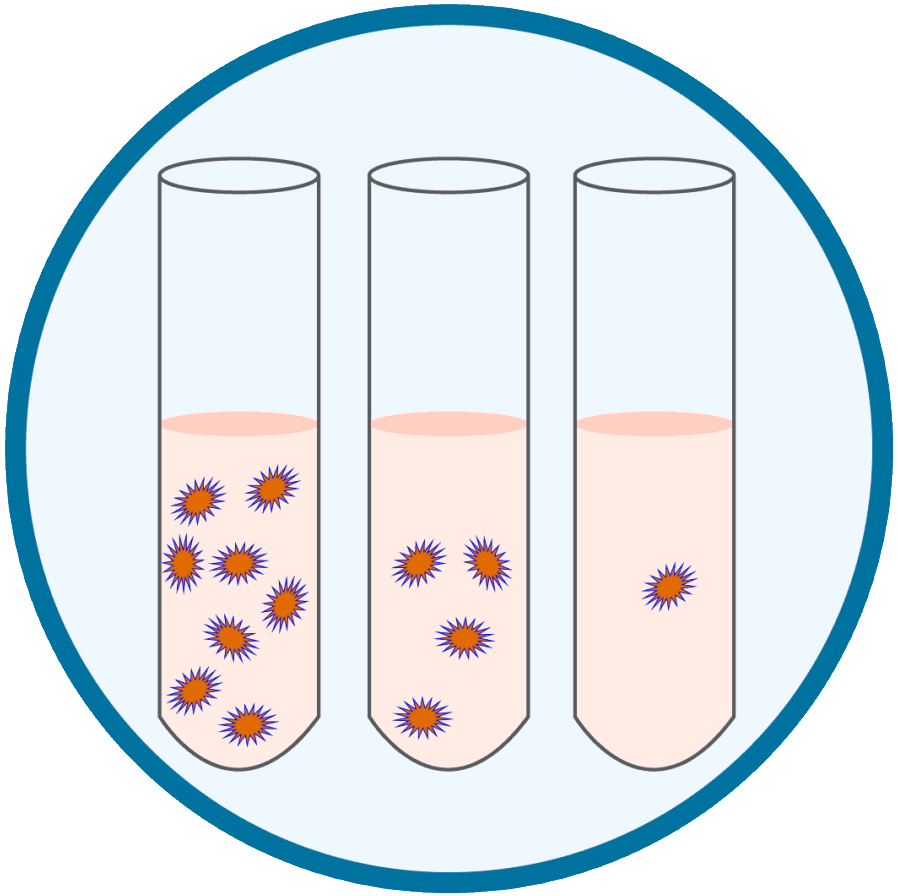
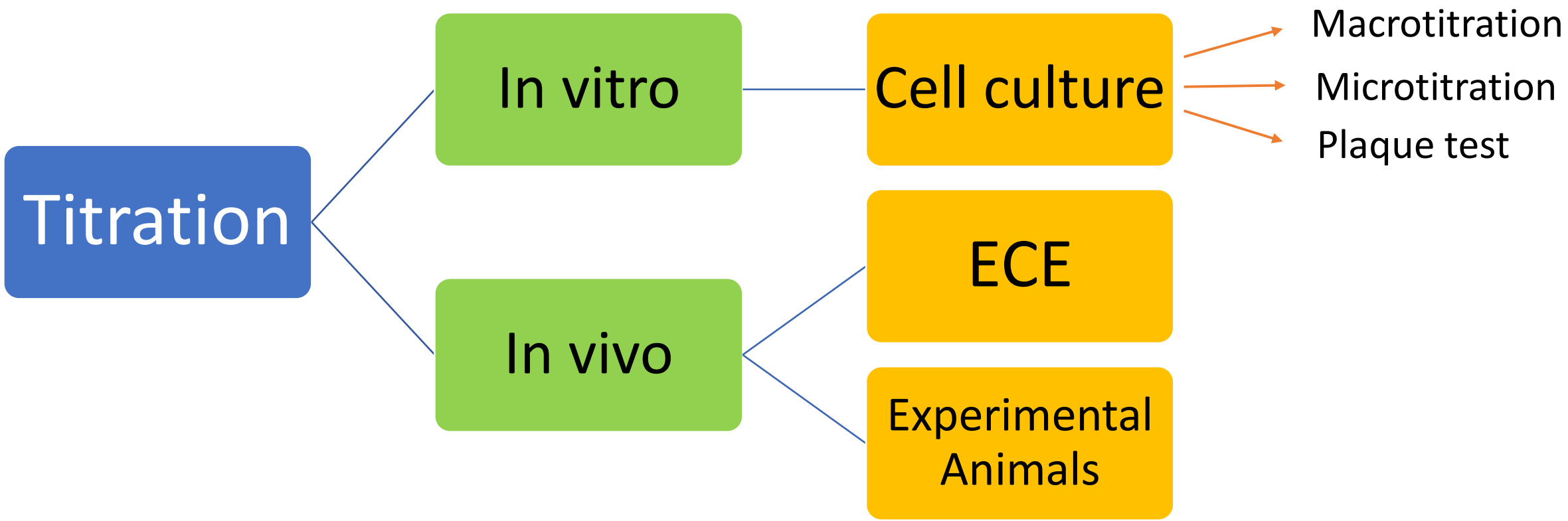
TITRATION IN VIRUSES



- **VIRUS TITER**: Numerical expression of the infectious power of a virus suspension
  - It expresses the **number of infectious virions** contained in a specific volume of virus suspension.

- Detection of infectious power of a virus is called TITRATION of that virus.



There are two types of titration metods according to the host system.

- In vitro performed in a test tube, culture dish, or elsewhere outside a living organism.
- In vivo performed in a living organism.

UNITS

- $TCID_{50}$: Tissue Culture Infective Dose 50=50%
  - Virus dilution that causes infection in the half of the host systems (cell culture) used in the test is determined and this expresses the infective power of the virus.

- EID_{50}: Egg Infective Dose 50=%50
- LD_{50}: Lethal Dose 50=%50 (for experimental animals)
- MID_{50}: Minimum Infective Dose 50=%50 (for experimental animals)

