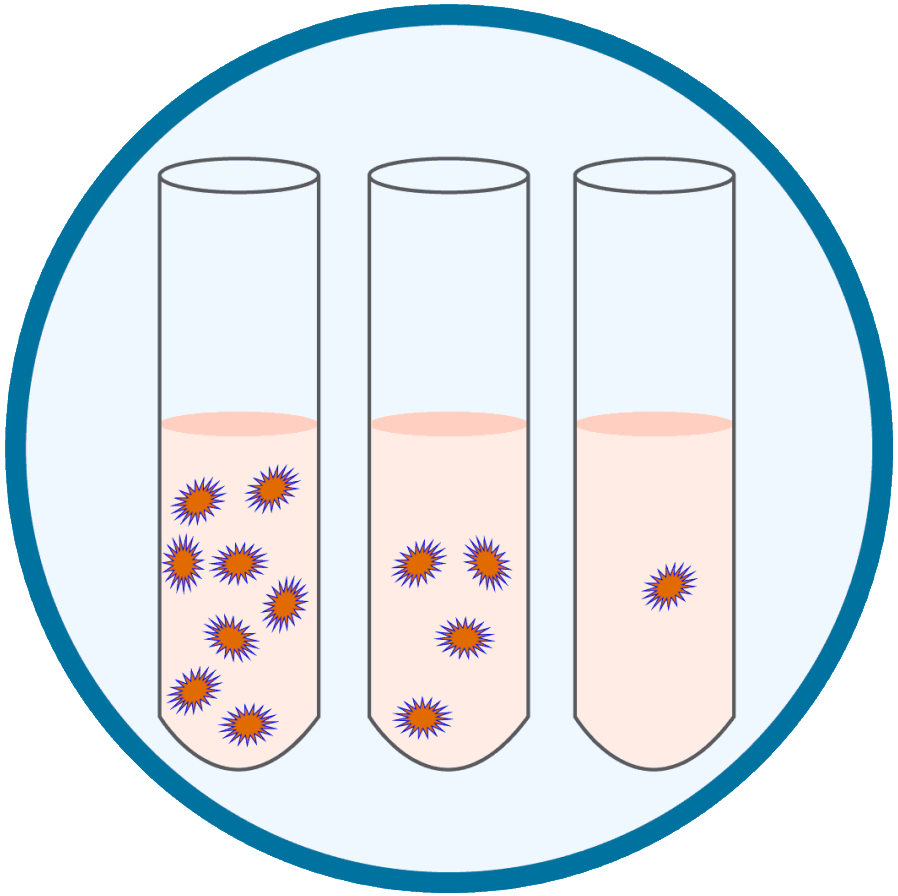
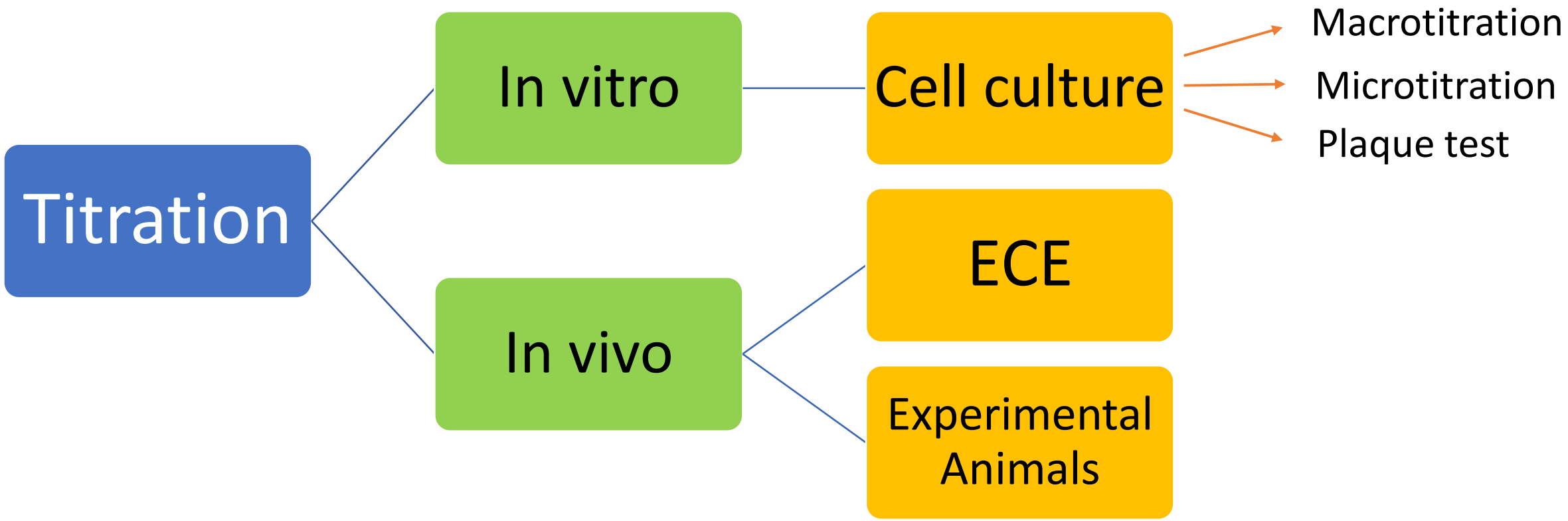
TITRATION IN VIRUSES



- **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.


Titration
In vitro
In vivo
Cell culture
ECE
Experimental Animals
Macrotitration
Microtitration
Plaque test

