PLAQUE REDUCTION TEST

Definition:

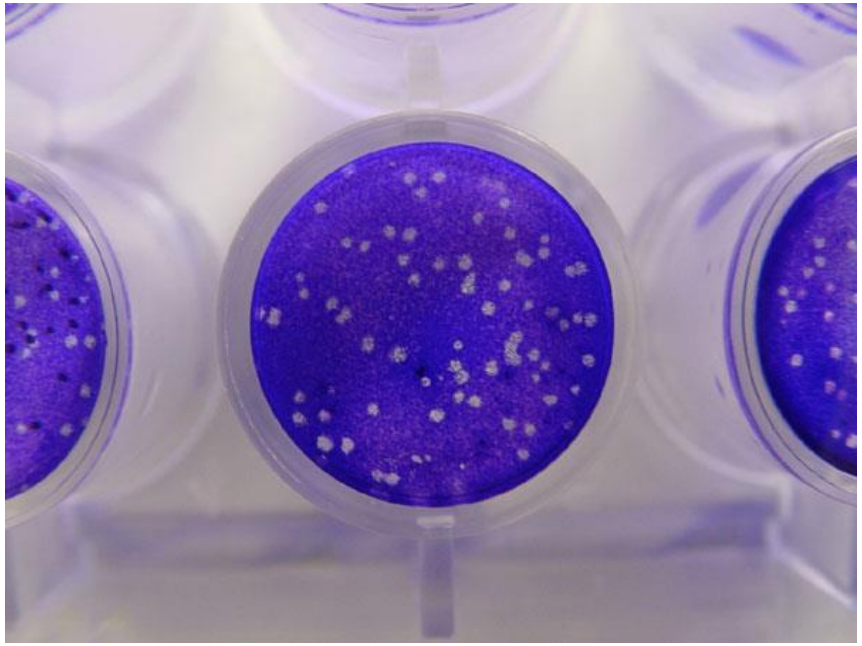
- This method detects the presence of antibodies or interferon in serum based on the reduction or complete inhibition of the plaque-forming ability of a virus.

Applications of the Test

- Detection of virus-specific antibodies
- Detection of interferon activity

What is Plaque?

- Plaques are areas that originate from a single virus particle and are characterized by gaps or altered cells that are clearly separated from normal cells in monolayer cell cultures.

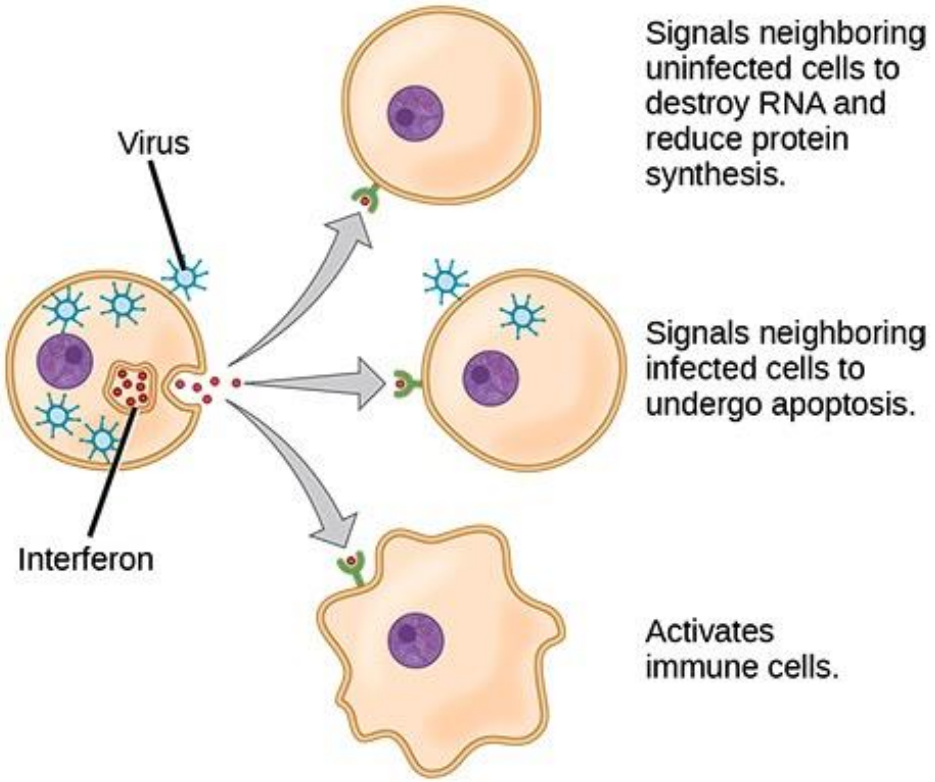


https://tr.m.wikipedia.org/wiki/Dosya:Plaque_assay_macro.jpg

- Interferon is a biological product in protein structure secreted by virus-infected cells.

