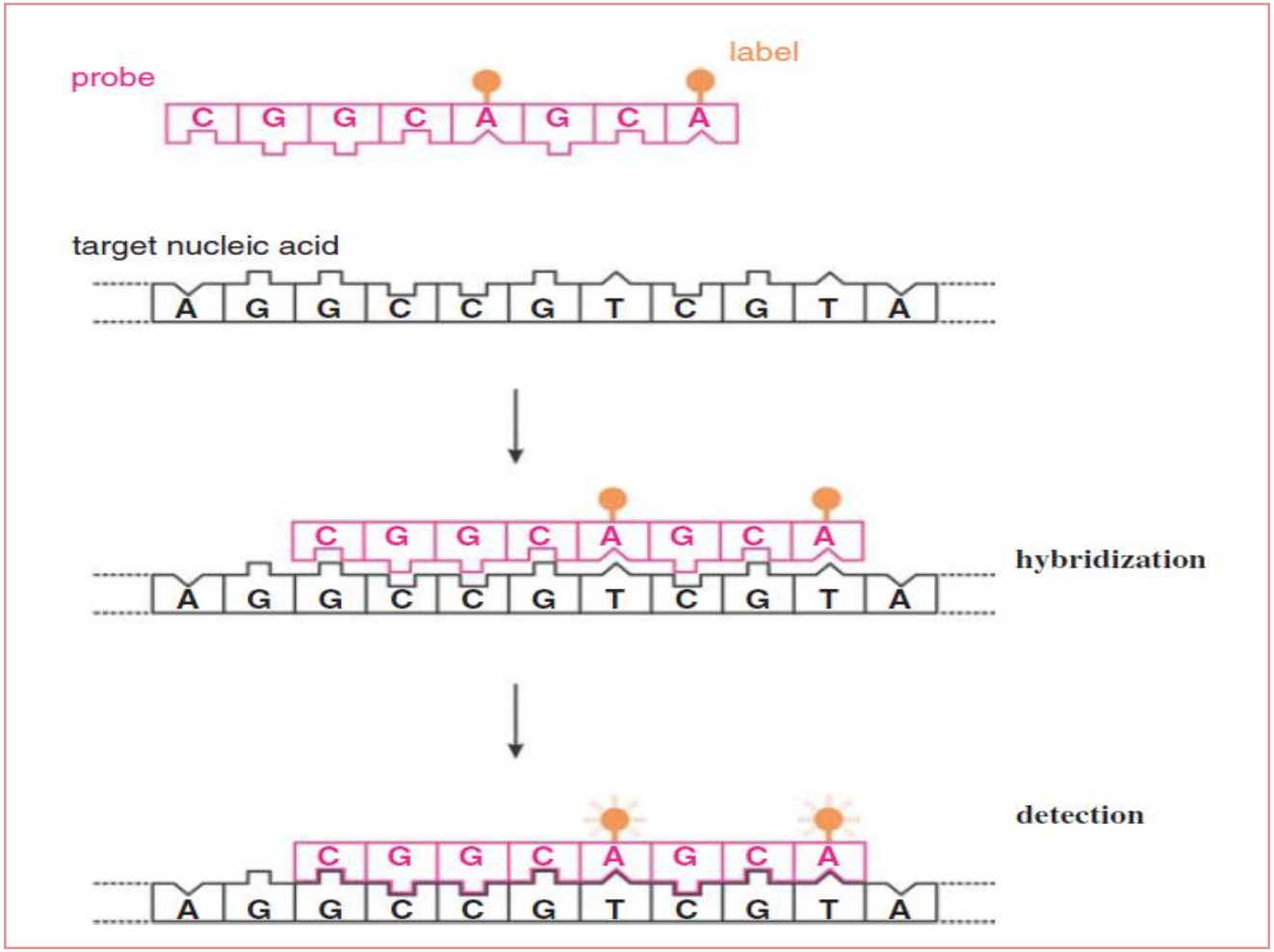


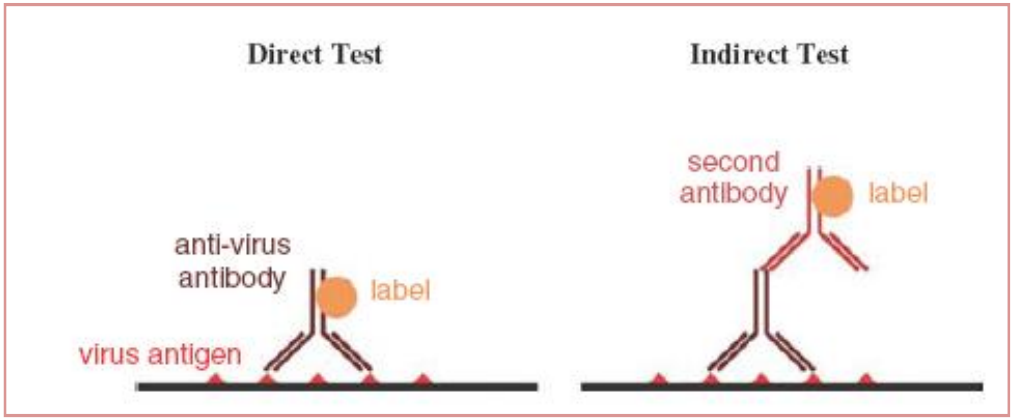
Detection of Virion

- *Detection of infectivity using cell cultures*
- *Detection of virus antigens: ELISA*
- *Detection of virus nucleic acids*
  Hybridization: Labeled probe with Fluorophor (FISH)= Fluorescent In Situ Hybridization
- *Polymerase chain reaction (PCR): use specific target and master mix (DNA pol., 2 primers, $MgCl_2$, Buffer, DW).*
- *Heamadsorption & Heamagglutination*

Hemagglutination

- Hemagglutination: Some viruses will cause clumping of RBC cells due to attachment to more than one cell and this can be used as the basis of an assay.
- Reverse passive hemagglutination: A sensitive serological test in which red blood cells are coated with virus-specific antibody and used to test for the presence of antigen. If virus antigen is present, the red blood cells are agglutinated.


probe
label
C G G C A G C A
target nucleic acid
A G G C C G T C G T A
C G G C A G C A
A G G C C G T C G T A
hybridization
C G G C A G C A
A G G C C G T C G T A
detection


Direct Test
Indirect Test
anti-virus
antibody
label
virus antigen
second
antibody
label

Detecting of Virus Growth

- CPE: Alteration in the microscopic appearance of cells in culture following infection with a virus. May consist of rounding up, cell detachment, cell fusion, production of inclusion bodies, Chromosome disorder, infection and spread), (Syncytia= multinucleate giant cells)
- Cell transformation (tumor)
- Interference (Rubella virus has not CPE, Echovirus and Coxsaki virus have specific CPE, if adding Echovirus to cell culture can not produce CPE, then it has rubella virus.
- Inhibition of acid production (Phenol Red)
- Candling test for egg

Virus Assay

- The quantity of viruses in a sample can be determined either by counting particle numbers or by measurement of the infectious unit concentration.
- Most normal virions are probably infective, many will not infect host cells because they do not contact the proper surface site.
- Thus the total particle count may be from 2 to 1 million times the infectious unit number depending on the nature of the virion and the experimental conditions.

Virus Assay

- Virus particles can be counted directly with the electron microscope.
- The most popular indirect method of counting virus particles is the hemagglutination assay (titer). Many viruses can bind to the surface of red blood cells.

- If the ratio of viruses to cells is large enough, virus particles will join the red blood cells together, forming a network that settles out of suspension or agglutinates.
- In practice, red blood cells are mixed with a series of virus preparation dilutions and each mixture is examined. The hemagglutination titer is the highest dilution of virus (or the reciprocal of the dilution) that still causes hemagglutination.