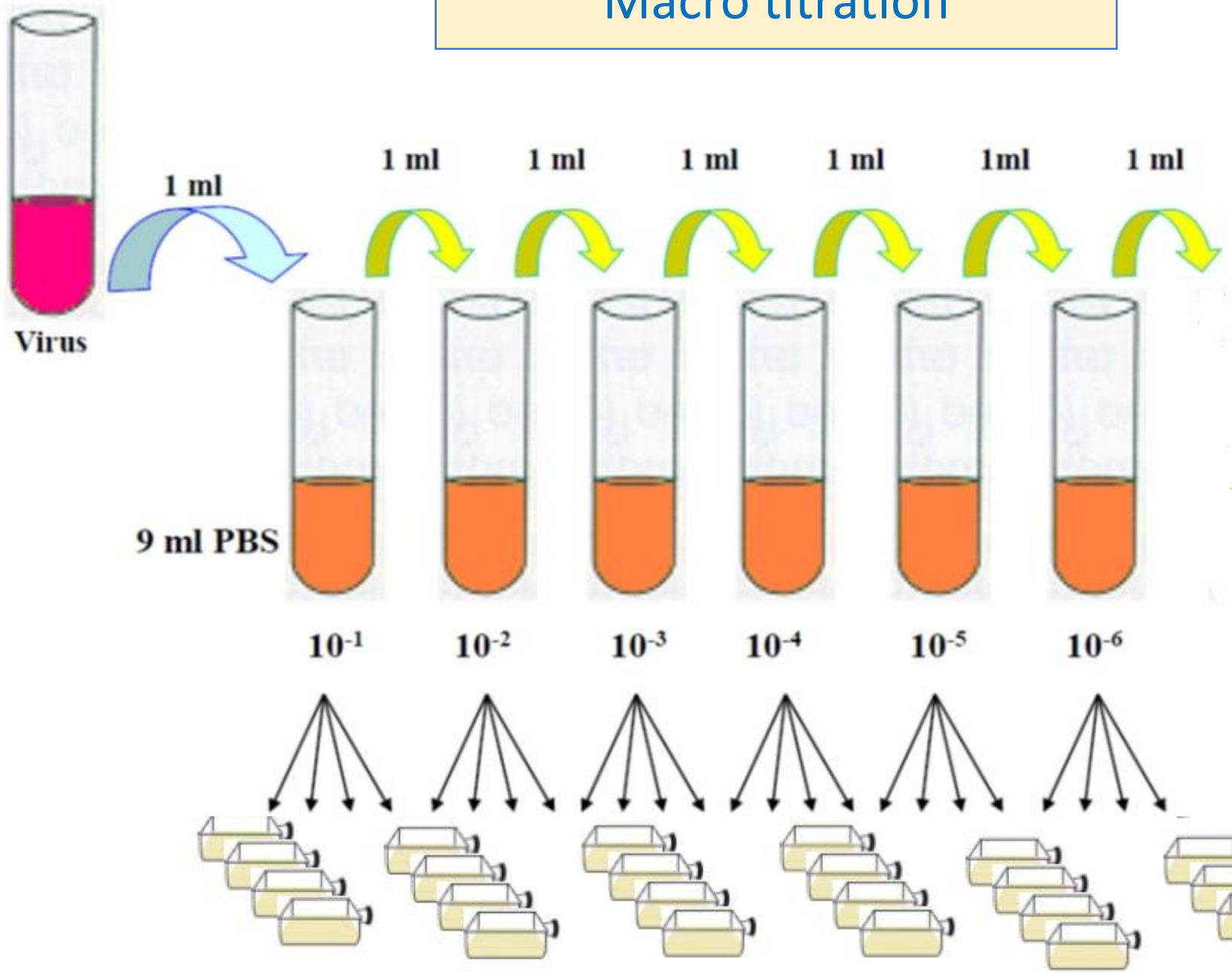
Why do we need to quantify viruses?

1. Standardization of viruses to be used in virological and serological studies (eg, neutralization test 100$TCID_{50}$)
2. Quantification and calculation the dose of virus for vaccine preparation
3. Evaluation of physico-chemical tests used in identification of viruses
4. Determination of inactivation kinetics
5. Purification of virus

Macro Titration

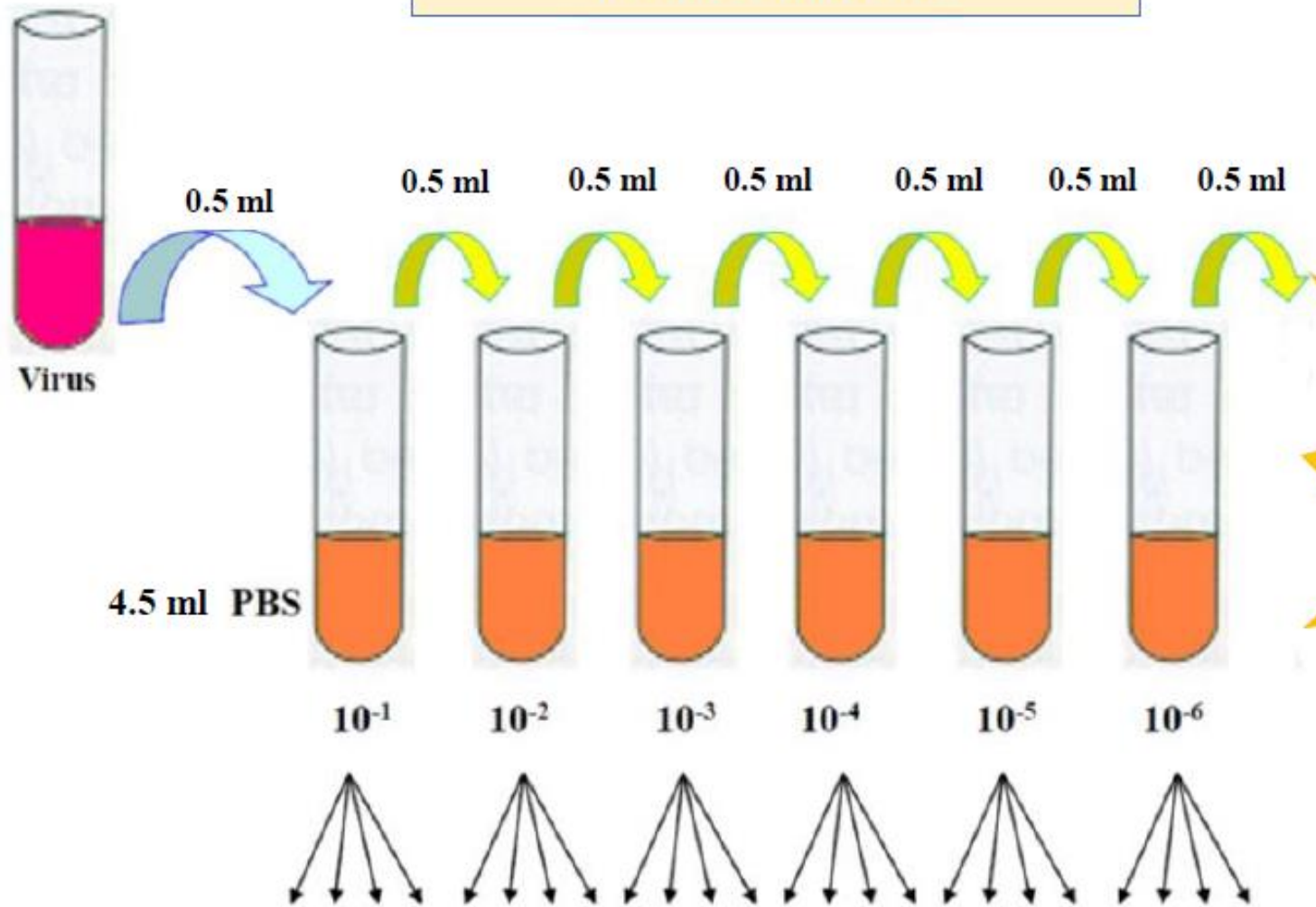
- Working in biosafety cabinet, prepare the diluting fluid which is PBS and dispense 9 ml in test tubes labelled 10^{-1} to 10^{-6} and keep the test tubes in rack immersed in plenty of ice.
- To make 10 fold (log_{10}) dilutions of the virus, dilute 1ml of virus in 9 ml of diluent (PBS) to get the initial dilution i.e. 10^{-1} (1/10).
- From 10^{-1} dilution pipette 1 ml and add to the next tube = 10^{-2} (1/100).
- It goes on $1/10^{-3}$, $1/10^{-4}$ etc. Dilutions are obtained.
- **Change your pipette** at each step (before pipetting) to avoid any carry-over of virus!
- 1ml from the last tube will thrown out.
- Each virus dilution are inoculated to four cell cultures flasks.
- Incubate in the 37^{o}C incubator and then evaluated according to CPE formation.
- The titer is calculated according to Reed-Muench or Spearman-Kaerber Method.

