TITRATION IN VIRUSES

TITRATION

- Infinite is the calculation of infectious power.
- Detection of a virus's infectious power is called the titration of that virus.
- There are two types of titration according to the host system used.
- Invitro titration: Tissue cultures
- A- Macro Titration
- B- Micro Titration
- C- Plate Titration
- 2. In vivo titration: ETY, Trial Animals

MACRO TITRATION

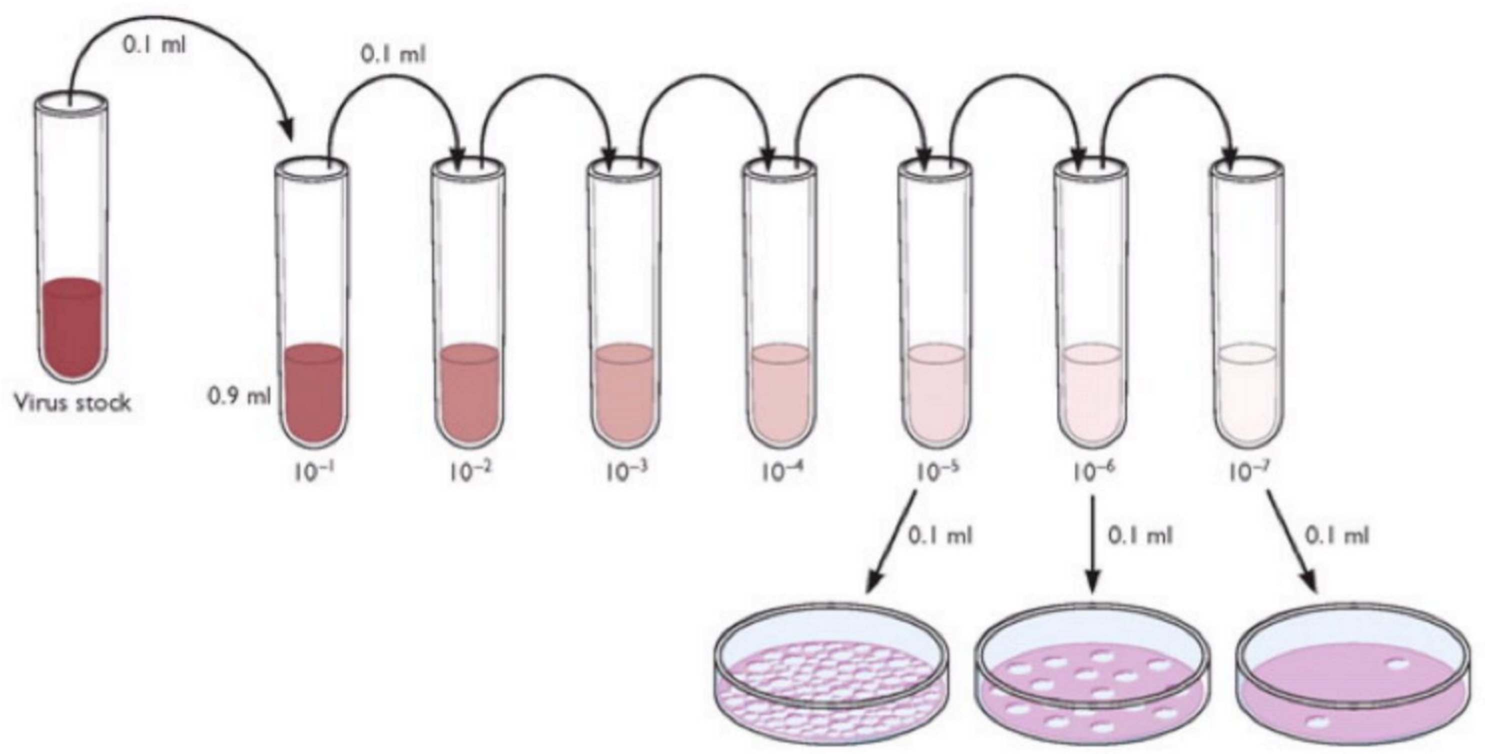
Working in microbiological safety cabinet, prepare the diluting fluid which is PBS and dispense 9ml in test tubes labelled 10^{-1} to 10^{-6} and keep the test tubes in rack immersed in plenty of ice.

To make 10 fold (log) dilutions of the virus material, dilute 1ml of virus in 9ml of diluent to get the initial dilution i.e. 10^{-1}. Sterile pipette 1 ml without 1/10 dilution. 1/100, 1/1000, etc. Dilutions are obtained. It is imperative to change the pipette every time.

1ml from the last tube. It's thrown out.

Four cell cultures are infected from each virus dilution.

They are removed from the 37 C incubator and evaluated according to CPE formation. The titer is calculated according to Reed-Muench or Spearman-Kaerber Method.



https://www.urmc.rochester.edu/MediaLibraries/URMCMedia/mbi/xenopus-laevis/documents/FV3-Production-and-Titration.pdf

MICRO TITRATION

1. The virus to be titrated is diluted with known method according to 10 fold (log)
2. In microtitration tablets, 4 wells are marked for each dilution and 4 wells for Cell Control and Virus Control.
3. 0.1 ml of each virus dilution is transferred to the relevant wells with micropipettes.

4 – Each of 4 wells separated for virus control is 0.05 ml. Pure virus, 0.05 ml virrus growing media

5 - 0.1 ml of cell media for each of 4 wells separated for cell control.

