

AGAR GEL IMMUNODIFFUSION  
(AGID) TEST

or

AGAR GEL PRECIPITATION TEST  
(AGPT)

# Description

- The test is based on the diffusion of antigen and antibody in the semi-solid medium formed with Agar and precipitation in areas where the antigen and antibody attach.
- The passive diffusion of soluble antigens and/or antibodies toward each other leading to their precipitation in a gel.

# DEFINITIONS

- **Precipitinogen:** an antigen that stimulates [precipitin](#) production or that reacts with antibody in an immunoprecipitation reaction.  
The antigen used in the agar gel test
- **Precipitin:** an antibody that reacts with its specific antigen to form an insoluble [precipitate](#).
- **Precipitate:** the line formed in positive reactions (Ag + Ab junction)
  - A solid or solid phase separated from a solution.

# Sensitivity

- AGID test; it is generally less sensitive than neutralization and KF test. However, it is recommended as a reference method for persistent infections like retroviruses, and also for the diagnosis of certain acute infections.

# What for do we use AGID test?

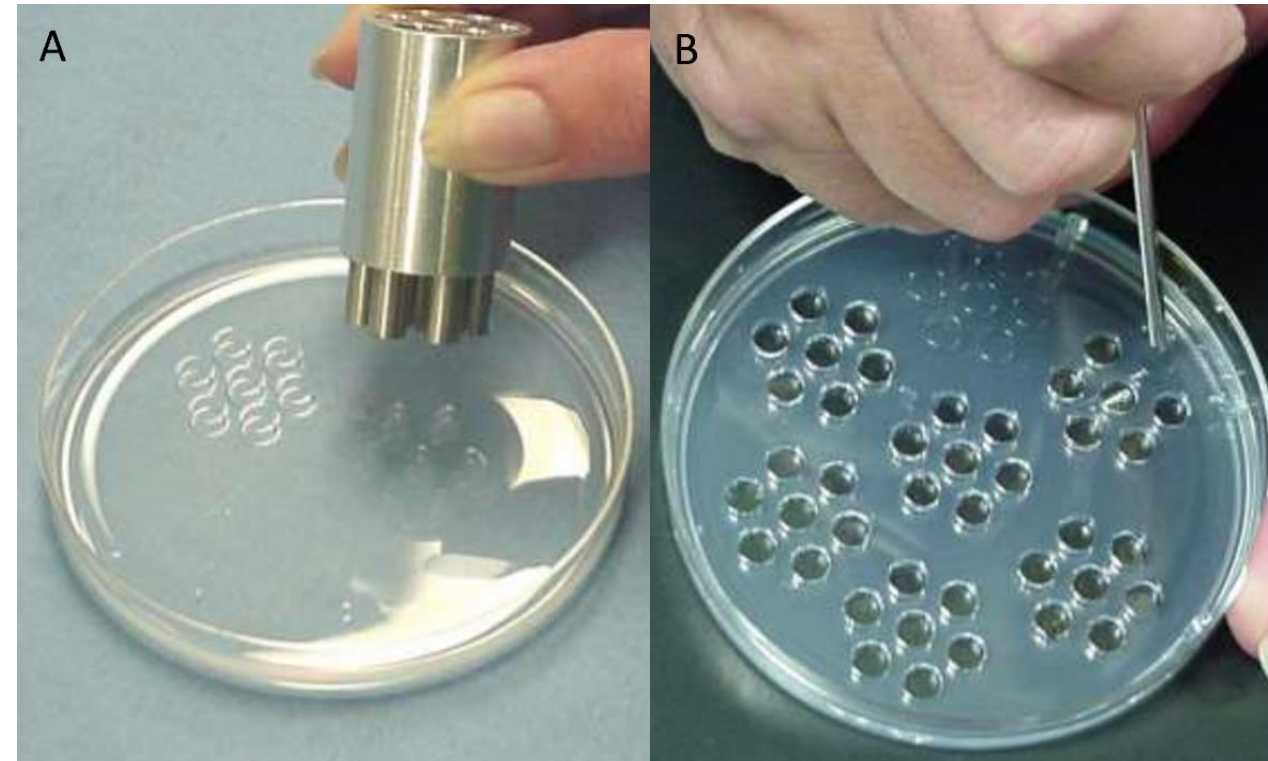
1. To detect Antibody or antibody titration
2. To detect viruses
  - Virus identification

# What do we need to perform the test?

- Agar
- Serum (known or suspected according to the purpose)
- Concentrated virus (known or suspected according to the purpose)

# Protocol

1. Once the agar is autoclaved, it is transferred to the petri dishes and wait for the agar to freeze.
2. Using a special drill, wells are drilled one at the center and 6 around it equidistant from the center.