Macro titration

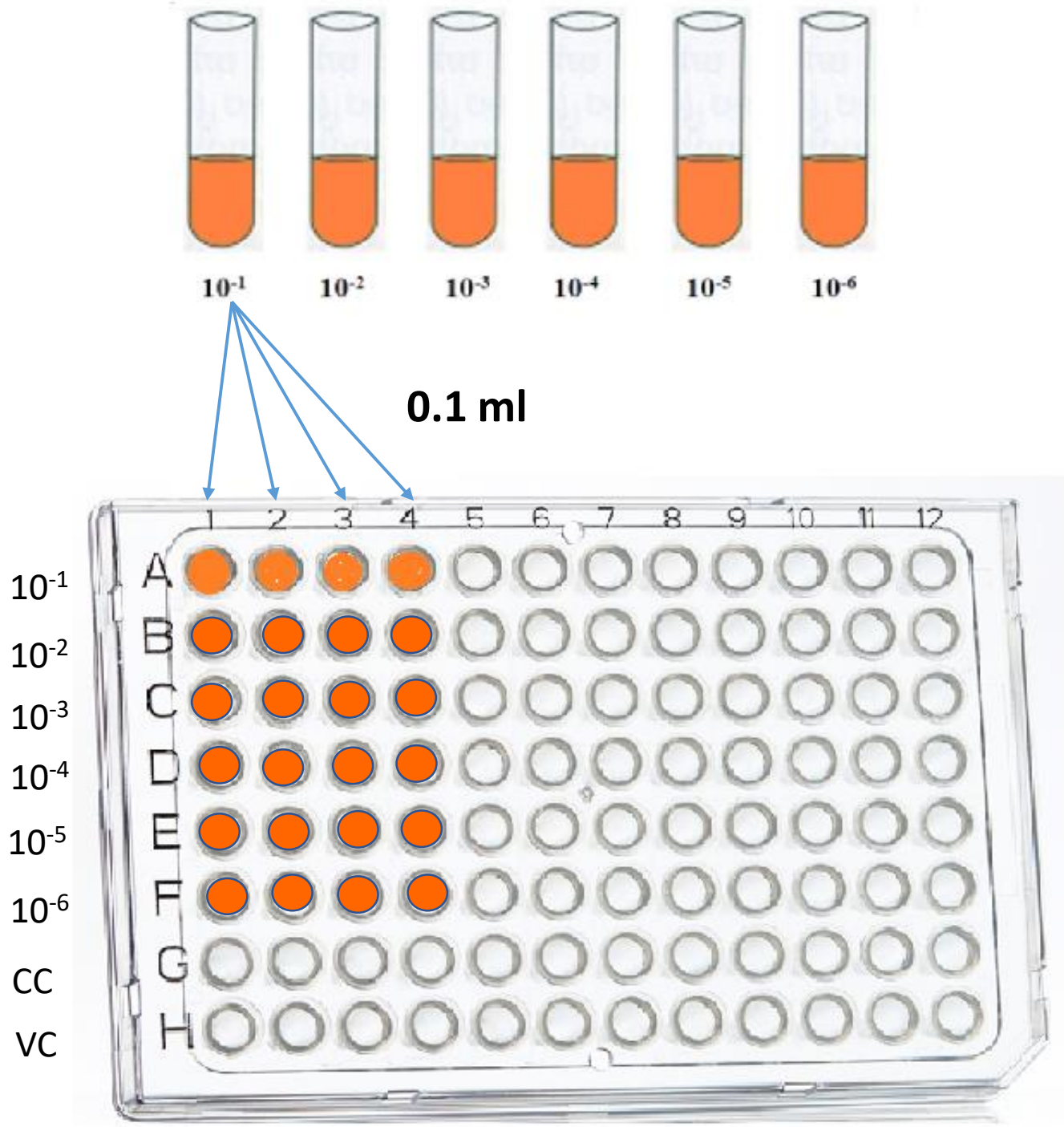


Remember to **change your pipette** at each step (before pipetting) to avoid any carry-over of virus!

Micro titration



Remember to **change your pipette** at each step (before pipetting) to avoid any carry-over of virus!


10-1
10-2
10-3
10-4
10-5
10-6
0.1 ml
1 2 3 4 5 6 7 8 9 10 11 12
A
B
C
D
E
F
G
H
10-1
10-2
10-3
10-4
10-5
10-6
CC
VC

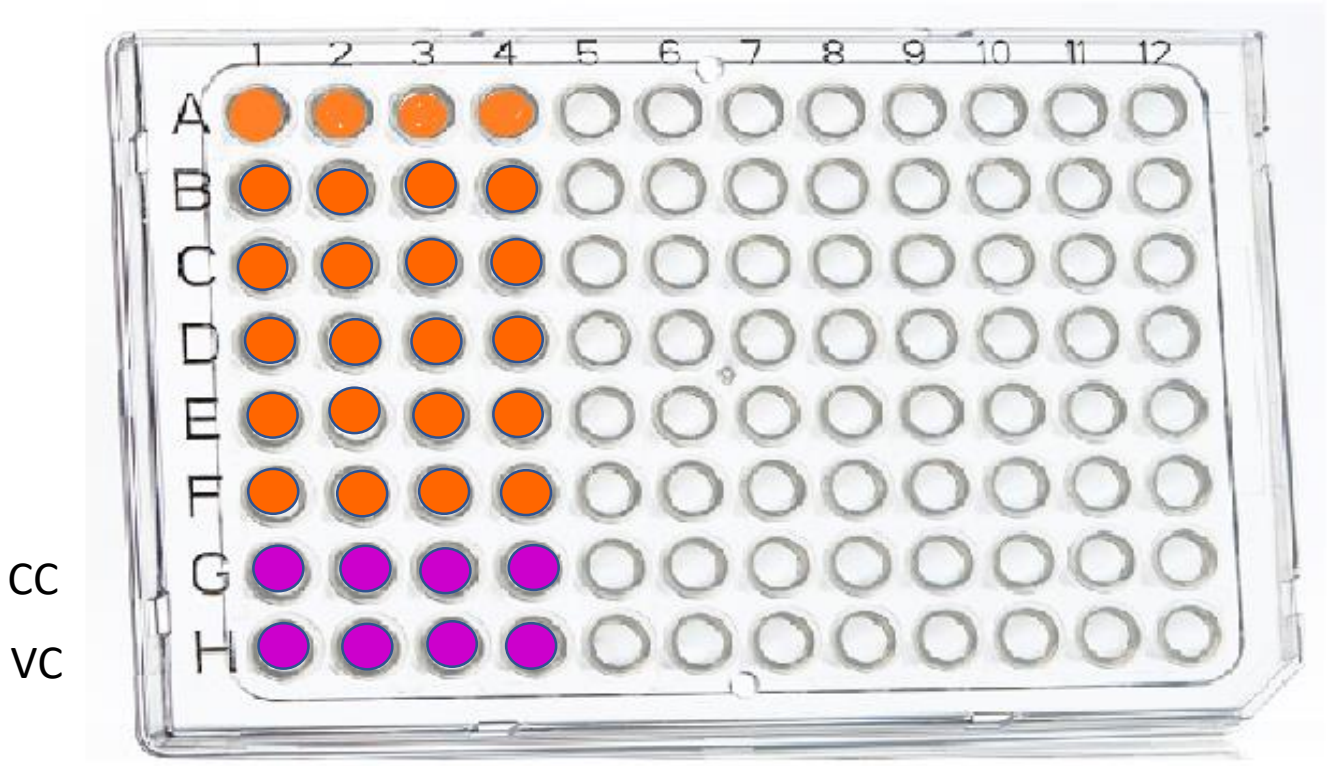
CC: Cell Control

- 0.1 ml Earle with Sera

VC: Virus Control

- 0.05 ml VIRUS
- 0.05 ml Earle without Sera

Finally, 0.05 ml (1 DROP) cell suspension (300,000 cells per ml to ALL WELLS (BY DROPPER!)