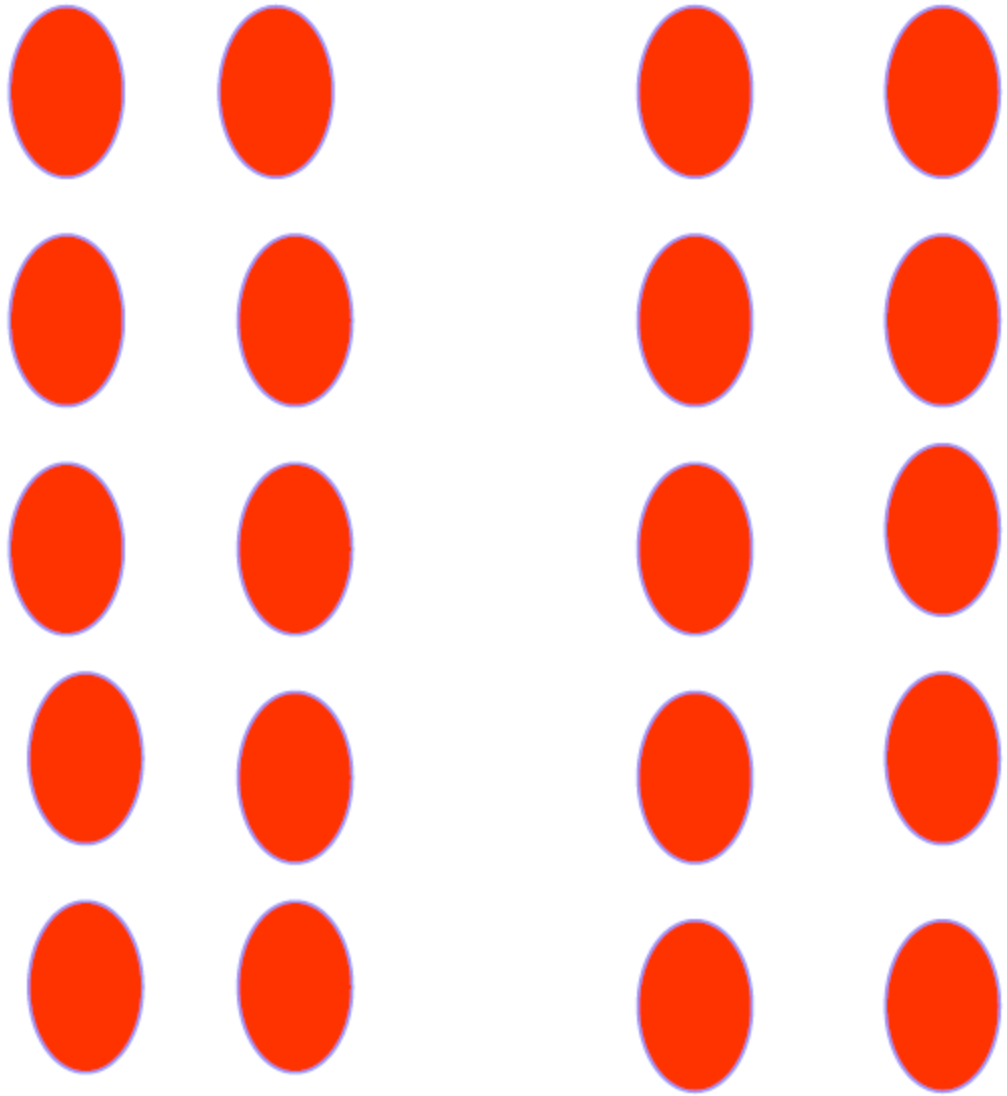
Interferon has antiviral activity. It does this by;