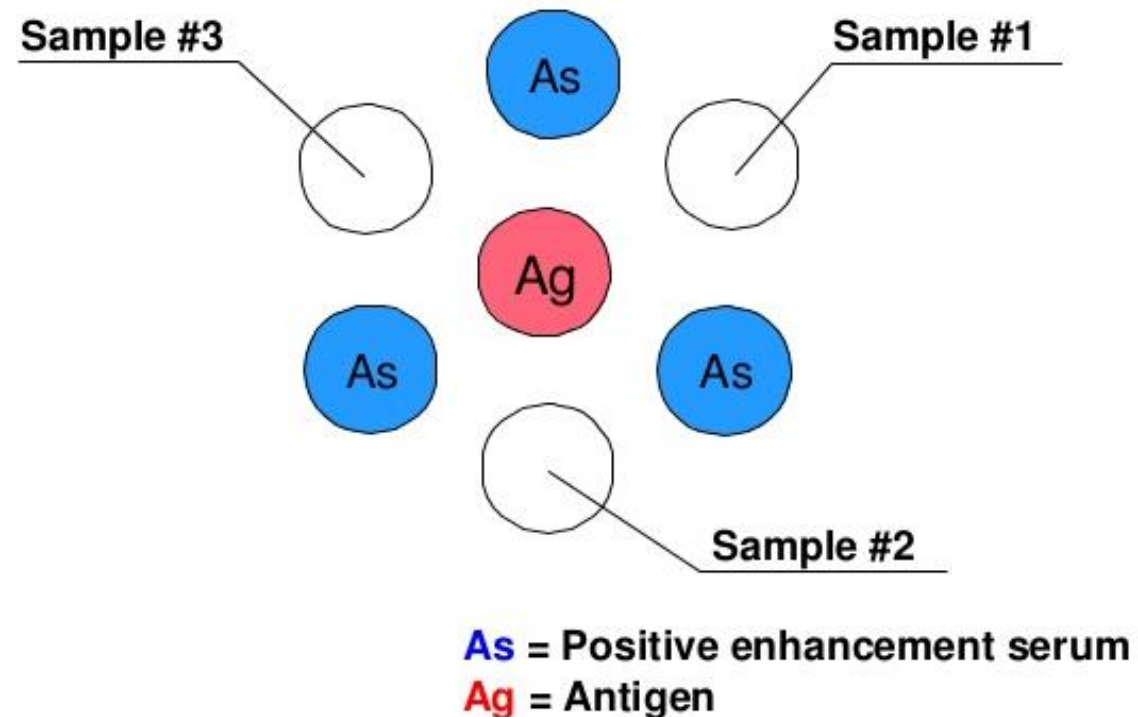
### 3. According to the purpose;

Add the known material (serum or antigen) to the center well

And

Add the suspected materials to the peripheral wells.