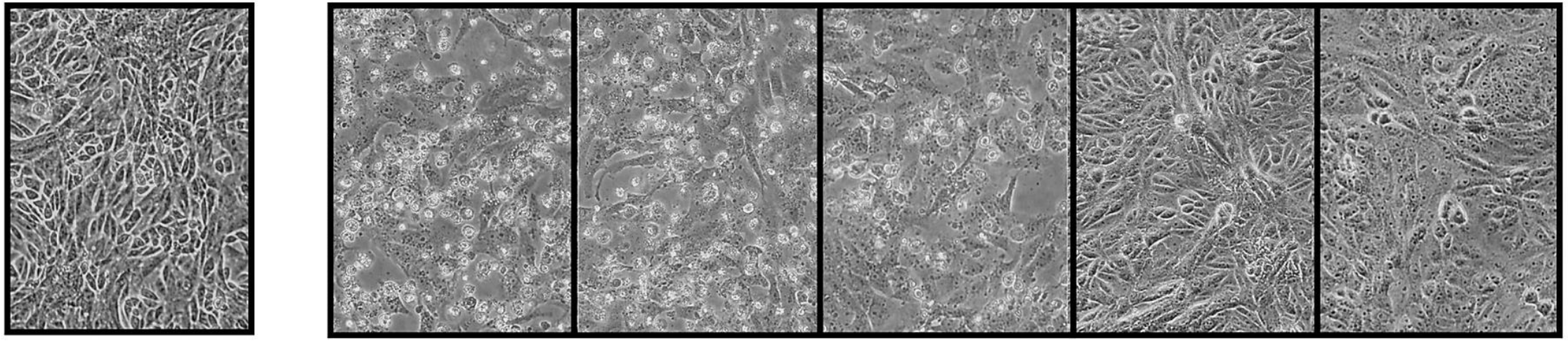
- Test is over

37°C ve %5 CO_2 incubator

We examine it every day under an invert microscope.

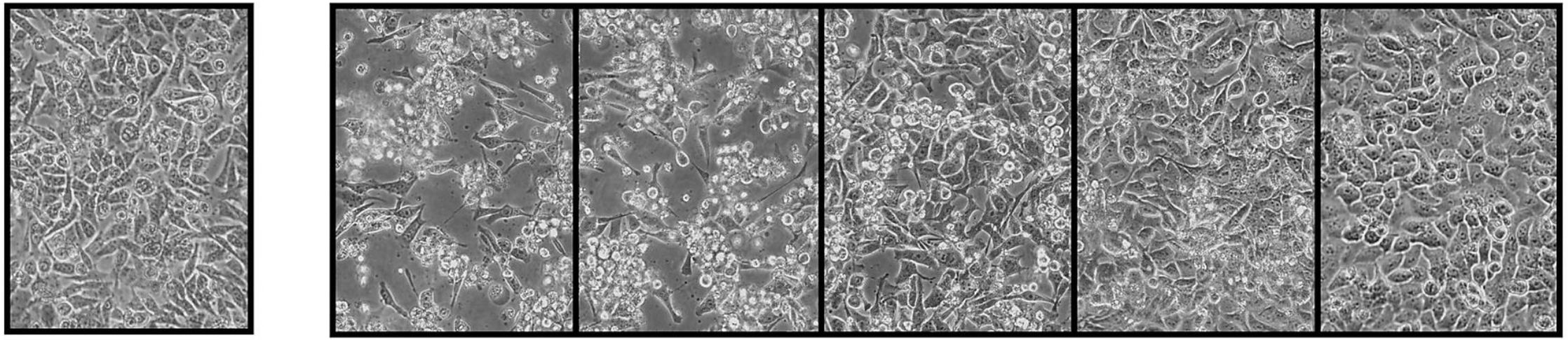
CPE

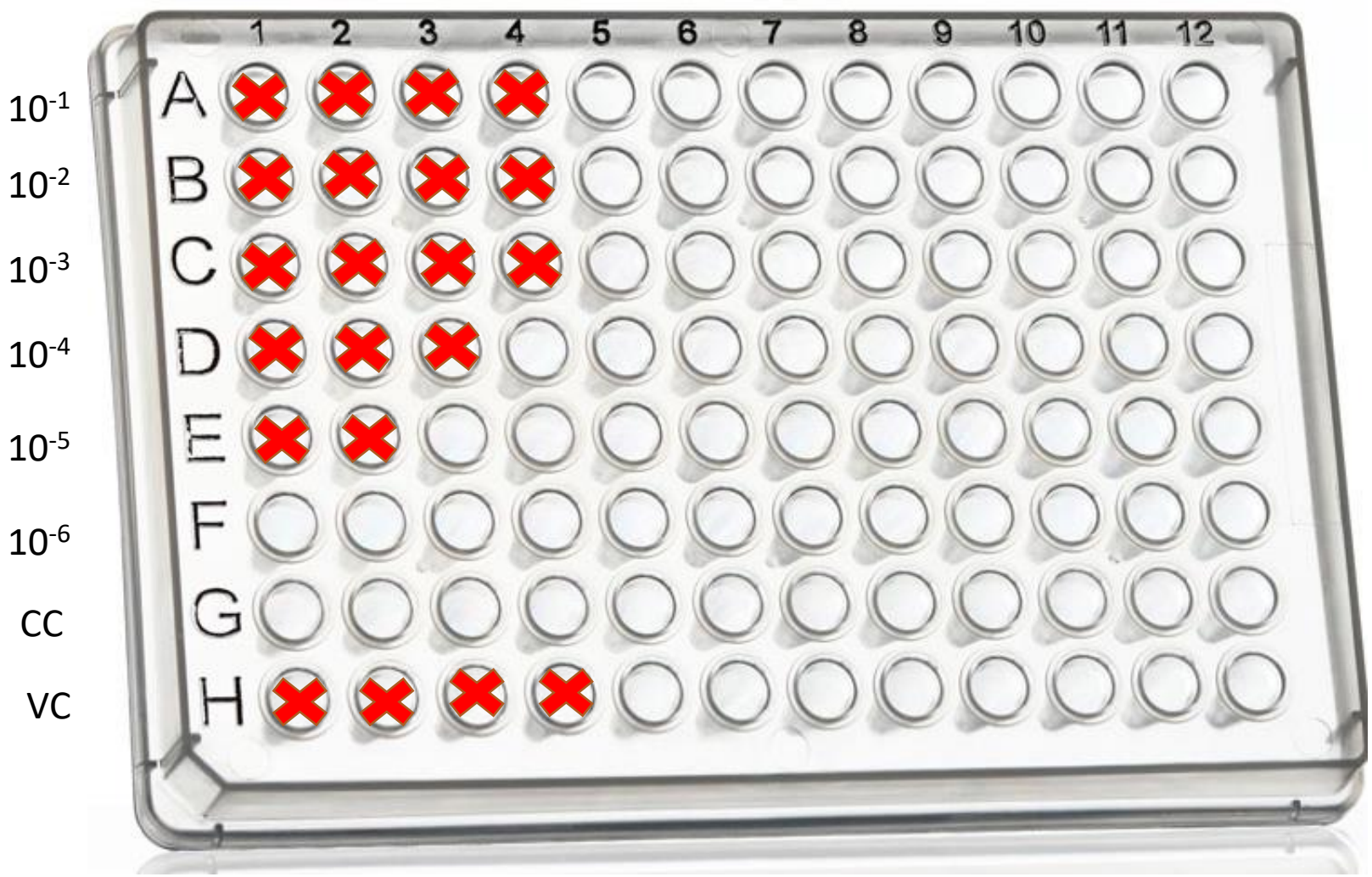
Cell Control	10^{-1}	10^{-2}	10^{-3}	10^{-4}	10^{-5}



Decrease CPE !!!!!!