UNITS

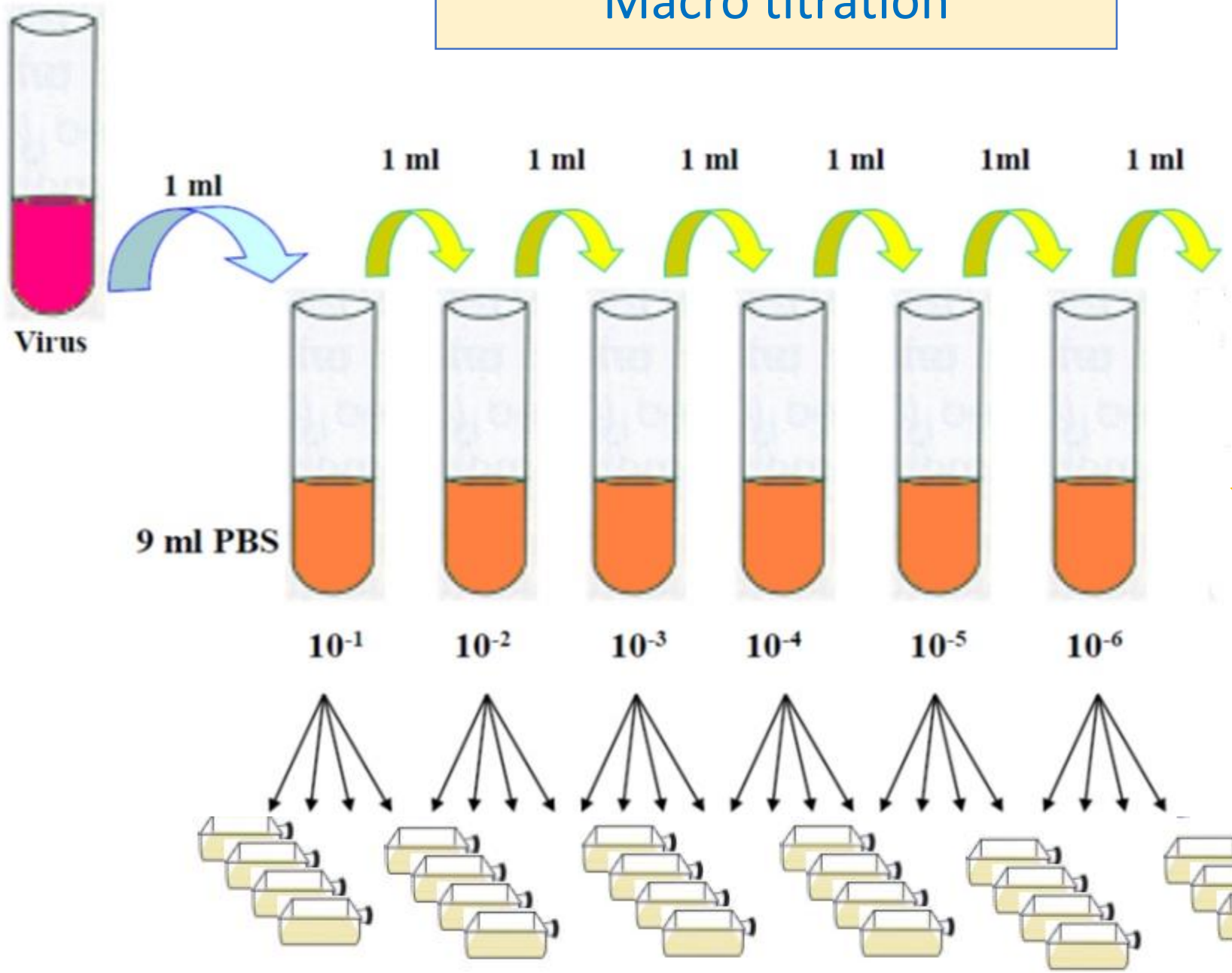
- $TCID_{50}$: Tissue Culture Infective Dose 50=50%
  - Virus dilution that causes infection in the half of the host systems (cell culture) used in the test is determined and this expresses the infective power of the virus.

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

Why do we need to quantify viruses?

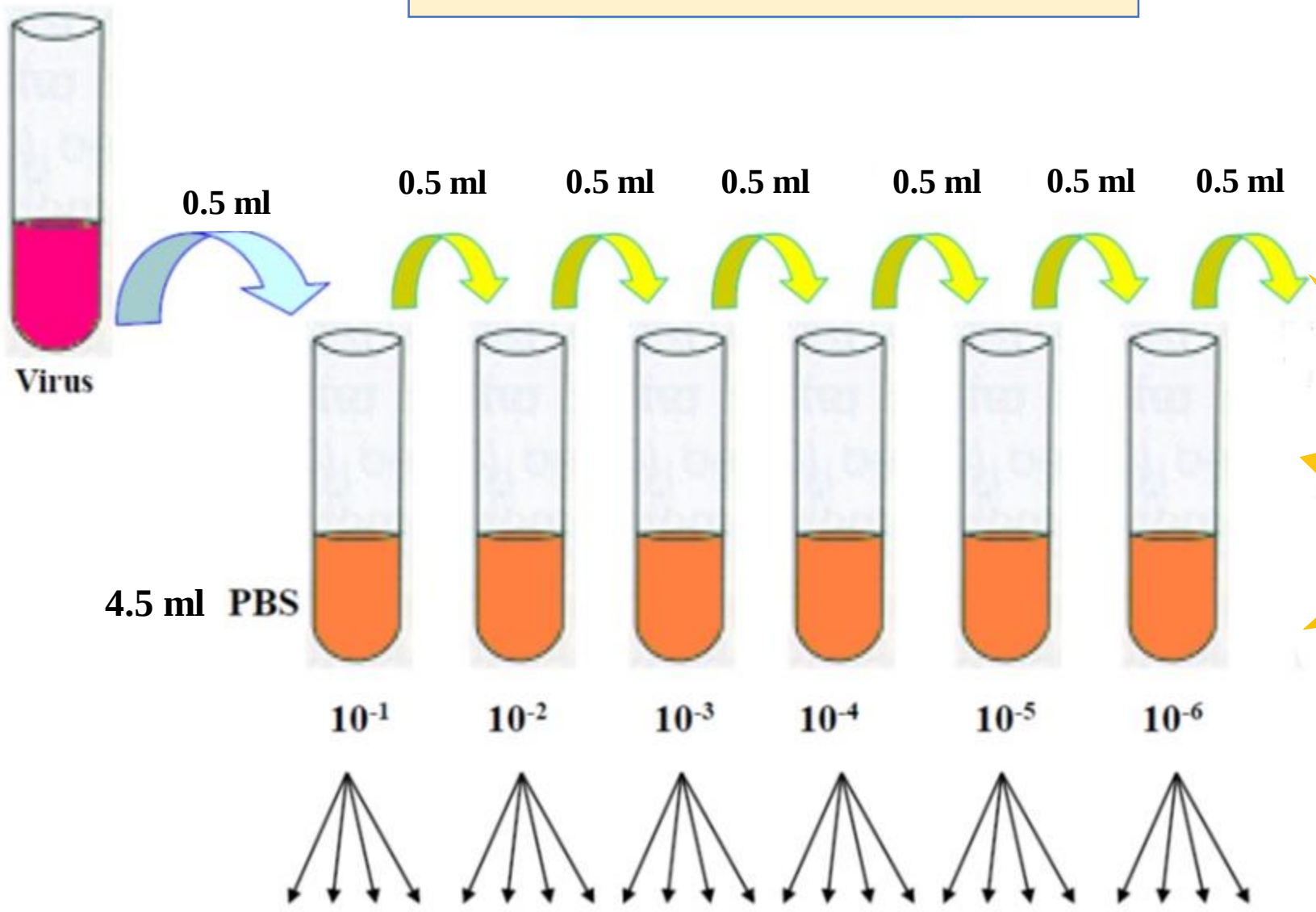
1. Standardization of viruses to be used in virological and serological studies (eg, neutralization test)
2. Quantification and calculation the dose of virus for vaccine preparation
3. Evaluation of physico-chemical tests used in identification of viruses
4. Determination of inactivation kinetics
5.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