## Placement of Reagents/Samples

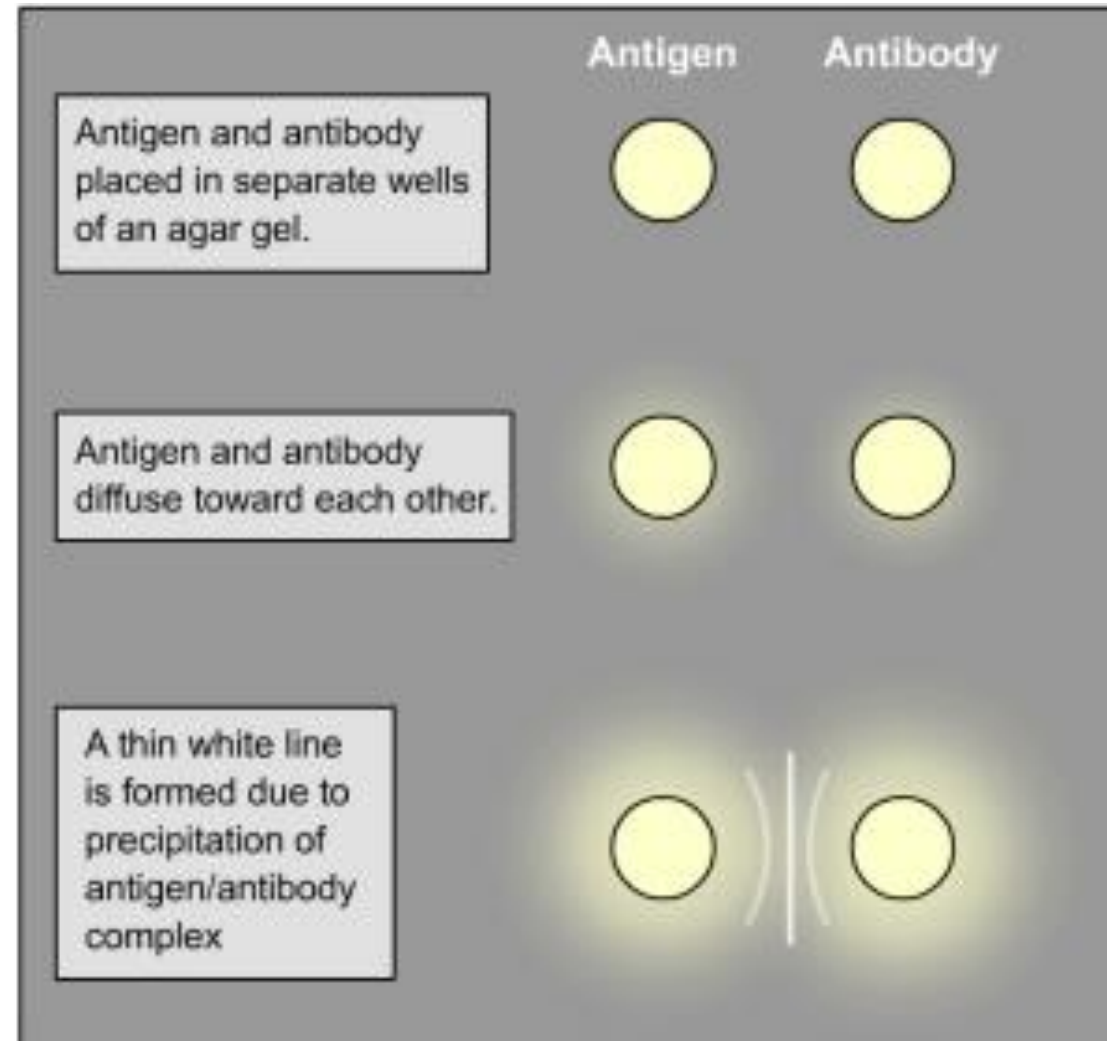




4. Following the incubation about 48-72 hours in an incubator, the results are evaluated under the light.