Cell Control	10^{-1}	10^{-2}	10^{-3}	10^{-4}	10^{-5}




1 2 3 4 5 6 7 8 9 10 11 12
A
B
C
D
E
F
G
H
10-1
10-2
10-3
10-4
10-5
10-6
CC
VC

Calculation

- $TCID_{50}$ Virus dilution forming CPE at least half of the systems inoculated.
- Spearman & Kaerber Methods

$$\text{Log10 DKID}_{50} = [\, X_o - d/2 + d \times (r/n) \,]$$

  - X0 = last CPE dilution seen in cpe in all wells
  - d = log10 of dilution coefficient $\longrightarrow Log_{10}10 = 1$
  - r = sum of all positives
  - n = number of wells used for each dilution
- Reed & Müench Methods

$$\text{Log10 DKID}_{50} = [\, X_o - d/2 + d \times (r/n) \,]$$

Sulandırma Basamağı	CPE/Göz sayısı
10^{-1}	4 / 4
10^{-2}	4 / 4
10^{-3}	4 / 4
10^{-4}	3 / 4
10^{-5}	2 / 4
10^{-6}	0 / 4
HK	0 / 4
VK	4 / 4

X_0 = last CPE dilution seen in CPE in all wells
d = $\log_{10}$ of dilution coefficient
r = sum of all positives
n = number of wells used for each dilution

$$\log10\ \text{DKID}_{50} = [\,(3 - ½ + 1 \times (9/4)\,]$$

$$\log10\ \text{DKID}_{50} = -4{,}75$$

$$\text{DKID}_{50} = 10^{-4{,}75} / 0.1\ \text{ml}$$

$$100\text{DKID}_{50} = 10^{-2{,}75} / 0.1\ \text{ml}$$

- Easy calculation

10^{0} 4/4 (not diluted virus)
10^{-1} 4/4
10^{-2} 4/4
10^{-3} 4/4
10^{-4} 3/4
10^{-5} 2/4
10^{-6} 0/4

$100TCID_{50} = 10^{-2,75}/0,1ml$

$TCID_{50} = 10^{-4,75}/0,1ml$

Total CPE : 21 21-2= 19 19 ÷ 4 = 4,75

↓ Constant

↓ used well number

Plaque Assay/Test

- Depending on the virus, the type of cells and the readout parameter indicating an infection, a variety of other virus titration assays are possible.
- For viruses that lyse the infected cell, for example, a plaque forming assay is commonly employed for quantification.

What is plaque?

- It identifies the limited virus reproduction areas in cell cultures.
- Plaque assays are the standard method that have long been used to determine the virus titer (i.e, infectious dose).
- Typically, 10-fold serial dilutions of the virus stock are inoculated into a monolayer-forming cell culture in a plate and incubated over several days.
- Areas with infected cells will be visible as holes or plaques either by the microscopy, or by colorimetric or fluorometric staining.
- Virus quantity is expressed as infectious units (IFU) / ml or plaque forming units (PFU) / ml.

https://doi.org/10.1016/B978-0-12-800838-6.00004-7

- Note that plaque assay is restricted to the viruses that induce cell lysis or death, such as picornavirus, influenza virus, and herpesvirus.

- The virus infected cell will lyse and spread the infection to adjacent cells. As the infection-to-lysis cycle is repeated, the infected cell area will form a plaque which can be seen visually.

- Three types of plaque

1. Lytic: middle of it is empty.
2. Degenerative: There are degenerated cells in the center.
3. Proliferative: There are dense cell proliferations in the centers.

1. Prepare 10 fold (log_{10}) dilutions of the virus.

2. Each virus dilution is inoculated into two cell culture produced in petri dishes or 24-wells plate.

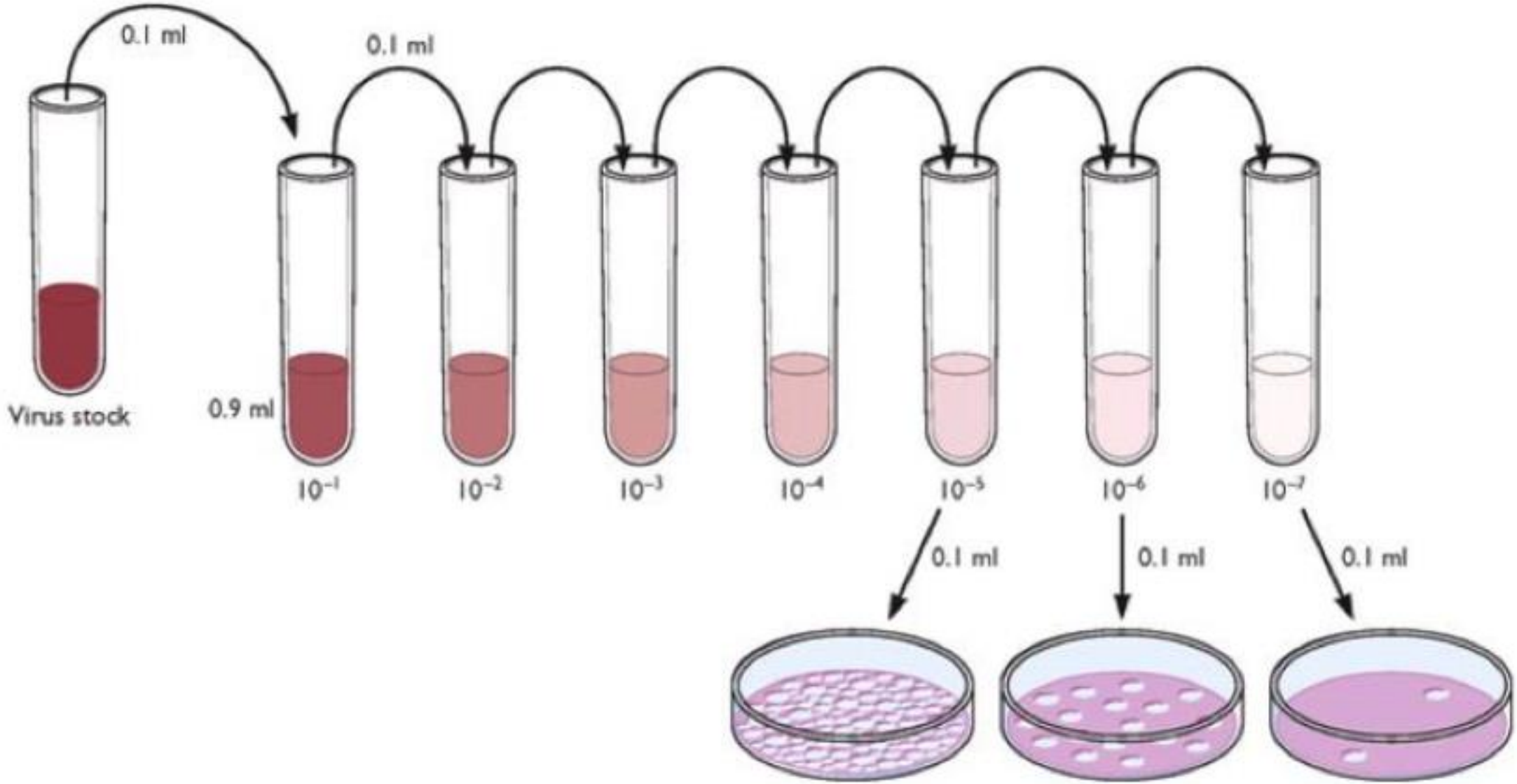
3. The virus is inoculated into the cells cultures with adsorption method.

