PLAQUE REDUCTION TEST

Definition:

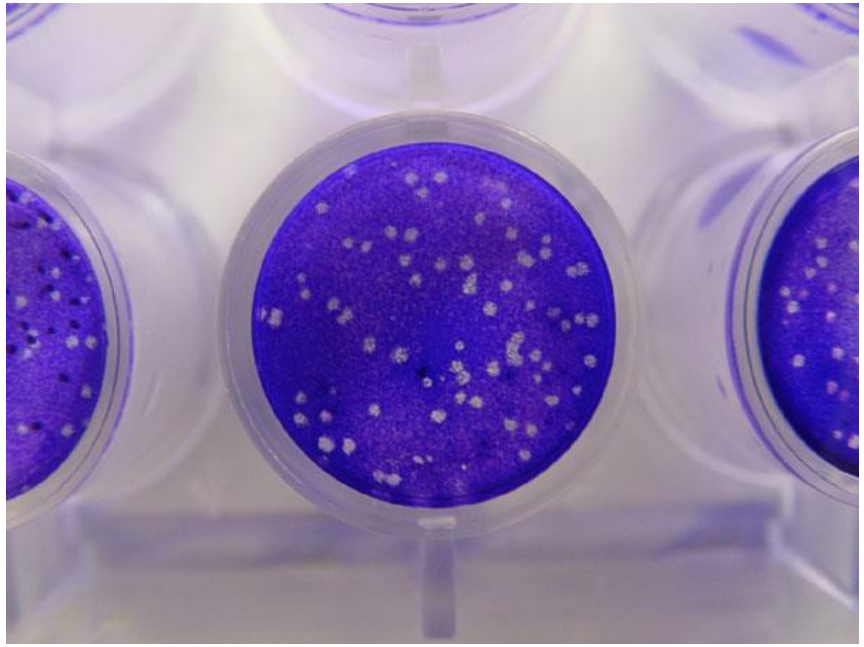
- It is a method used to detect the presence of antibodies or interferon in serum based on the decrease or disappearance of this ability of a virus that has the ability to form plaques.

Purposes of Use of the Test

- Determination of the presence of antibodies
- Detection of the presence of interferon

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

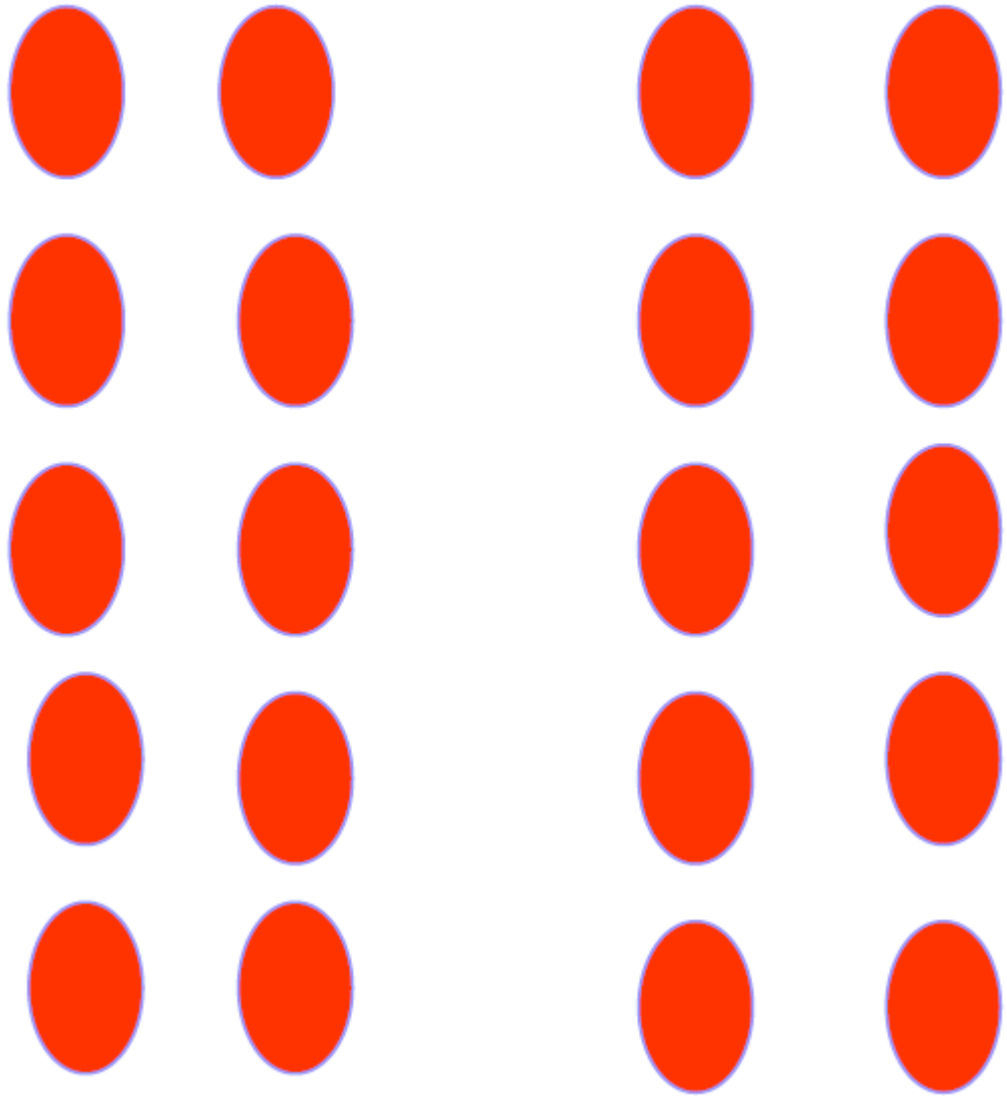
- Interferon is a biological product in protein structure secreted by virus-infected cells.