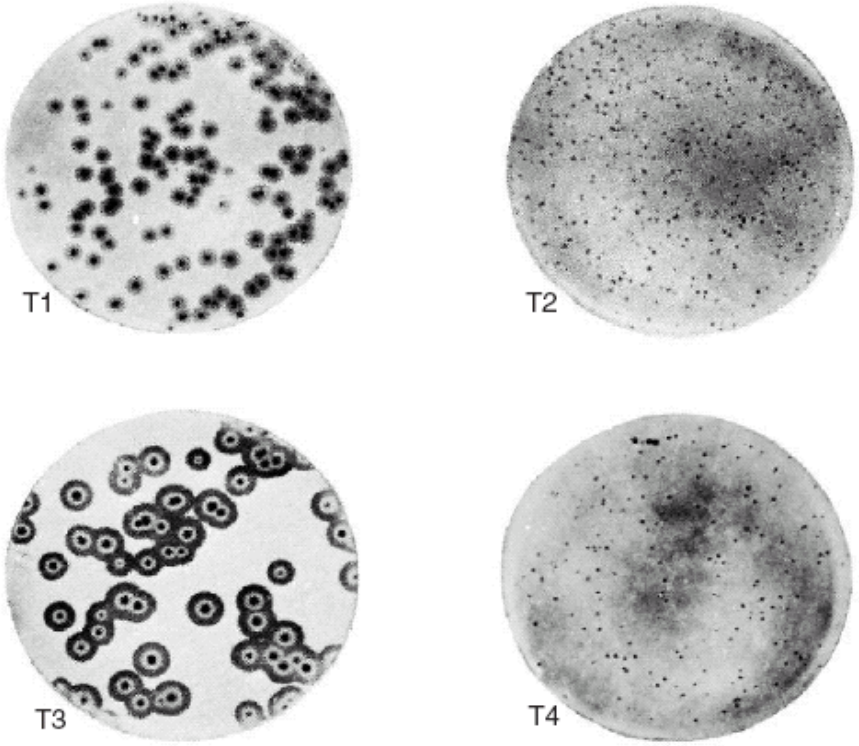
Virus Assay

- In the plaque assay, several dilutions of bacterial or animal viruses are plated out with appropriate host cells.
- Each plaque in a layer of bacterial or animal cells is assumed to have arisen from the reproduction of a single virus particle. Therefore a count of the plaques produced at a particular dilution will give the number of infectious virions or plaque-forming units (PFU).
- eg. Suppose that 0.10 ml of a 10^{-6} dilution of the virus preparation yields 75 plaques. The original concentration of plaque-forming units is:

$$\text{PFU/ml} = (75\ \text{PFU}/0.10\ \text{ml})(10^6) = 7.5 \times 10^8.$$

Virus Assay

- Viruses producing different plaque morphology types on the same plate may be counted separately.



Quantal Assay

- ID 50: 50% Infectious Doses (EID50 for egg): that dose of a given infectious agent which, when given to each of a number of experimental test systems or animals, brings about the infection of 50% of the systems/animals under given conditions.
- TCID50: 50% Tissue Culture Infectious Dosees
- LD50: 50% Lethal Doses (ELD50 for egg): that dose of a given lethal agent which, when given to each of a number of test animals, kills 50% of those animals under given conditions.
- Use Karber (5-5) or Read-Muench (8-8) statistical methods