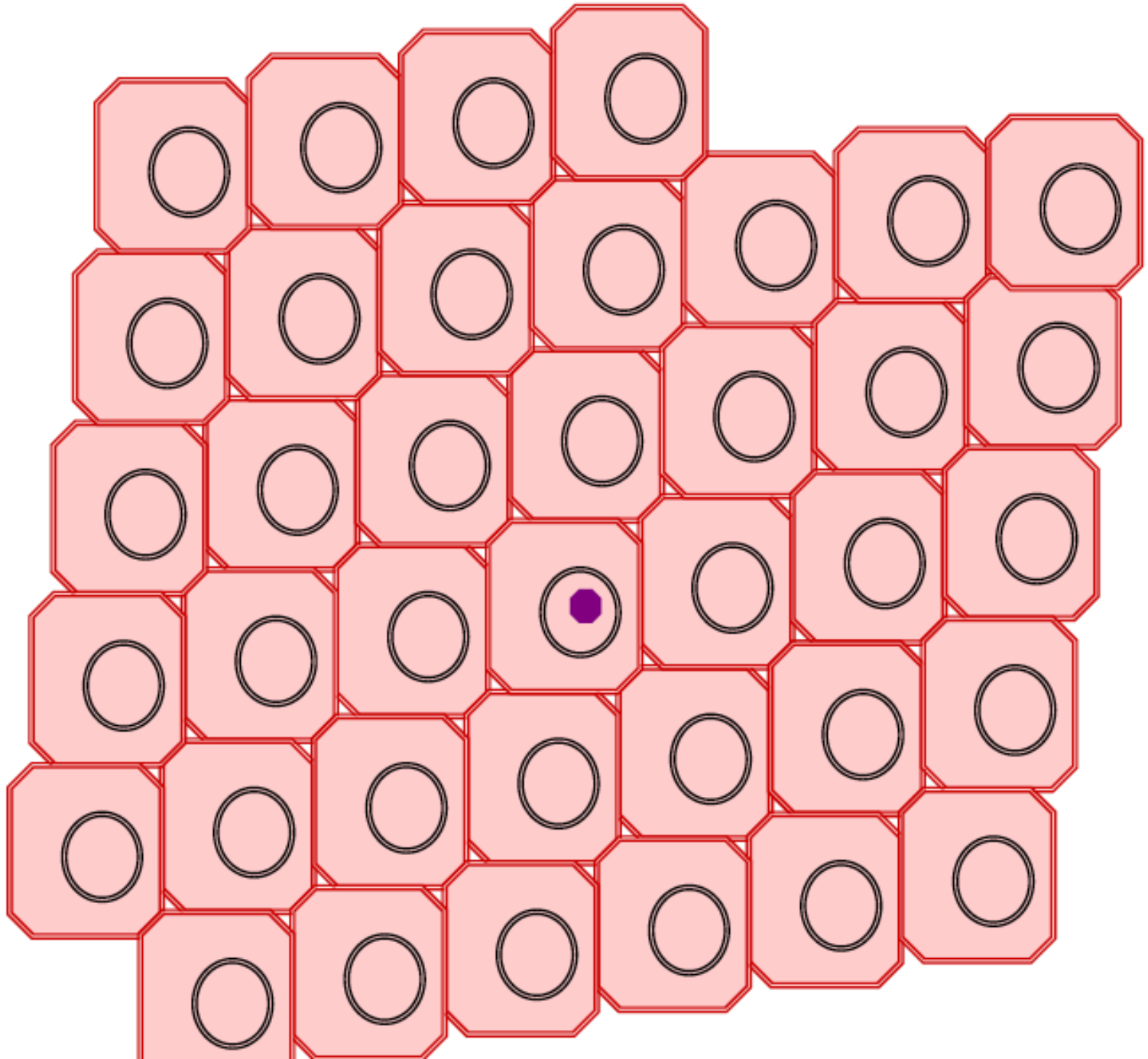
4. After incubation (1 hour), 2xEarle+1,8-2% Noble Agar is used as a virus growing media.

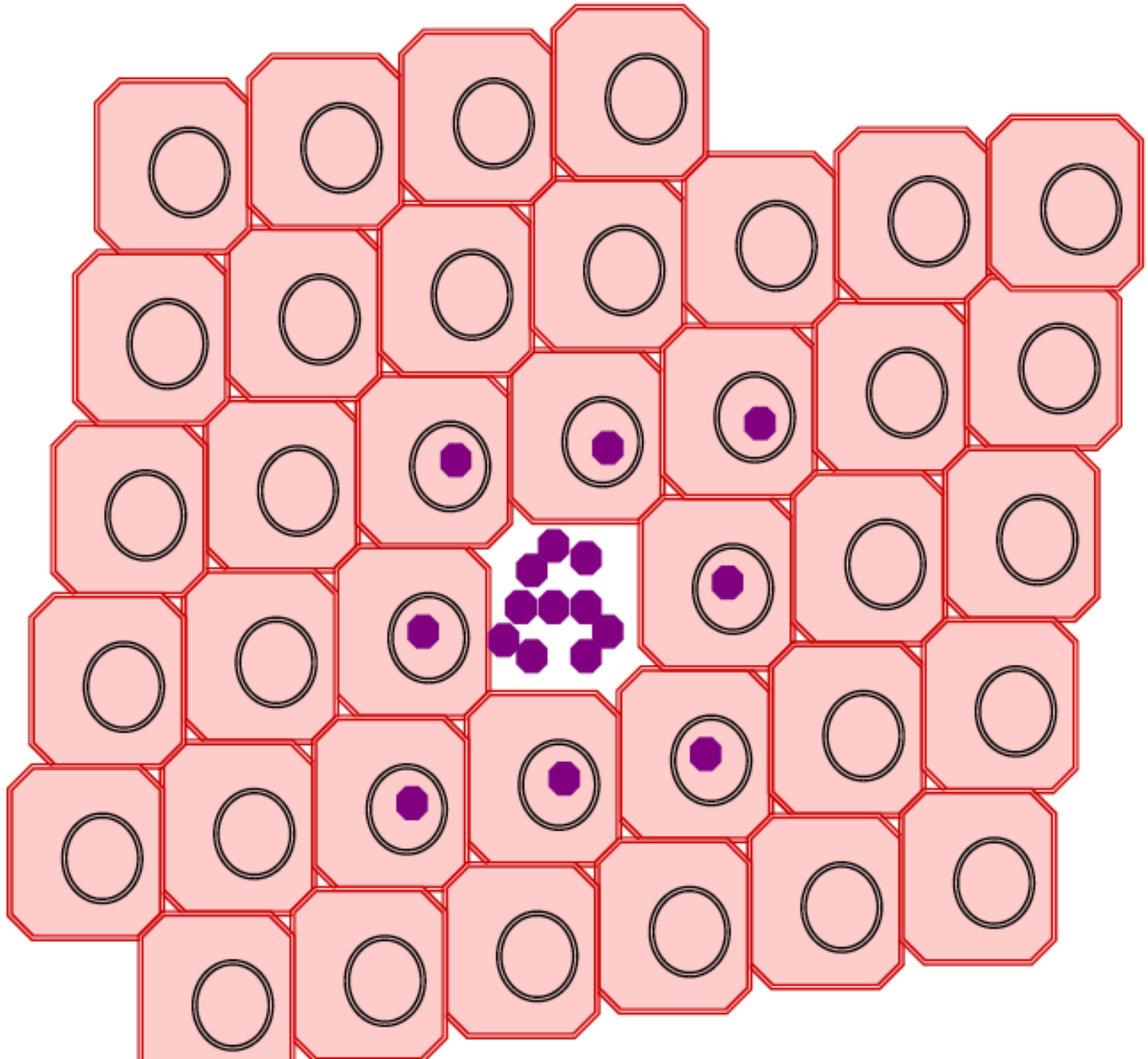
5. Incubate the plate(s) to a humidified incubator at 37°C and with CO_2.