Macro titration

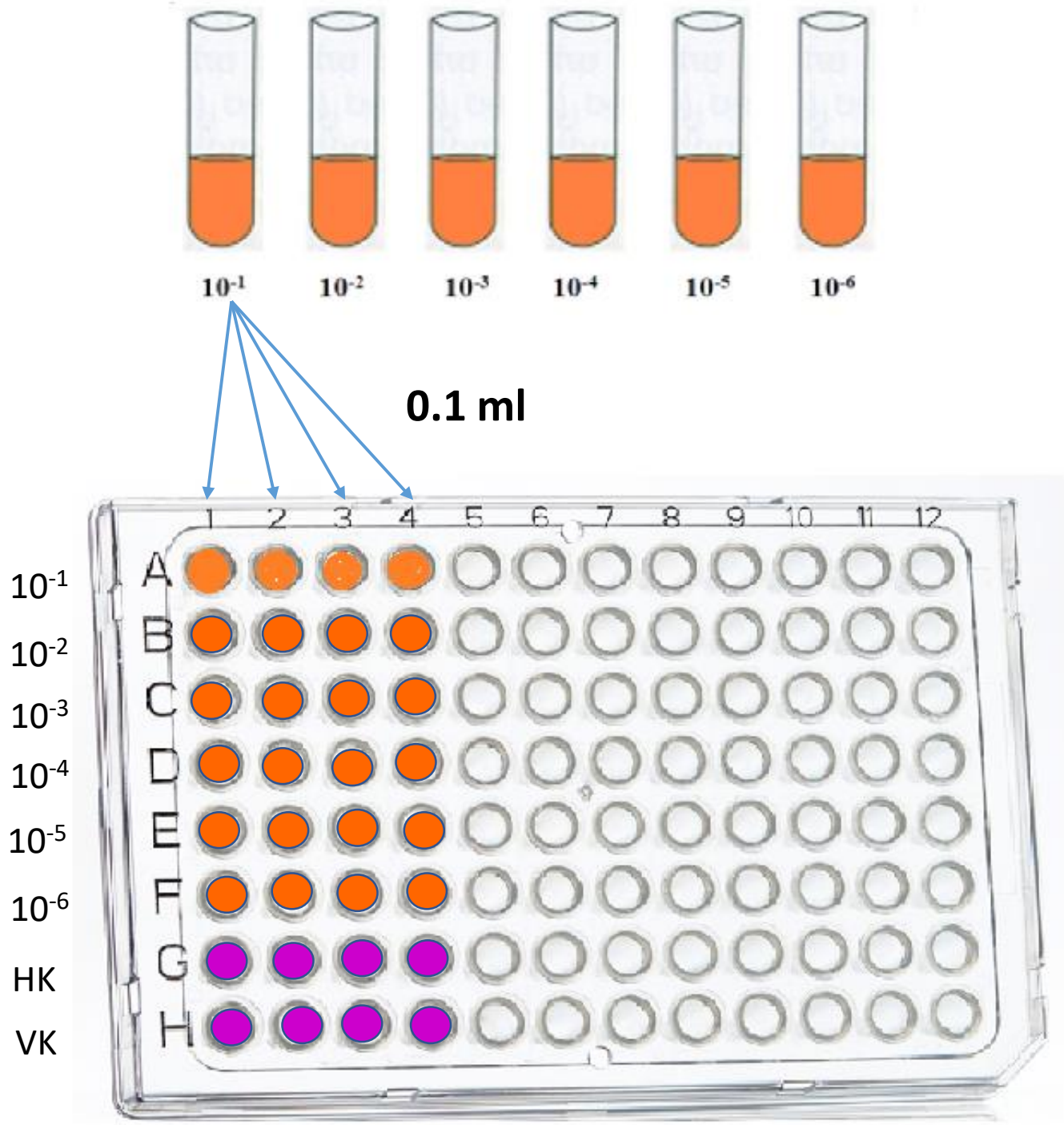


Remember to **change your pipette** at each step (before pipetting) to avoid any carry-over of virus!