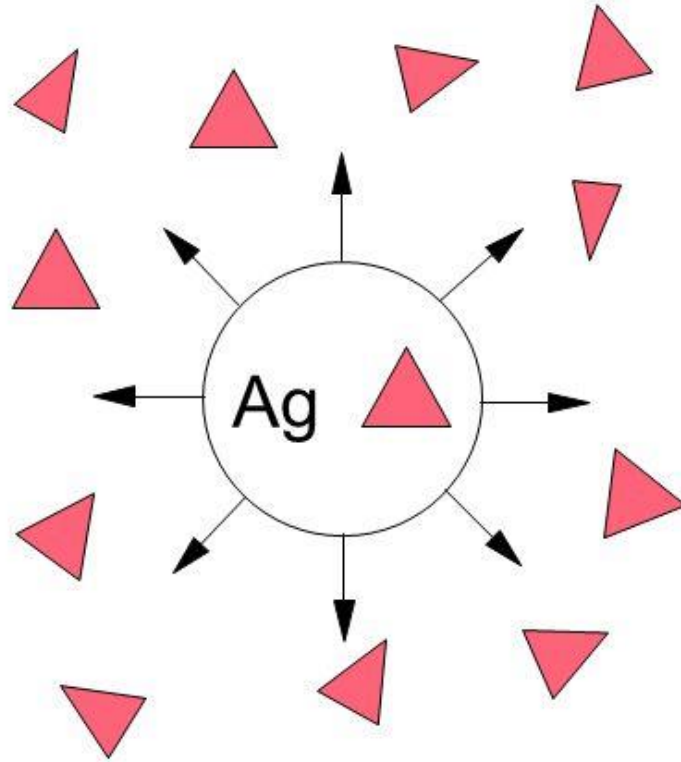


# Mechanism

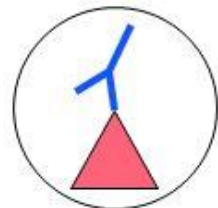
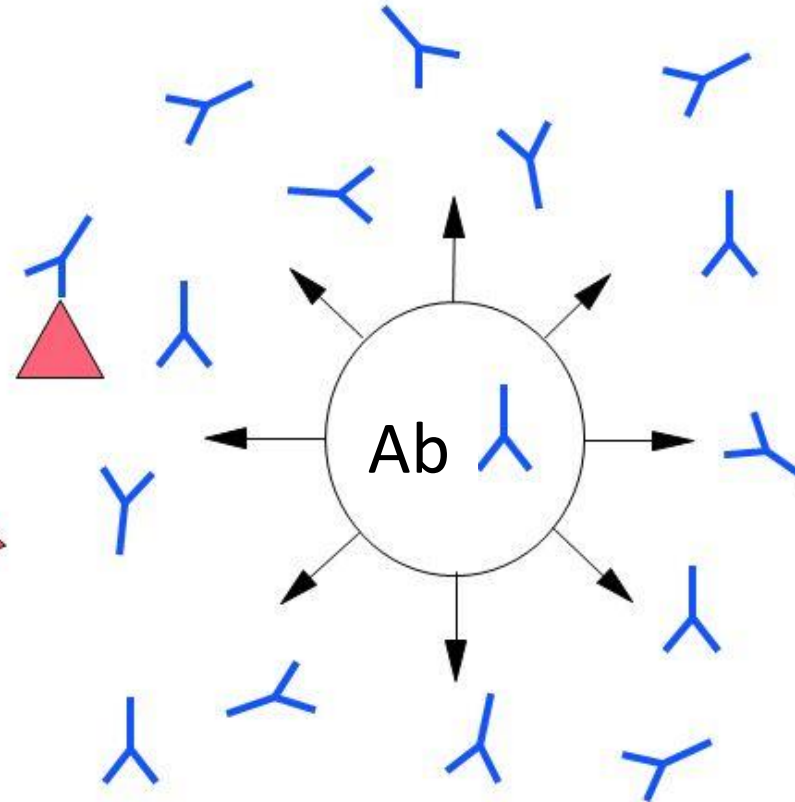


# Diffusion of Reagents

Antigens diffuse in all directions within the gel.