1. By degrading cell surface receptors,
2. By blocking the synthesis of some enzymes important in virus replication

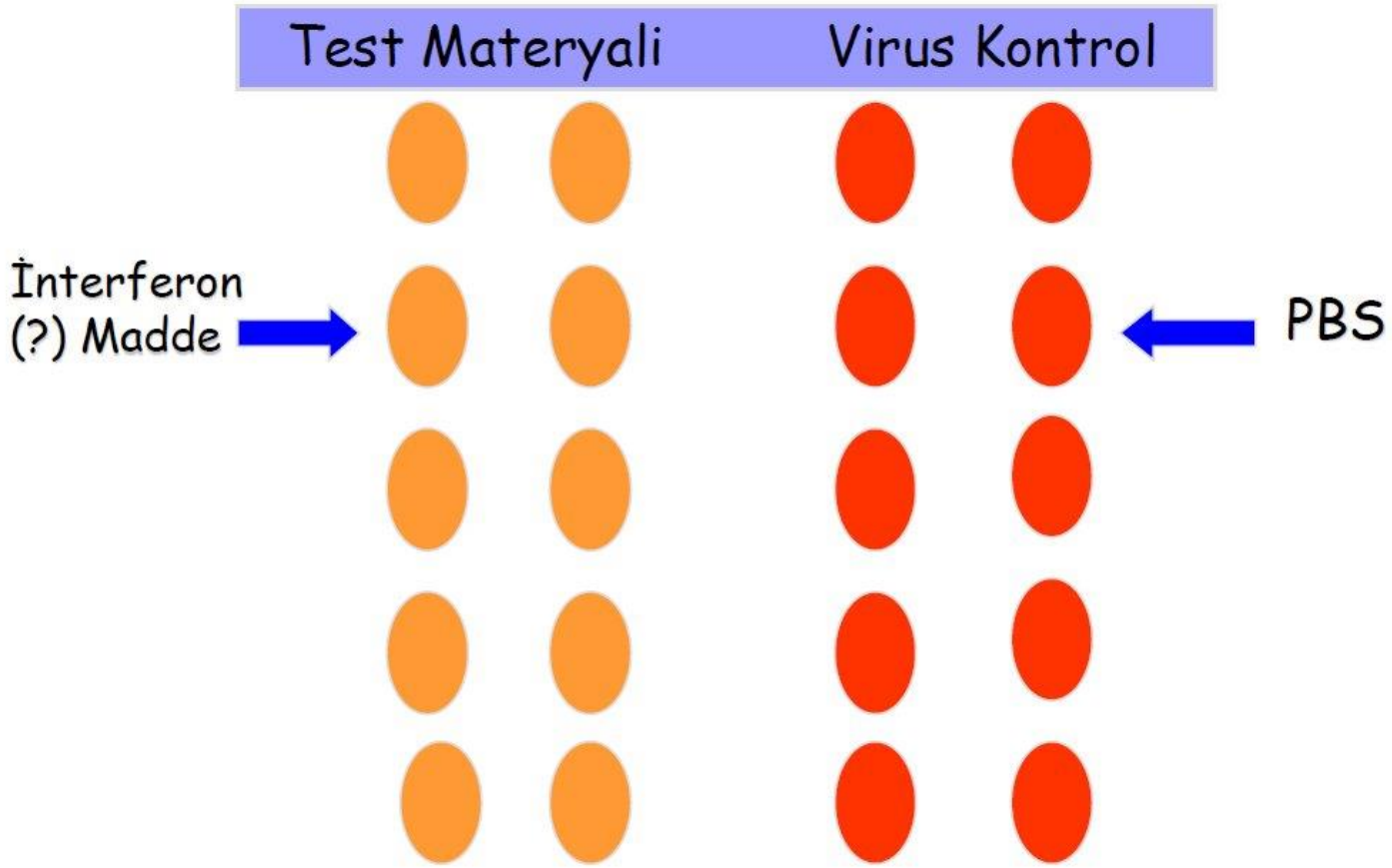


To detect interferon,

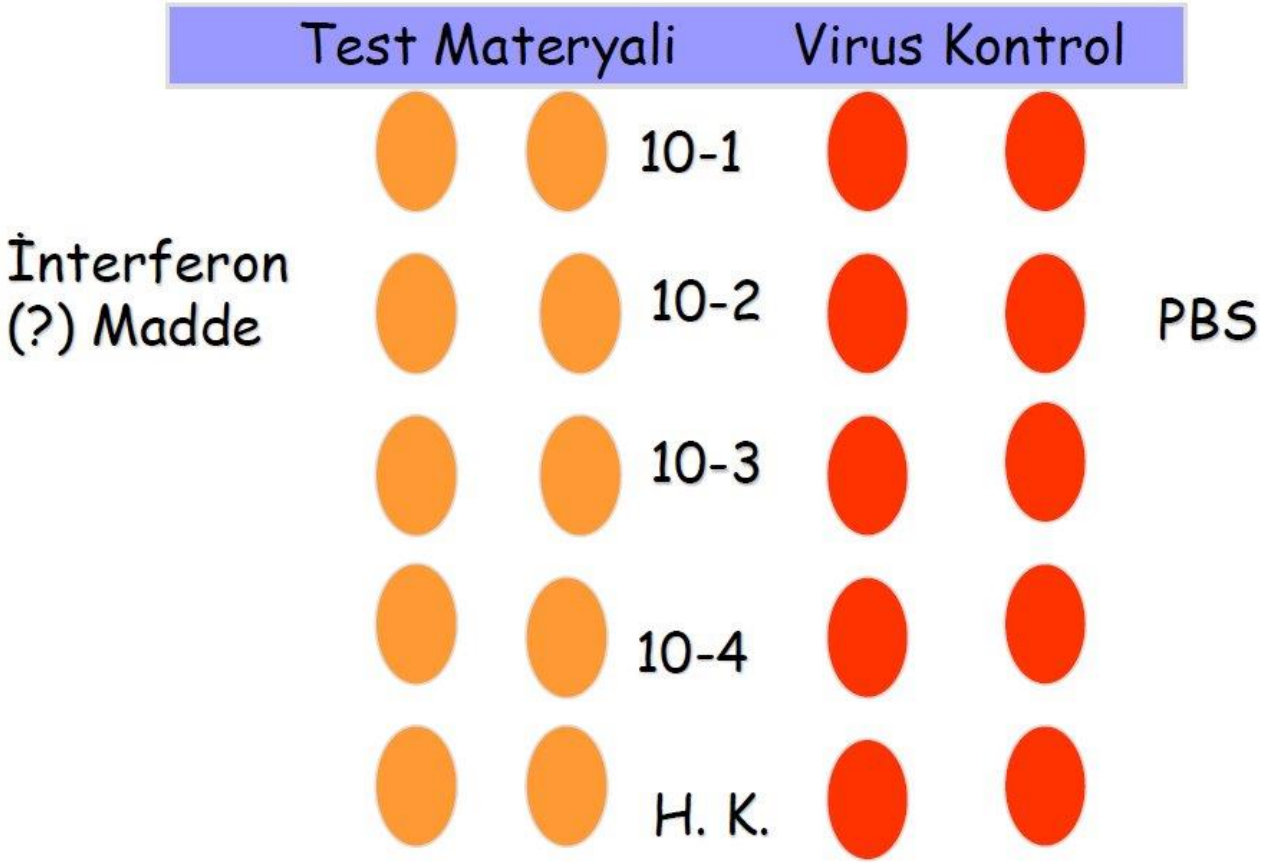
- Cells are grown in a series of petri dishes.
  - test material (interferon suspected ?)
  - the control virus.



- Interferon (?) was added to the cells in the petri dishes used for test material and PBS was added to the petri dishes used for Virus Control.
- Cells are incubated at 37°C.
- (Note: If the suspect substance is interferon, surface receptors will be degraded in the treated cells during this time).



- Prepare virus dilutions based on log10.
- Following the incubation period, each virus dilution is inoculated onto cells treated with the material suspected to contain interferon and onto untreated cells (PBS treated).
- Incubated at 37°C for 1 hour (for adsorption)



- At the end, the petri dishes were washed with PBS, virus growth medium was added on the cell cultures and the cells were placed in an incubator (37°C) for 2-3 days.

Virus growth medium:

1-2% Noble agar + 2X EARLE

(%50) + (%50)

- 2X EARLE: It contains twice as concentrated chemicals in the same volume.

Why do we use 2XEarle?

- Because 2X Earle medium is mixed with noble agar in equal volume, the resulting virus growth medium will have the same chemical content as normal Earle medium.