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
HK
VK

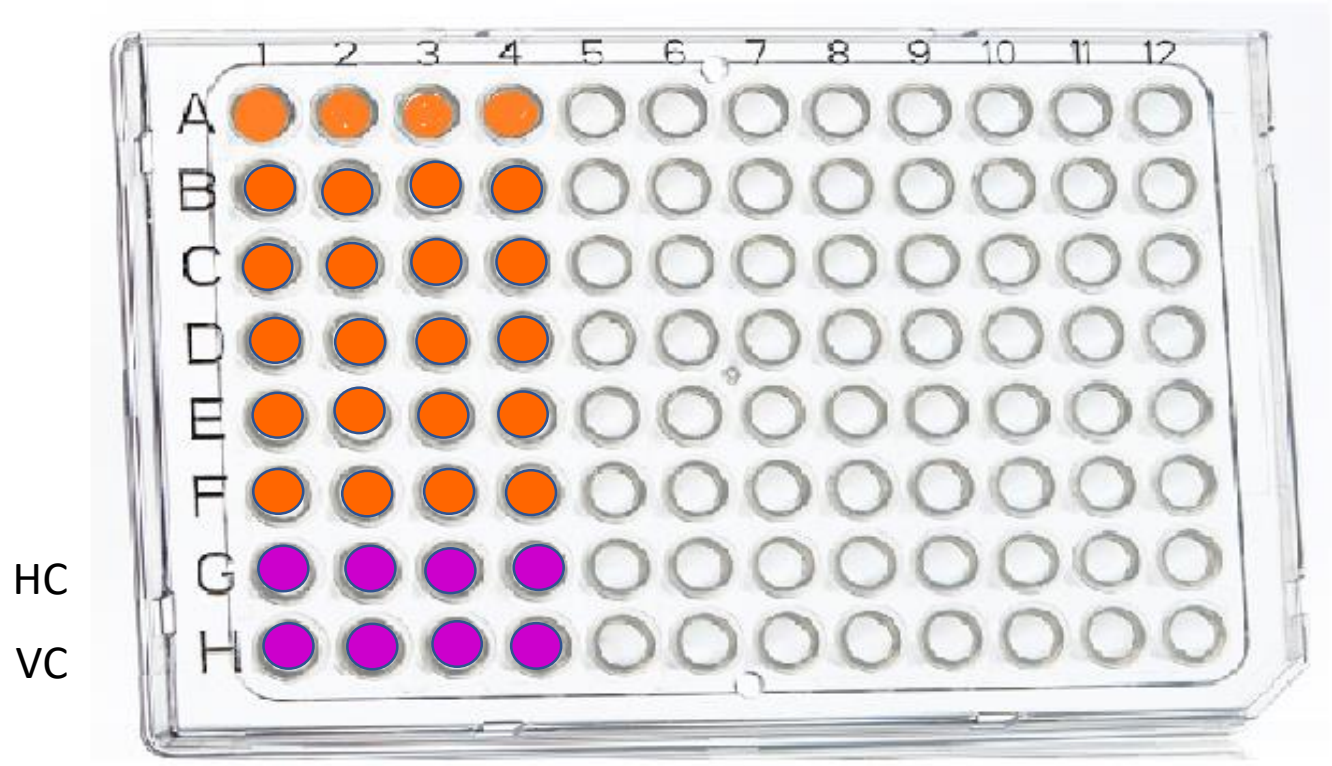
CC: Cell Control

- 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 to ALL WELLS (BY DROPPER!)



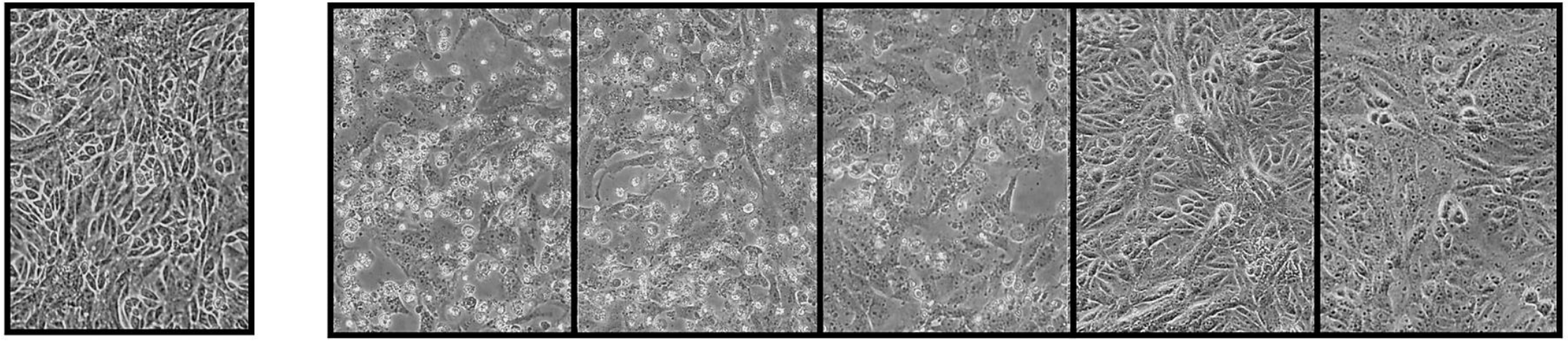
- Test is over

37°C ve %5 CO_2 incubator

We examine it every day under an invert microscope.