Interferon has antiviral activity. It does this by;

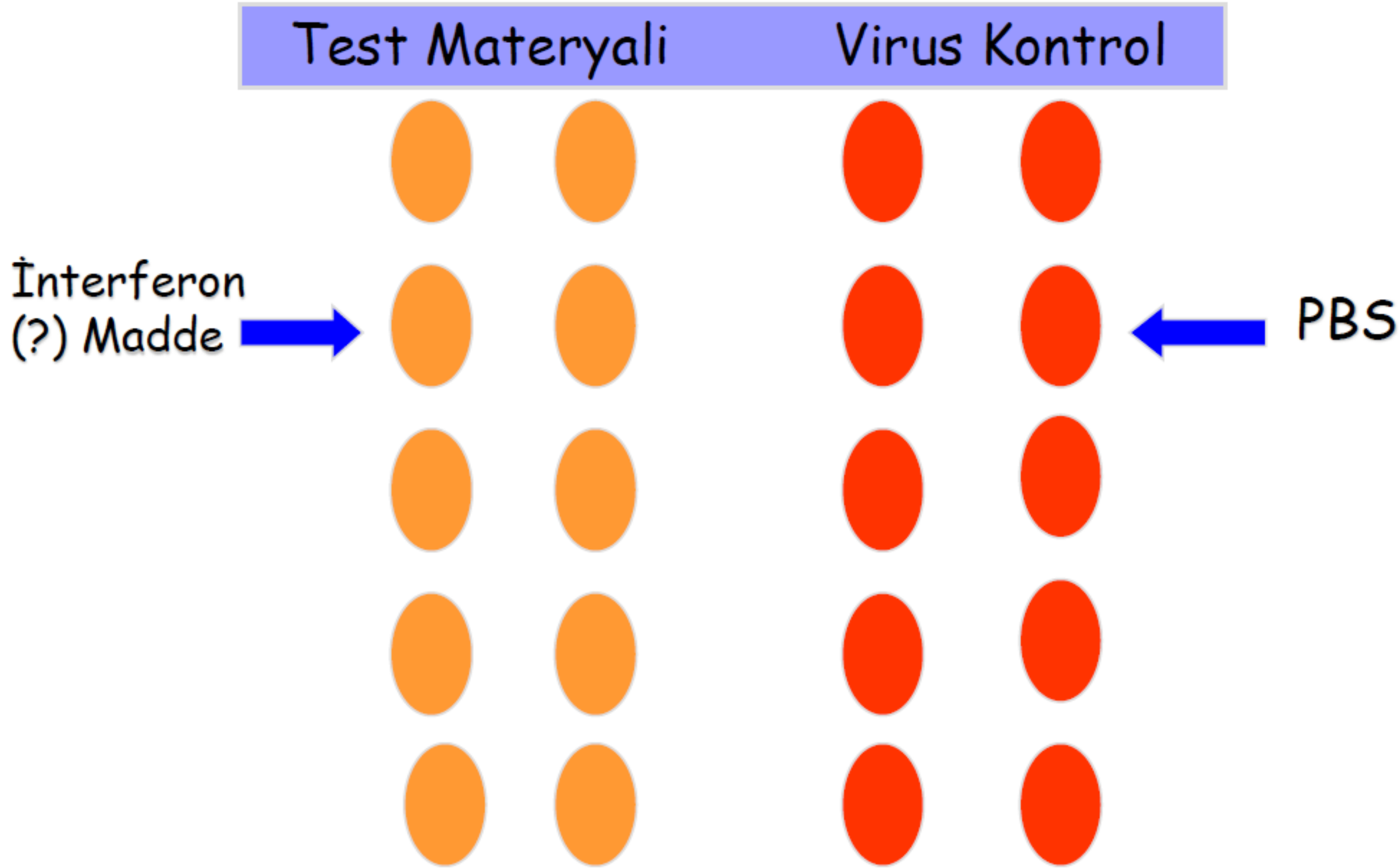
1. By degrading cell surface receptors,
2. By blocking the synthesis of some enzymes important in virus replication