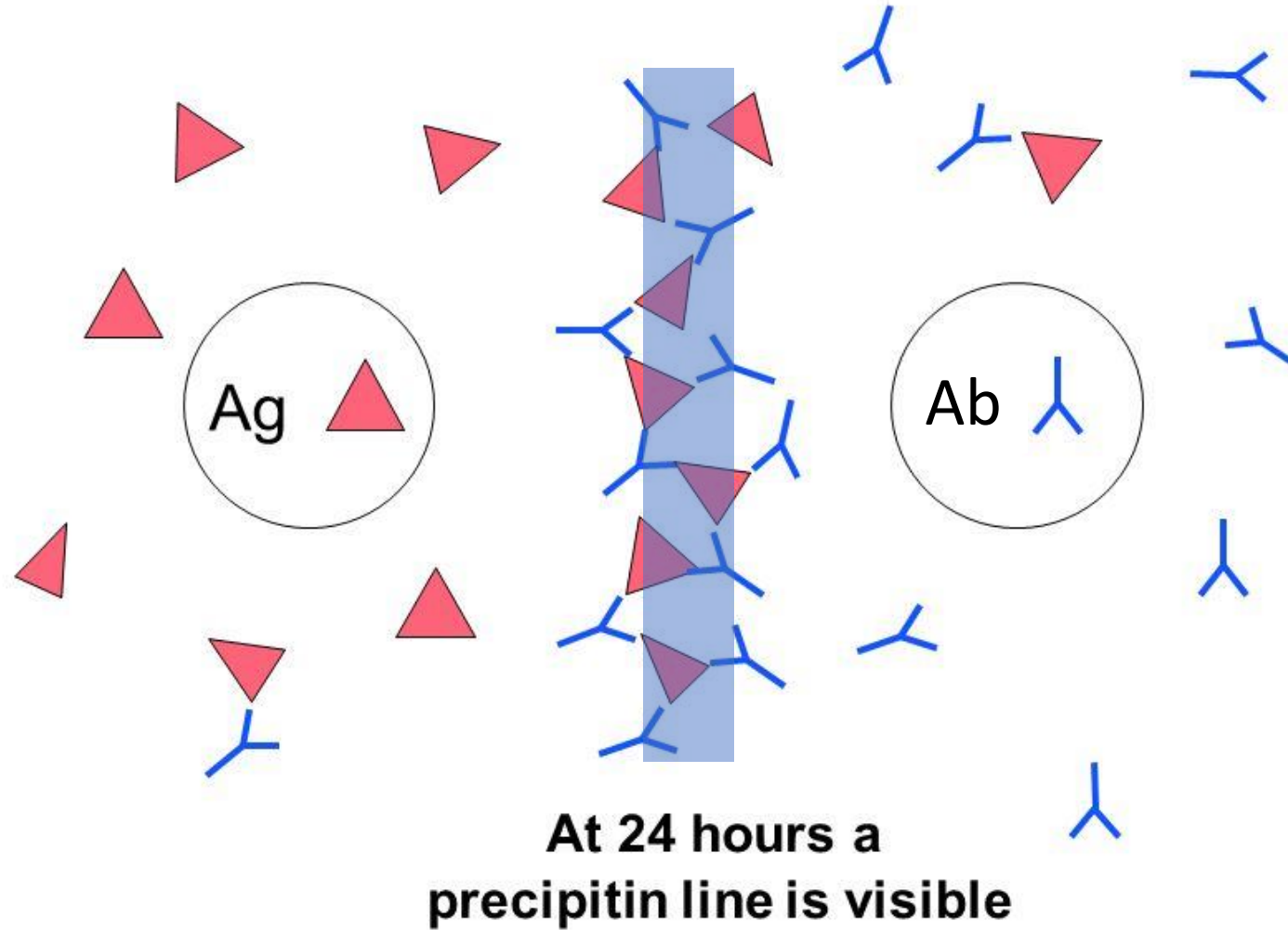


Antibodies diffuse in all directions within the gel.