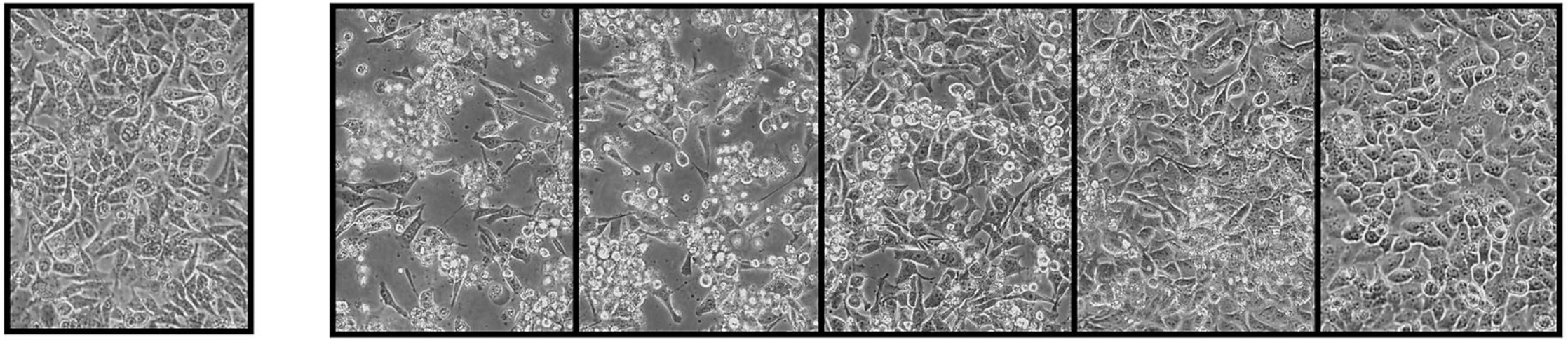
CPE

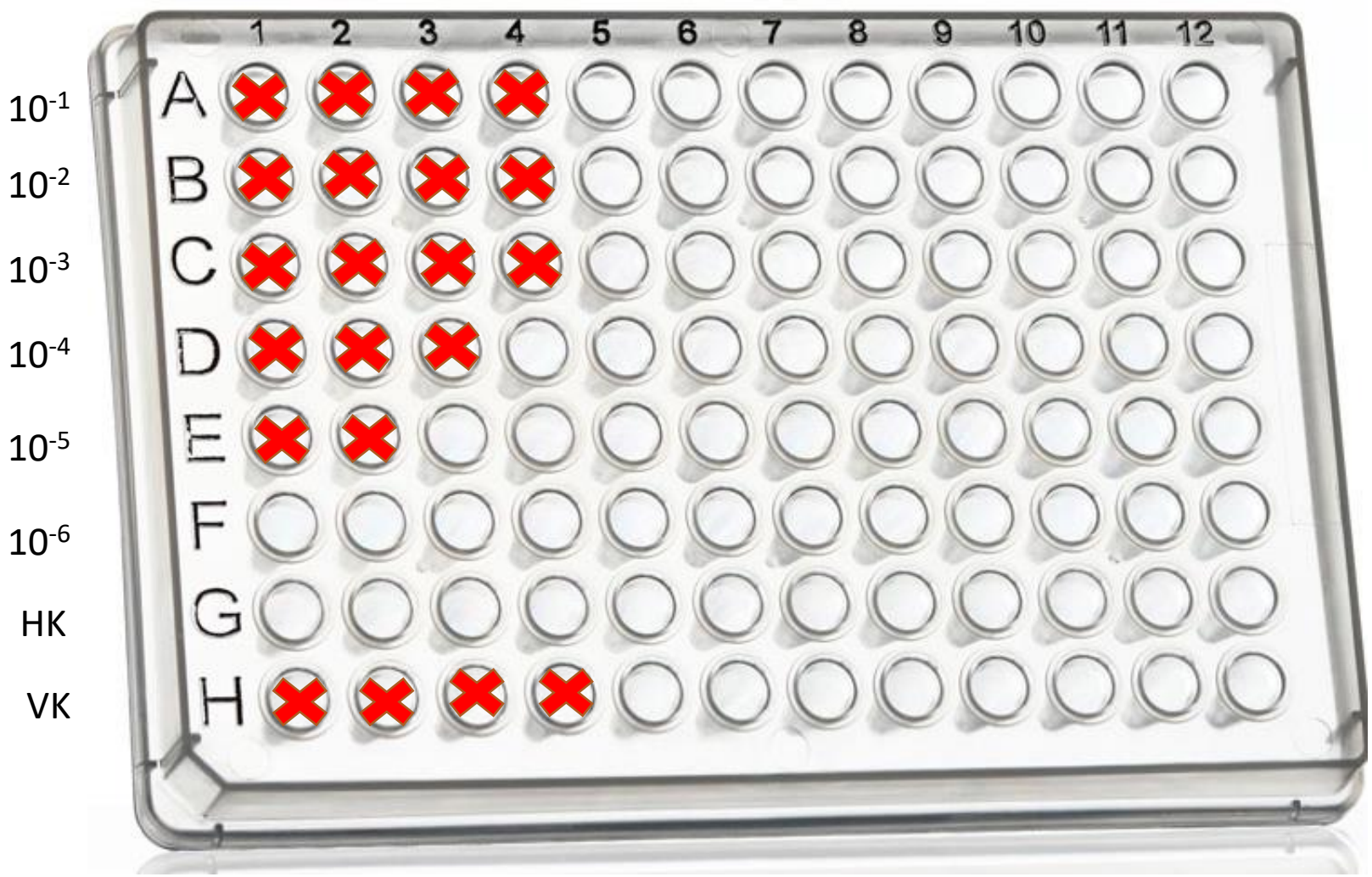
Cell Control	10^{-1}	10^{-2}	10^{-3}	10^{-4}	10^{-5}



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
HK
VK

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 → $Log_{10}10 = 1$
  - r = sum of all positives
  - n = number of wells used for each dilution
- Reed & Müench Methods