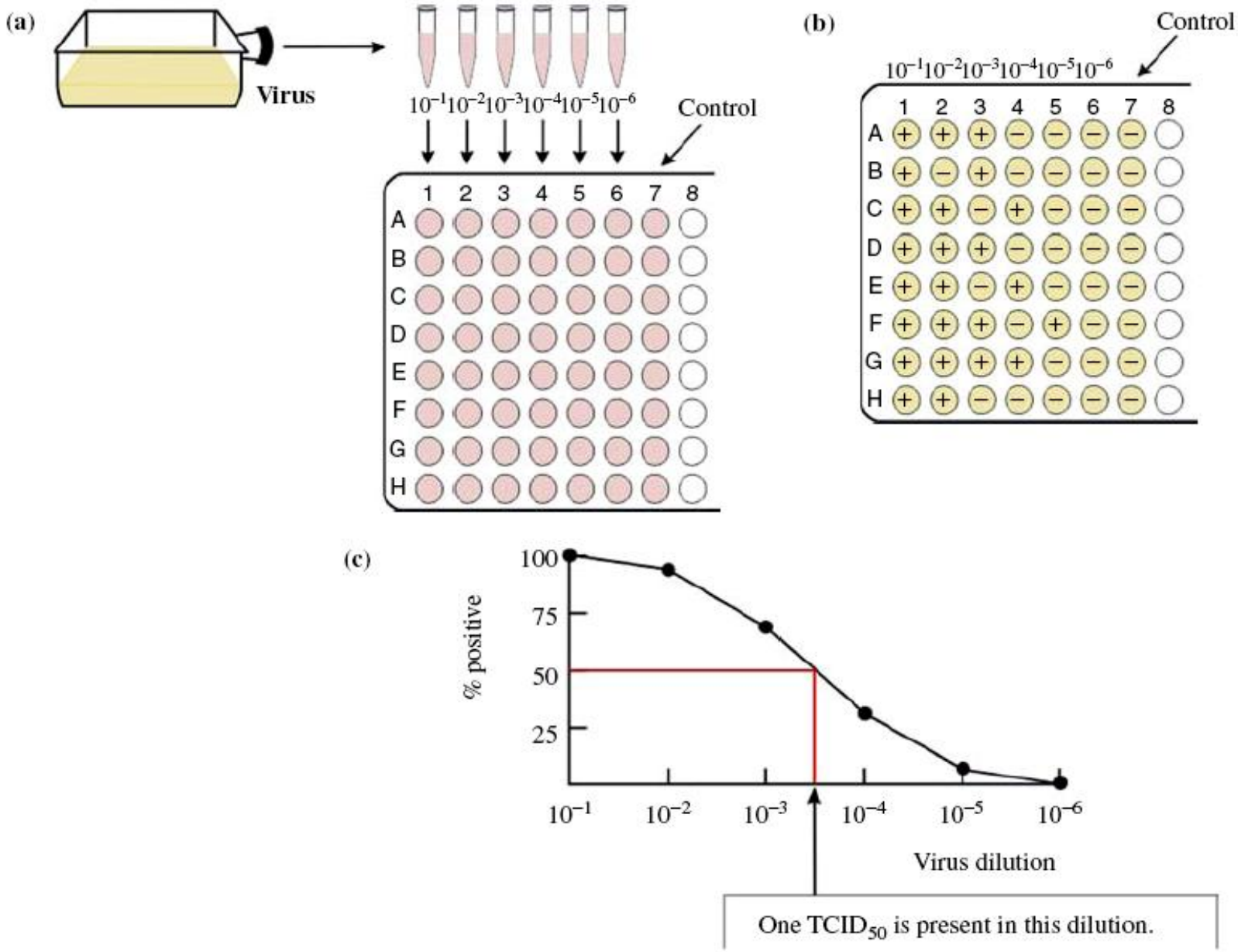


Figure 2.15 *Example of a $TCID_{50}$ assay.* (a) Tenfold dilutions of the virus were inoculated into cell cultures grown in the wells of a plate. Each well received 1 ml virus suspension, except for the control wells, which each received 1 ml diluent. (b) After incubation the cell culture in each well was scored '+' or '–' for CPE. (c) The results of the assay.

END-POINT DILUTION ASSAY: specimen data

Column A: dilution of virus	Column B: mortalities in 5 test animals	Column C: cumulative mortalities	Column D: cumulative survivors	Column E: C/(C + D)%
10^{-6}	0/5	0	9	0
10^{-5}	2/5	2	4	33.3
10^{-4}	4/5	6	1	85.7
10^{-3}	5/5	11	0	100

Reed–Muench method. The logarithm (to base 10) of the 50% end-point dilution lies between −5 and −4, and is obtained by linear interpolation – appropriate values from column E being substituted in the following equation:

$$\log_{10} 50\% \text{ end point} = -5 + \frac{50 - 33.3}{85.7 - 33.3}$$

The logarithm of the 50% end point is thus $10^{-4.68}$; since the antilog of 4.68 is ca. 48000 a dose from the 1/48000 dilution of the sample will contain the LD_{50}.

Kärber method. Substitutions are made in the following equation:

$$\log_{10} 50\% \text{ end point} = L_1 - L(S - 0.5)$$

in which L_1 = logarithm of lowest dilution tested; L = log interval between dilutions; S = sum of the proportion of positive reactions at each dilution; 0.5 is a constant. In the accompanying table, $S = 0 + 0.4 + 0.8 + 1.0 = 2.2$; hence, the required value is $10^{-4.7}$.

Virus Assay: Quantitaitive

- Quantitative PCR: use real-time PCR