To detect interferon,

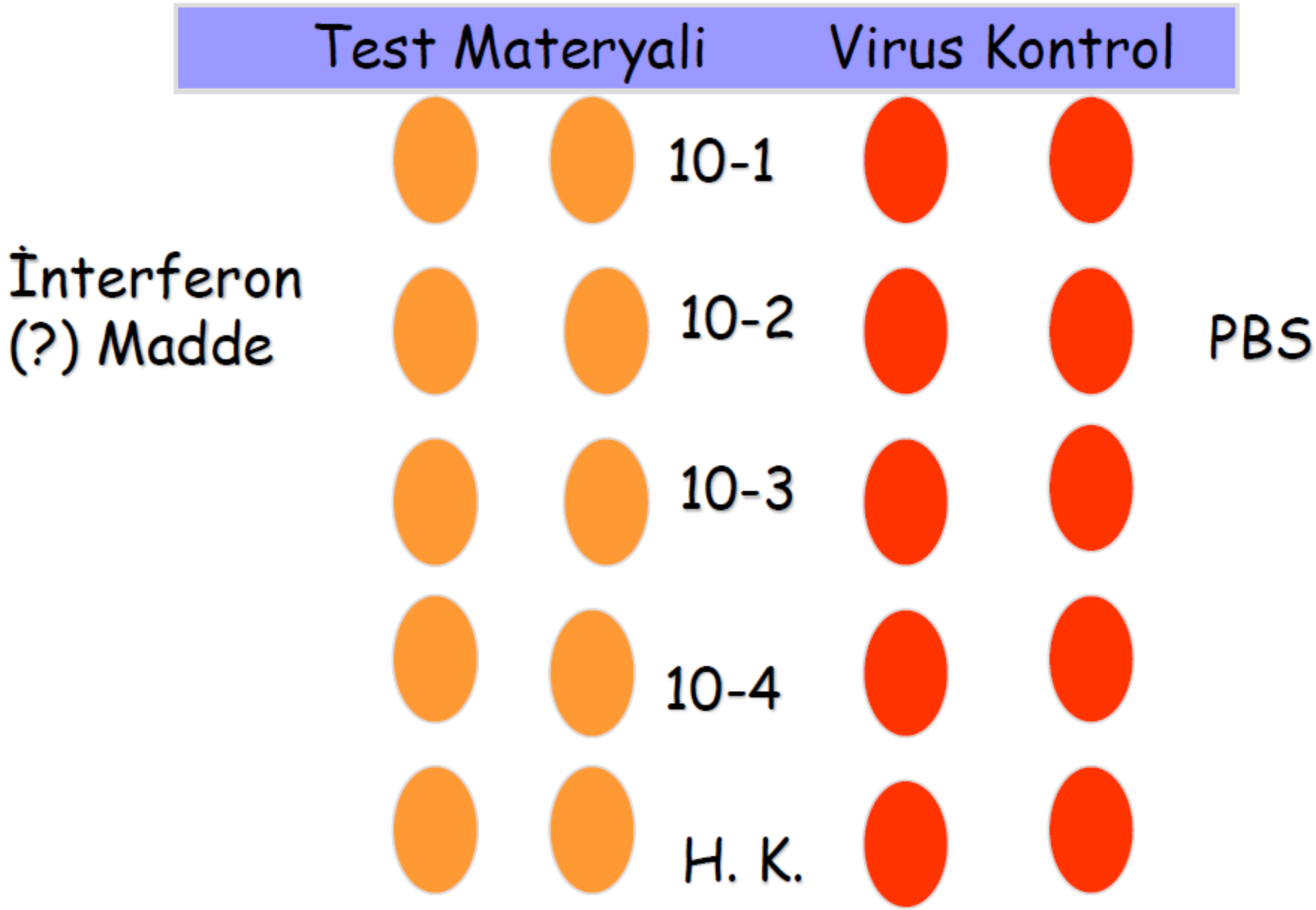
- Cells are grown in a series of petri dishes.
- Some of these petri dishes will be used for the test material (interferon suspected ?) and some for the control virus.



- 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).


Test Materyali
Virus Kontrol
İnterferon
(?) Madde
PBS

- Prepare virus dilutions based on log10 and inoculate at least 2 petri dishes of each virus dilution, treated and untreated with interferon suspected substance, following the incubation period.
- Incubated at 37°C for 1 hour (for adsorption)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PBS

- At the end of the period, 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).
- Petri dishes are incubated for 2-3 days.

Virus growth medium:

1-2% Noble agar + 2X EARLE

(%50) + (%50)

- 2 X EARLE: 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 enable the formation of areas of infection caused by the replication of a single virus particle through the semi-solid medium created by agar.

Evaluation of the test

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

- Plaques formed in petri dishes treated with test material and used as virus control are counted.
- A decrease in the PFU value relative to the control indicates that the test material is interferon.