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

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

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

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

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

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

- 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} 1/4

$100TCID_{50} = 10^{-3}/0,1ml$

$TCID_{50} = 10^{-5}/0,1ml$

Total CPE : 22 22-2= 20 20 ÷ 4 = 5

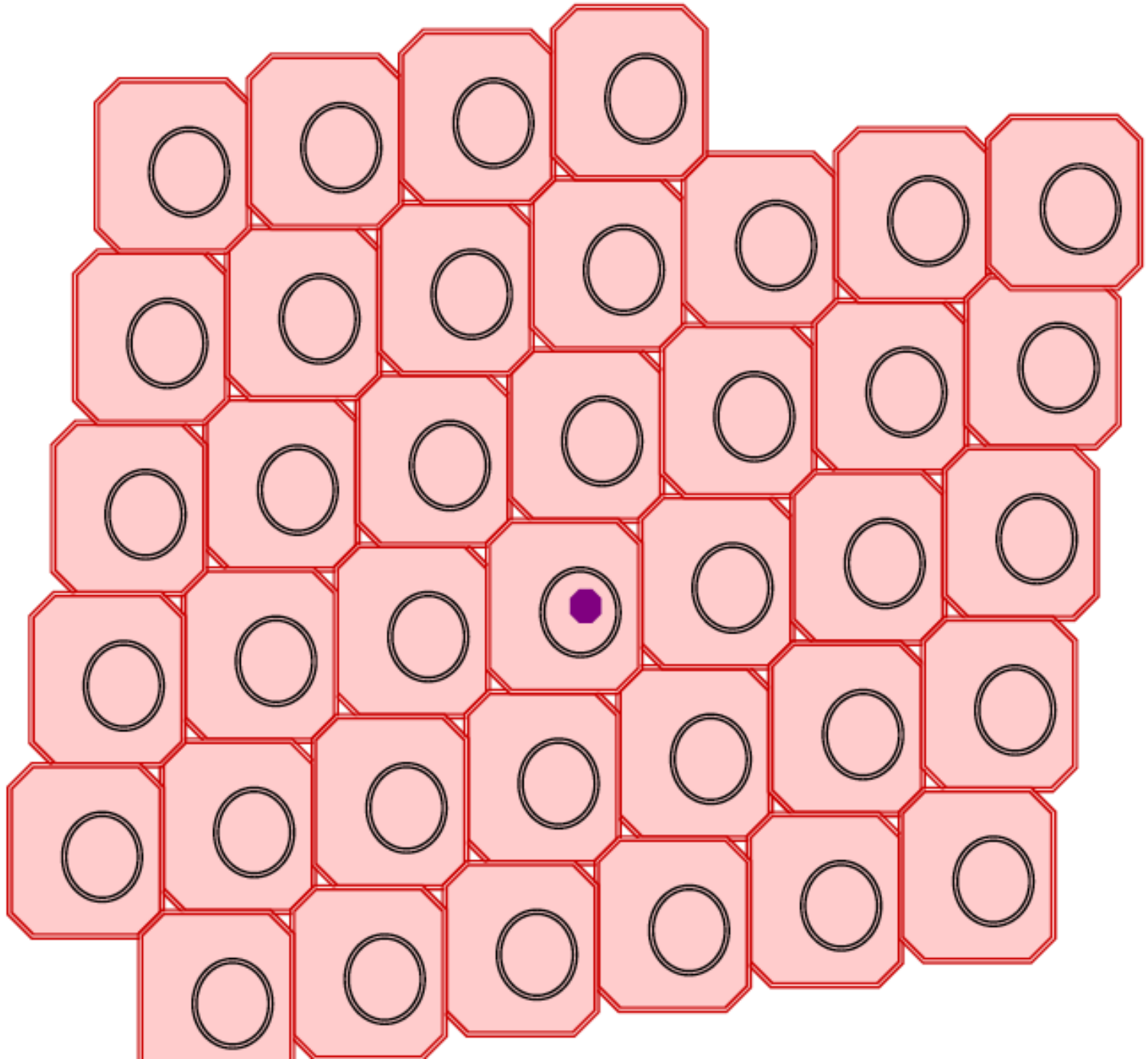
↓ Constant ↓ used well number

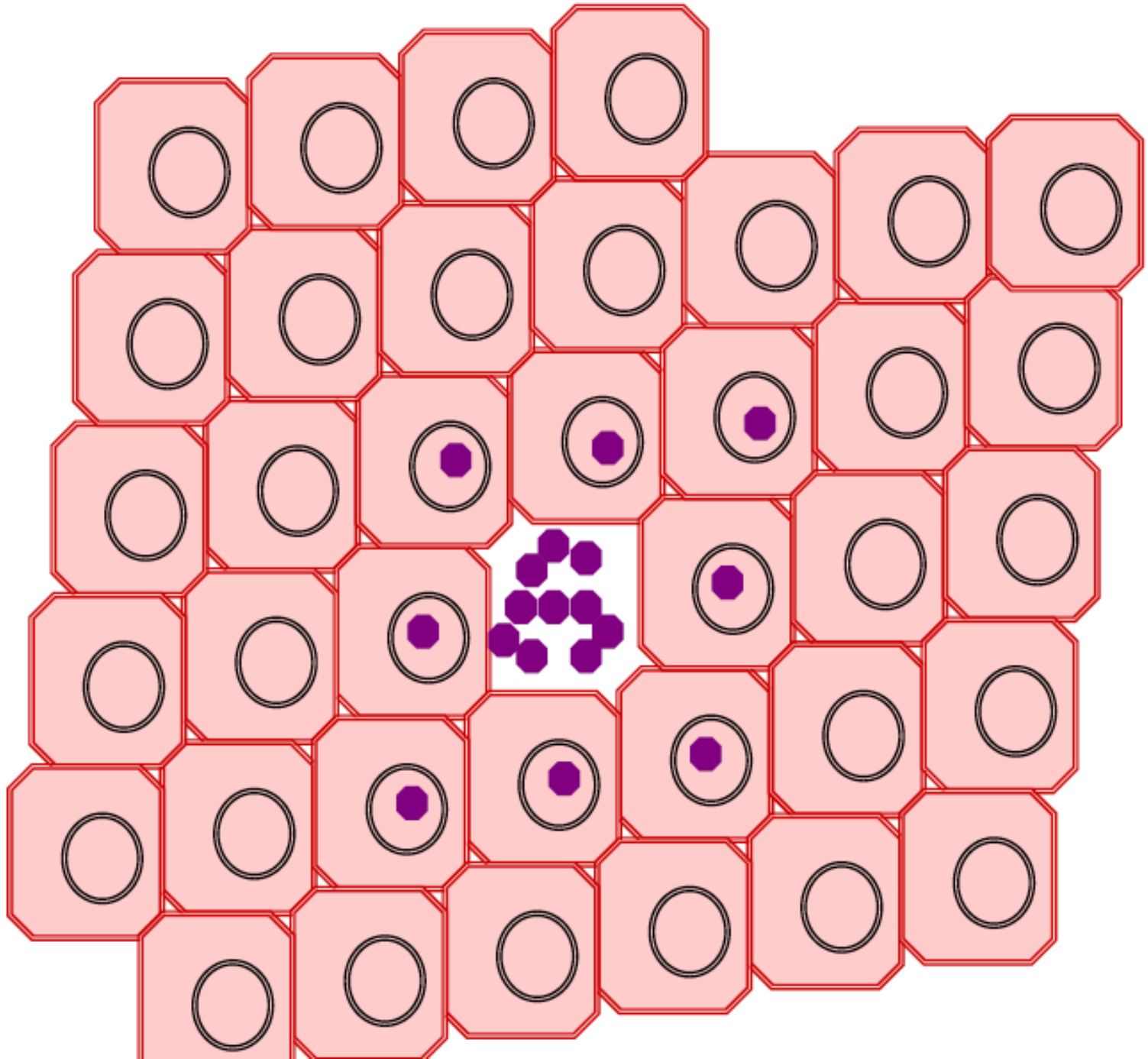
Plaque Assay/Test

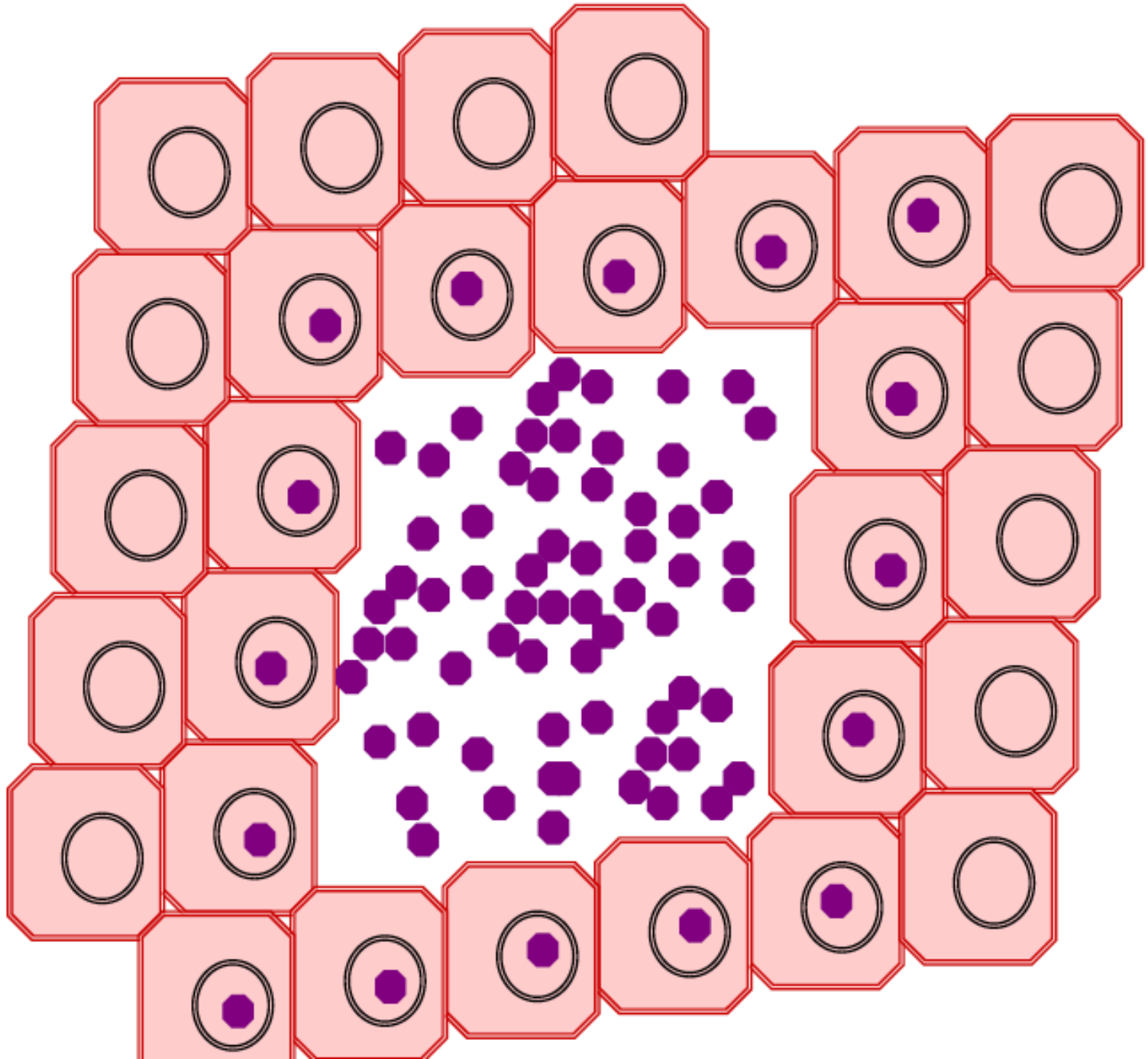
- What is plaque?
- It identifies the limited virus reproduction areas in cell cultures.
- Plaque assays are the standard method that have long been used to determine the virus titer (ie, infectious dose).