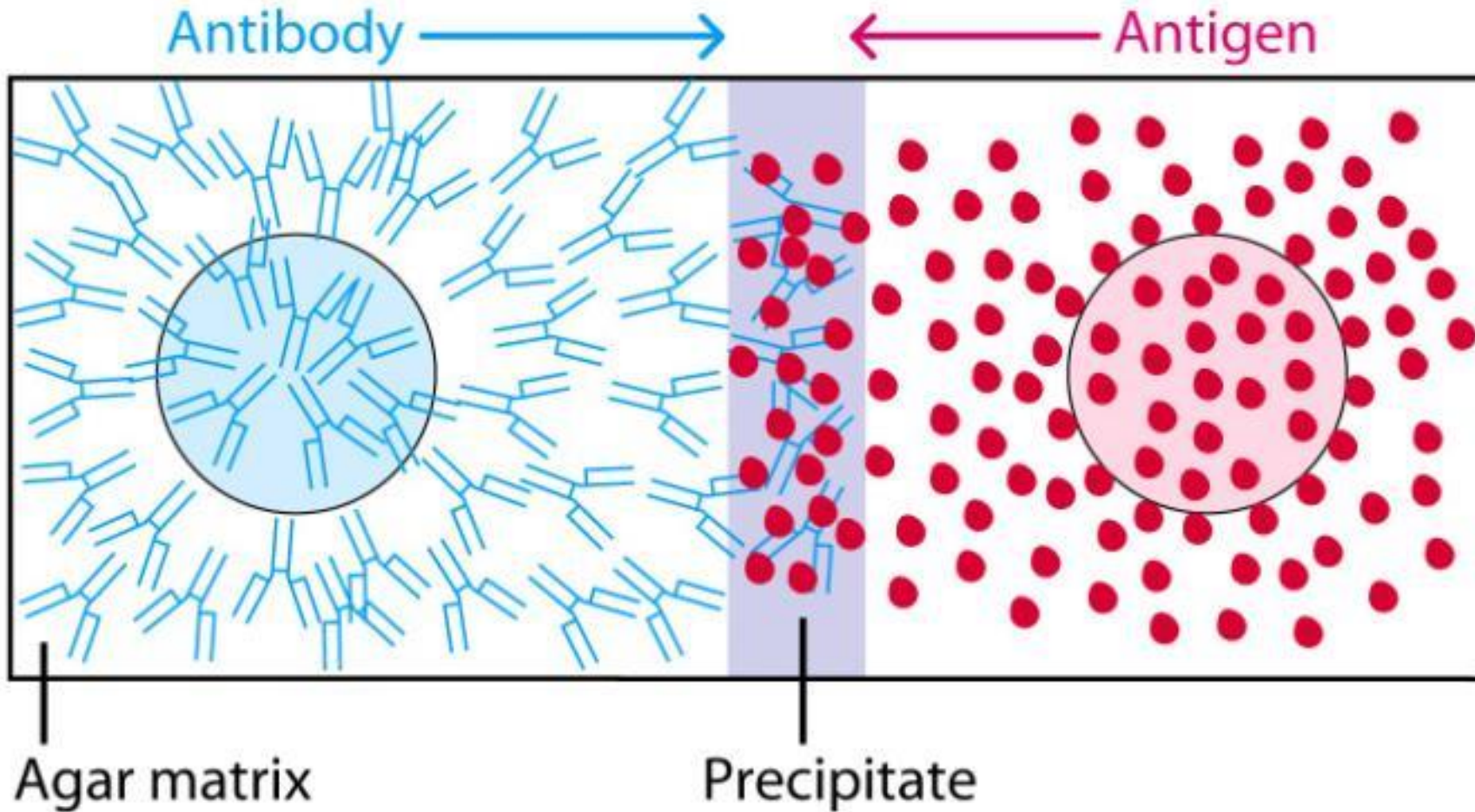


**Seen as a precipitin line when concentrations are optimal**

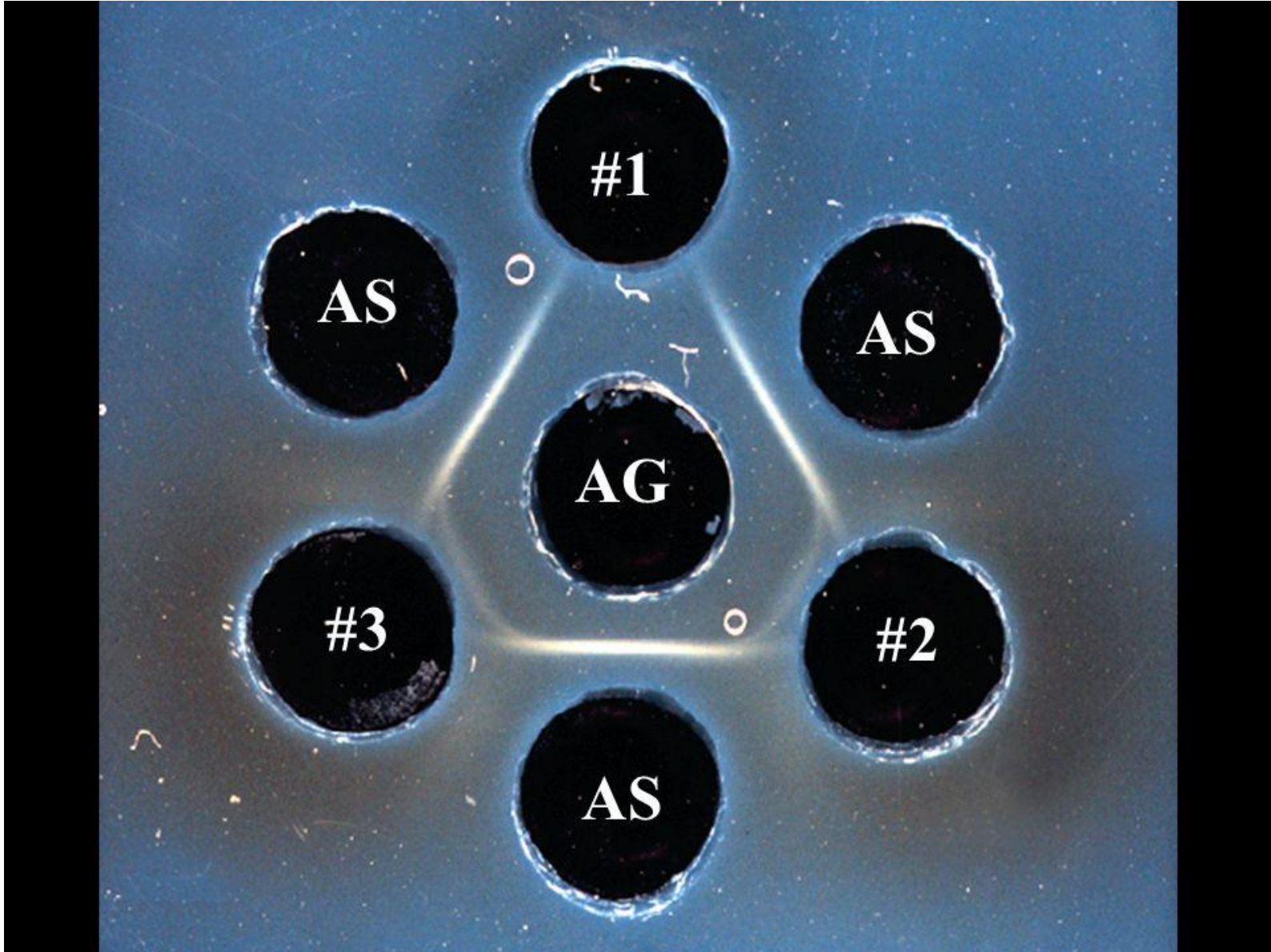
# Diffusion of Reagents



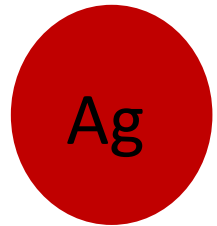
## DOUBLE IMMUNODIFFUSION



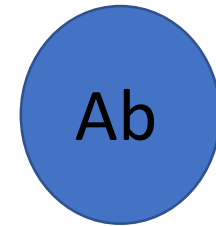
Both Ab and Ag diffuse radially from the wells  
As equivalence is reached a visible ring of precipitation is formed