6 - Cell suspension with 300,000 cells per milliliter is dropped as 0.05 ml to each well by dropper.

7 - Microtitration tapes are covered with Sterile Bands and taken up in 37 C dishes and evaluated.

PLAK TITRATION

- Plate titration is used to isolate and purify the viruses as well as to titrate the viruses.

Plaque: It is called restricted gaps caused by virus multiplication in infected cell cultures.

A- Lytic Plates: middle of it is empty.

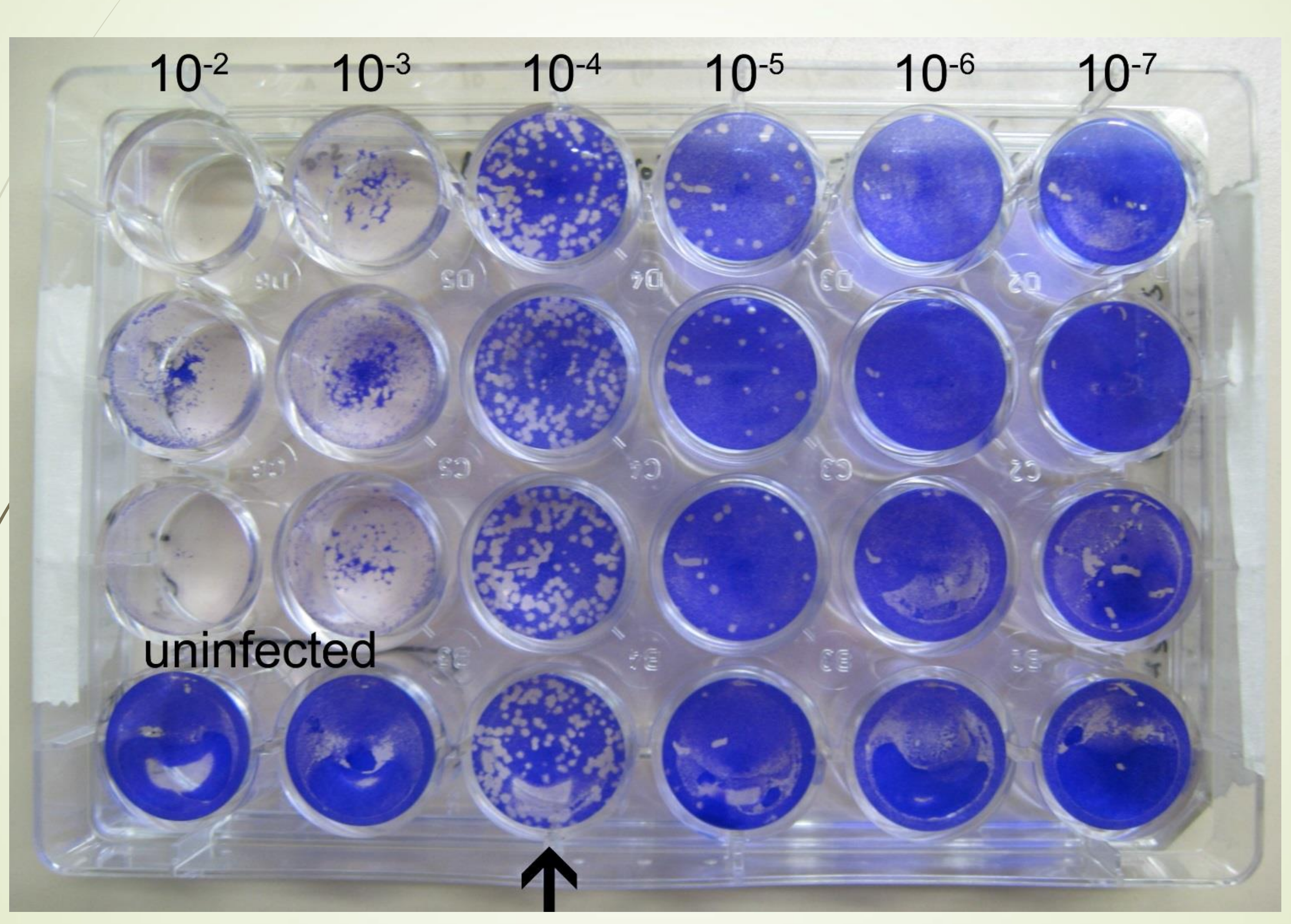
B- Degenerative Plaques: There are degenerated cells in the center.

C- Proliferative Plates: There are dense cell proliferations in the centers.

Confirmation of plaque test

1. Plaques should not be seen on uninfected controls.
2. The virus used should have a plaque formation ability.
3. Plaque formation should be stopped with immun sera.
4. Plaque tests made with the same virus should give the same result.

https://bio-protocol.org/e1295



PLAK TITRATION TEST

1. make 10 fold (log) dilutions of the virus.
2. For each dilution, 2 cells are marked from the cells produced in petri dishes.
3. The virus is cultivated in the cells of these petri dishes with adsorption.
4. After incubation, 2x Early - 1,8-2% Noble Agar is used as a virüs growing media.
5. Move the plate(s) to a humidified incubator at 37°C and with CO2.
6. Sometimes Neutral Red is used to dye plates for better viewing.
7. Plate number Plate Formation Unit (PFU) is calculated.