Why do we use agar?

- Agar is used to create a semi-solid overlay that restricts viral spread, allowing localized areas of infection, known as plaques, to form from the replication of individual virus particles.

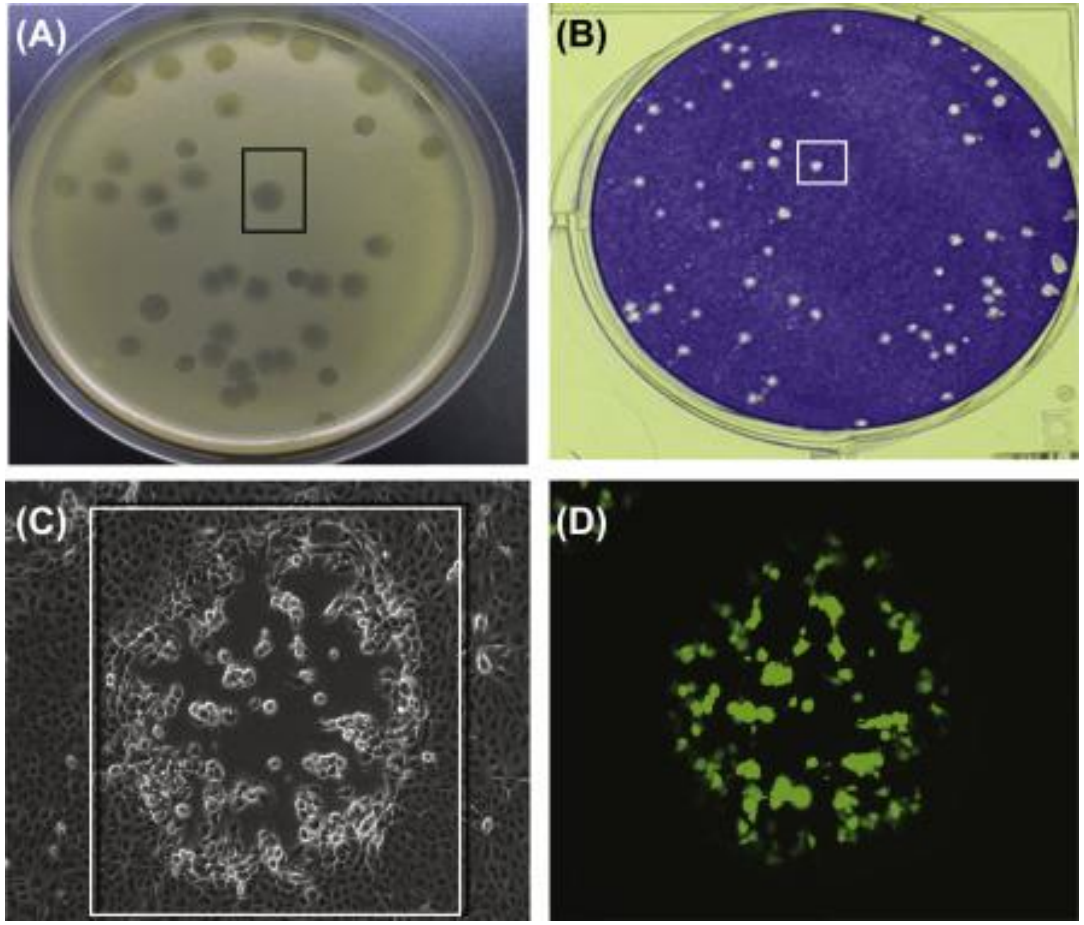


Figure Viral plaques.
(A) Phage plaques on bacterial lawn; (B) Monkeypox plaques on a lawn of Vero E6 cells stained with crystal violet; (C) African green monkey BS-C-1 cells infected with EGFP expressing vaccinia virus WR strain bright field microscopy image, (D) fluorescent microscopy image of panel C. Single plaques are boxed with rectangles in panels A, B, and C.

Evaluation of the test

- In order to see the plaques macroscopically, 2XEarle+Noble agar mixture containing 0.01% neutral red or crystal violet is added to the petri dishes.
- Since neutral red and crystal violet are a vital dyes, plaques are not stained and unstained areas are seen on a background of the stained cells.

- Plaques formed in petri dishes are counted.
- A decrease in the PFU value relative to the control indicates that the test material is interferon.