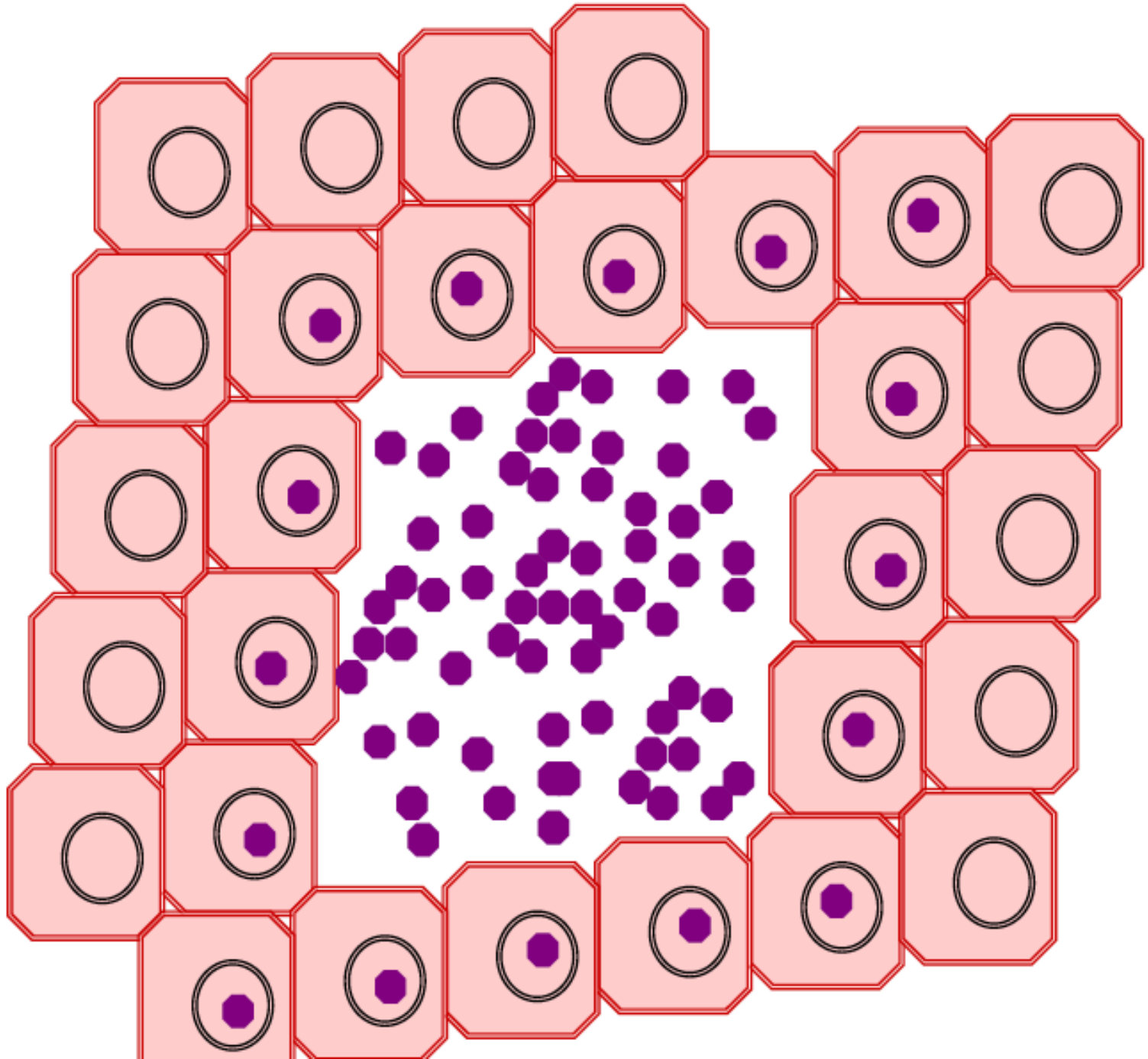
6. Sometimes Neutral Red, Crystal Violet is used to dye plates for better viewing.

7. Plate Formation Unit (PFU) is calculated.


0.1 ml
0.1 ml
Virus stock
0.9 ml
10^{-1}
10^{-2}
10^{-3}
10^{-4}
10^{-5}
10^{-6}
10^{-7}
0.1 ml
0.1 ml
0.1 ml