- It determines the number of plaque forming units (PFU) in a sample.
- Typically, 10-fold serial dilutions of the virus stock are inoculated into each plate.
- Note that plaque assay is restricted to the viruses that induce cell lysis or death, such as picornavirus, influenza virus, and herpesvirus.

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

- 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.







- 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.

- 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. dilute the virus according to $\log_{10}$

2. Inoculation is performed into two cell culture grown in petri dishes from each virus dilution.

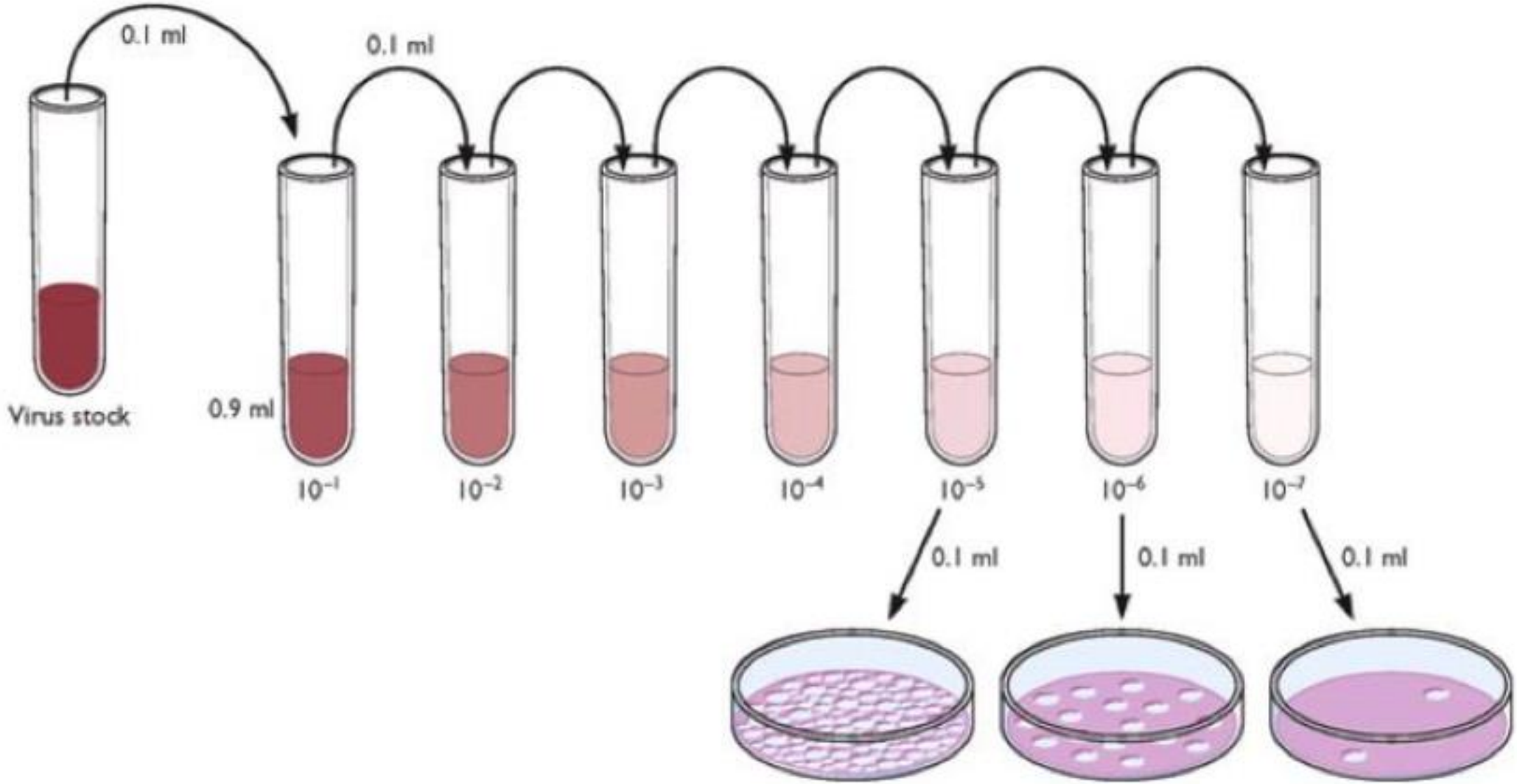
3. The virus is cultivated in the cells of these petri dishes with adsorption.