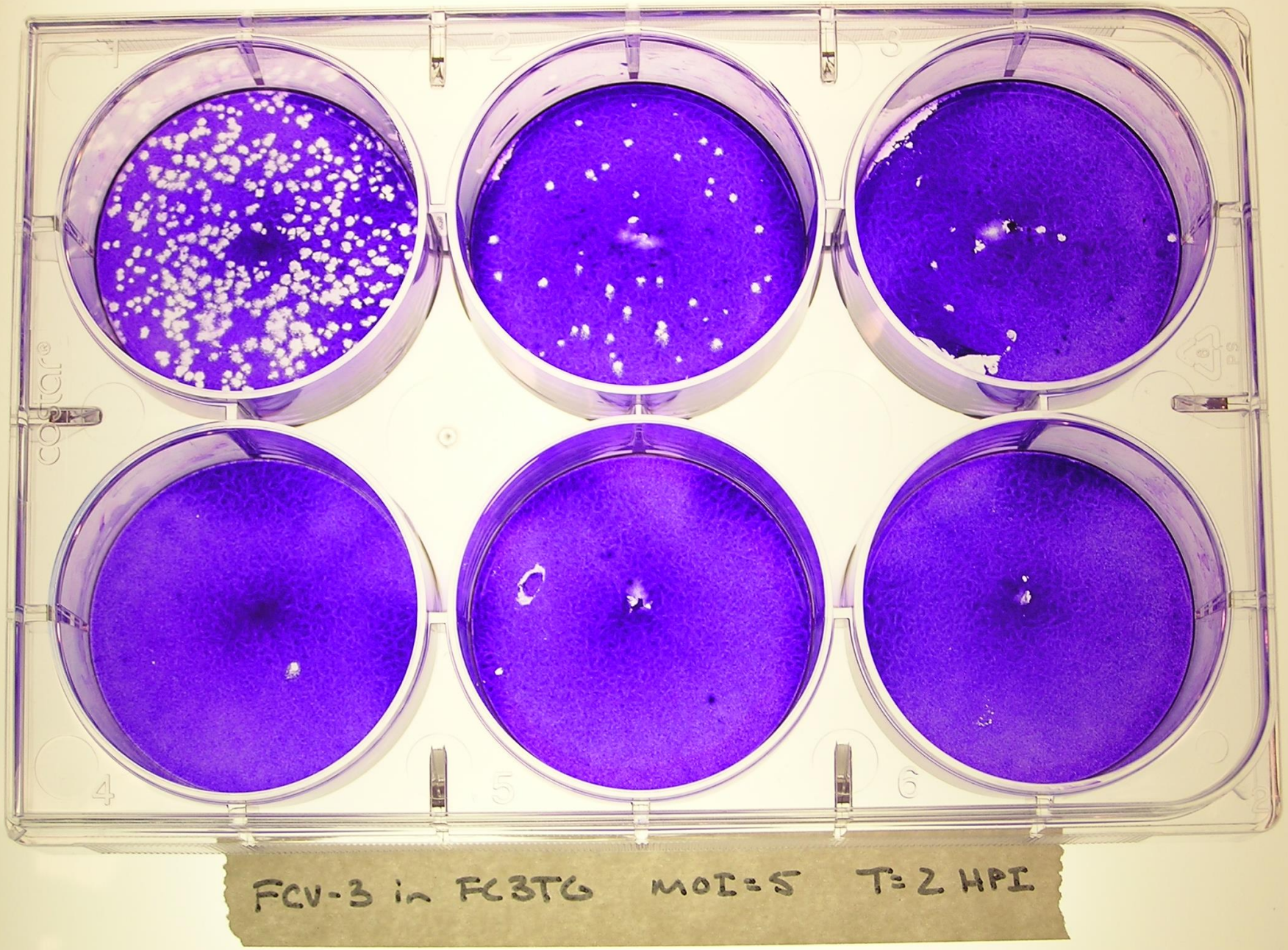




- The PFU/mL result represents the number of infectious particles in the sample, assuming that each plaque is caused by a single infectious virus particle.

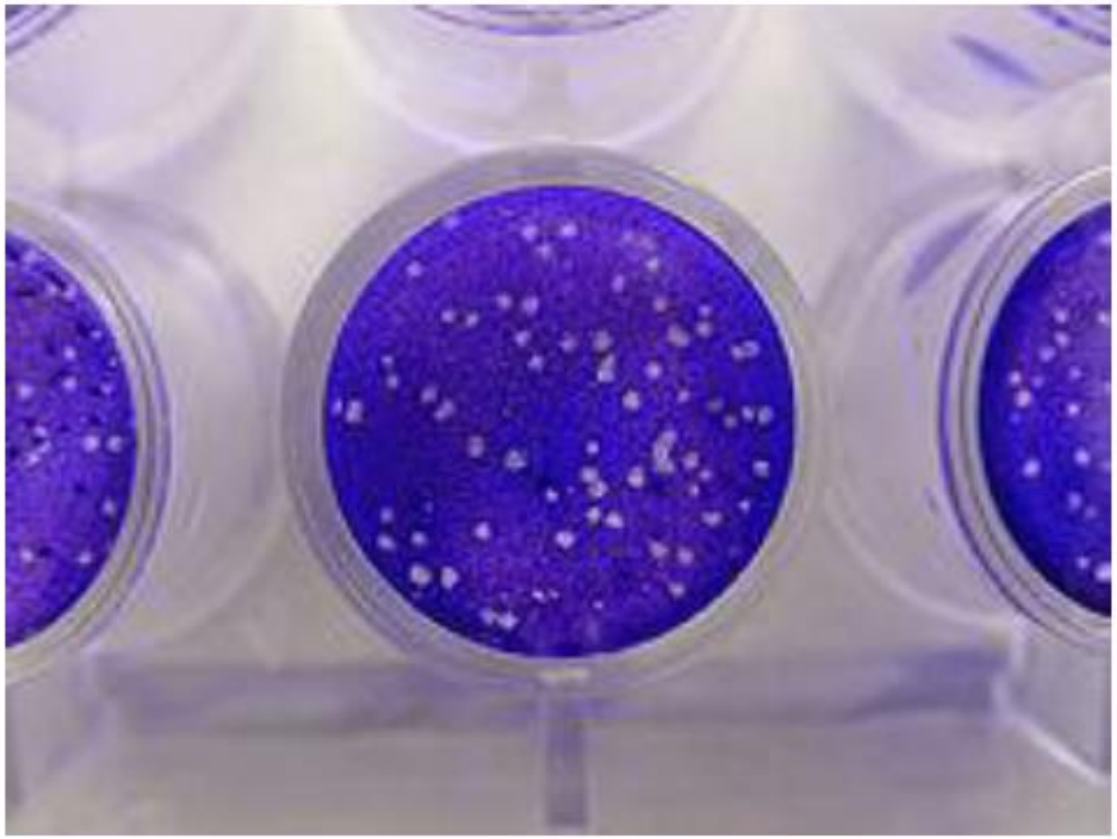
$$\text{PFU/ml} = \frac{\#\ \text{of}\ \text{Plaques}}{\text{Dilution Factor x Volume of Virus (in ml)}}$$

Example: $\frac{35\ \text{plaques}}{10^{-4} \times 0.1\ \text{mL}} = 3{,}500{,}000\ \text{pfu/mL}$ or $3.5 \times 10^{6}\ \text{pfu/mL}$

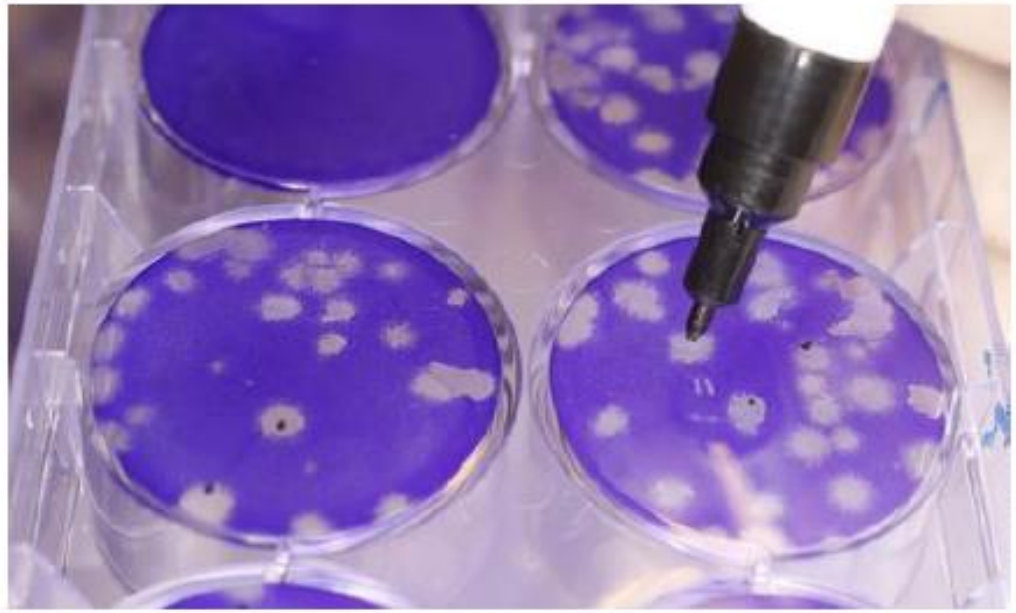


https://www.vet.cornell.edu/image/calicivirus-plaques-jpg-0

each of the clear holes in the blue monolayer of cells is called a plaque and represents an area of virus infection.



https://en.wikipedia.org/wiki/Virus_quantification



http://www.lumacyte.com/applications/rapidviraltiter/