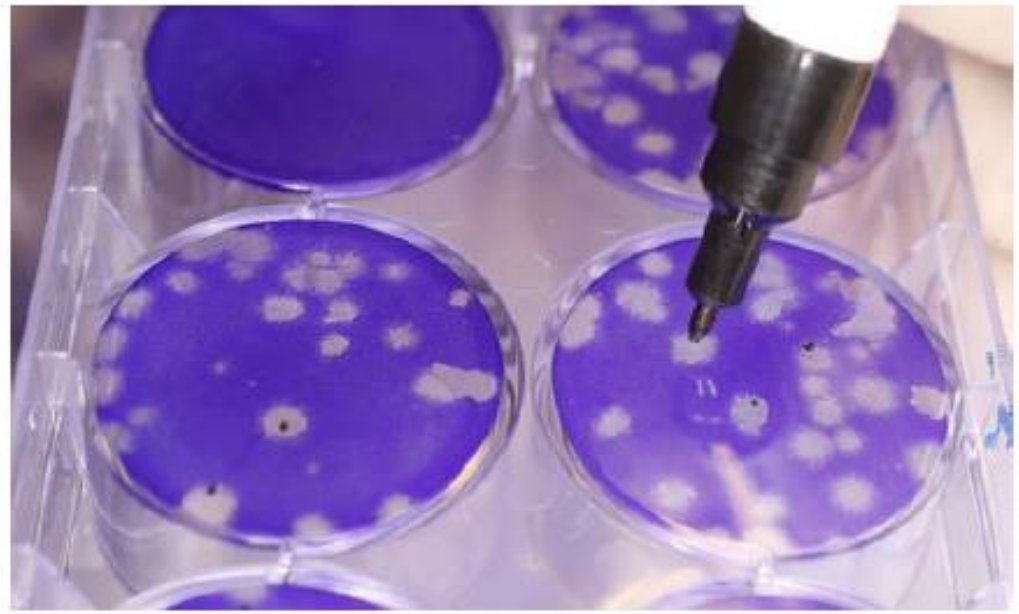
4. After incubation, 2x Earle -1,8-2% Noble Agar is used as a virus growing media.

5. Incubate the petri dishes in an incubator at 37°C and with CO_2.

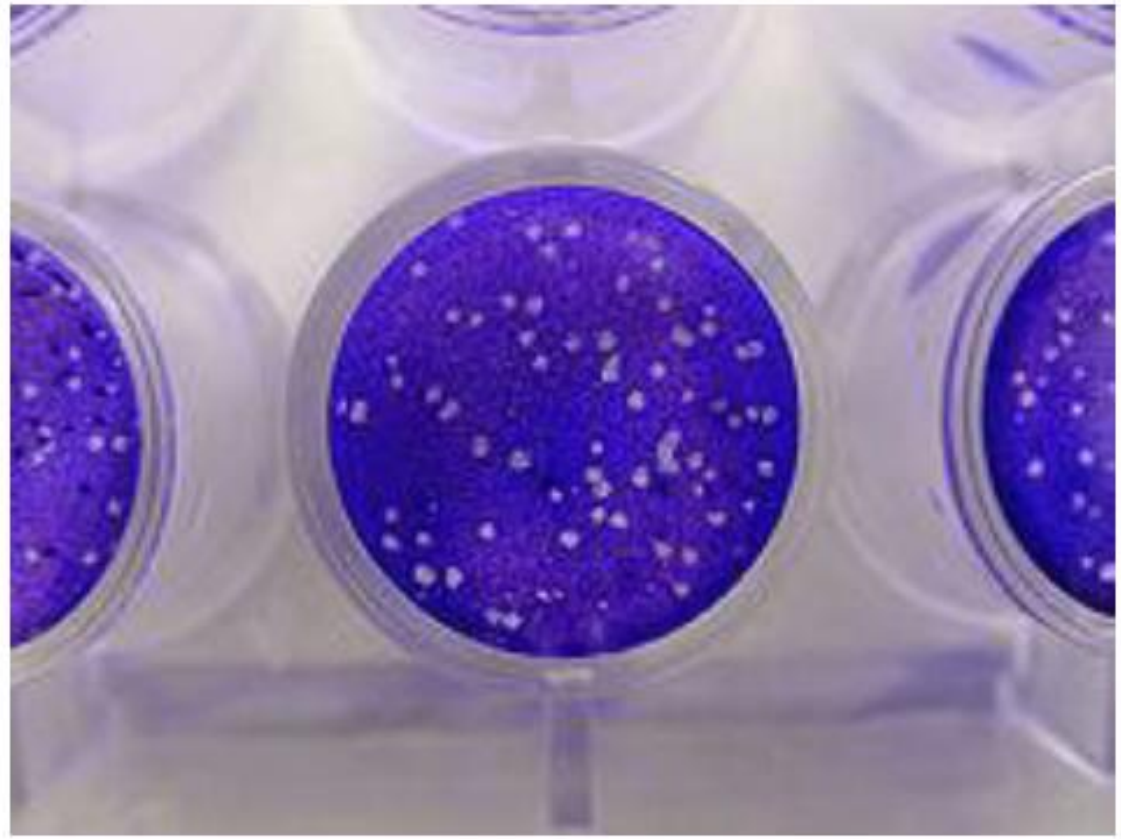
6. Crystal violet staining could be used to stain plates for better viewing.

7. Plaques are counted and the Plaque Formation Unit (PFU) is detected.


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



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



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