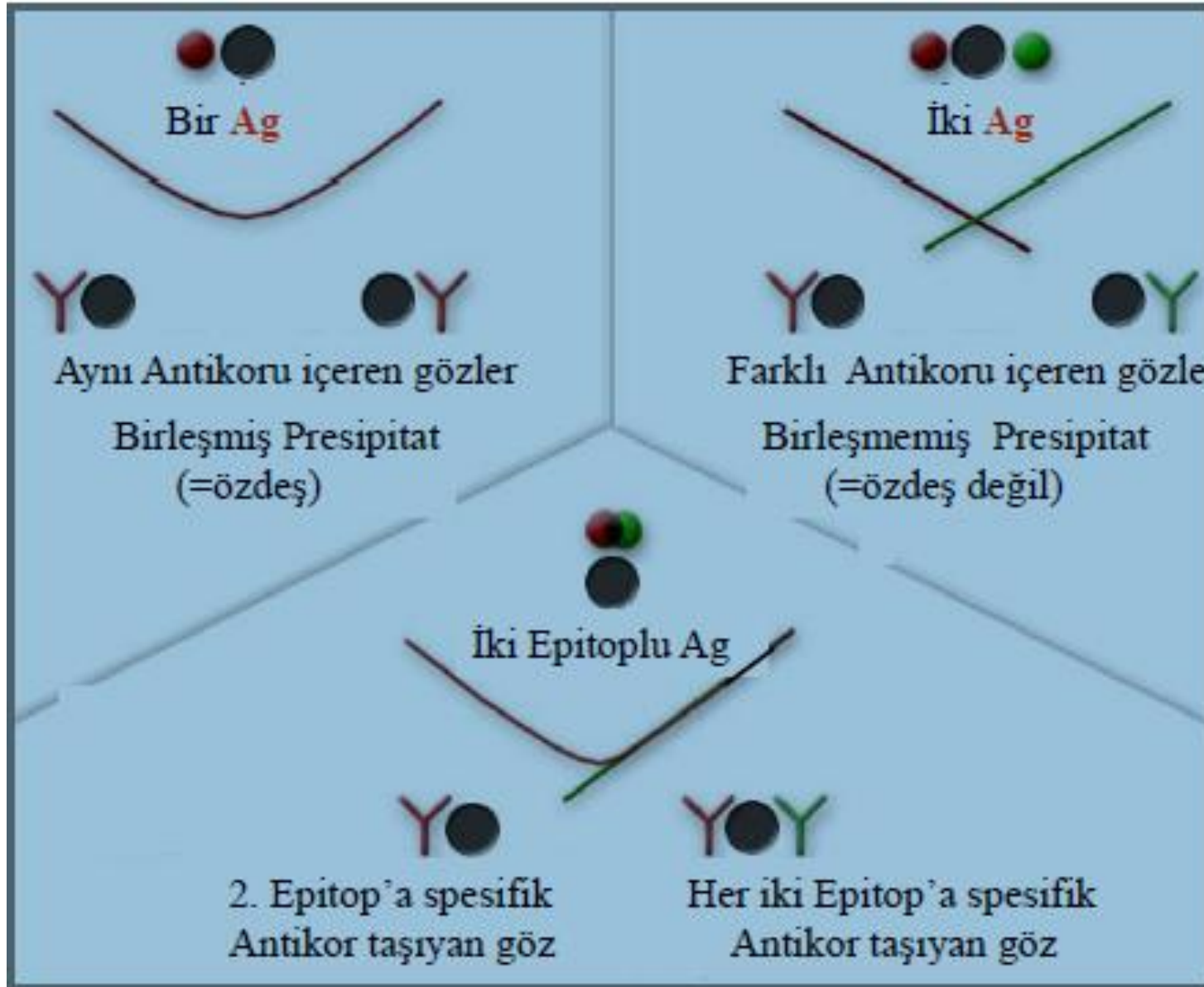


If **Ab** concentration is high, precipitate forms close to **Ag**

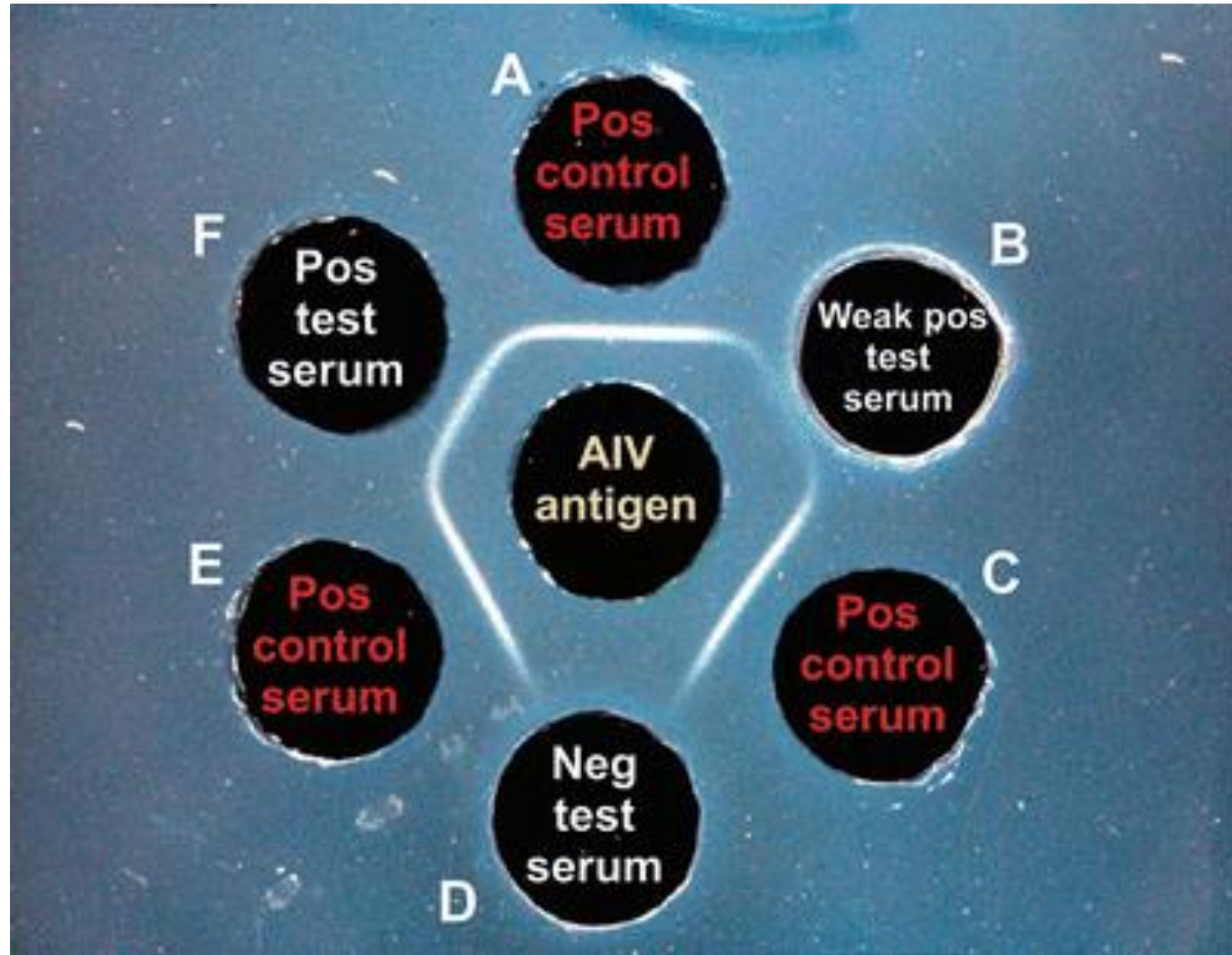


If **Ag** concentration is high, precipitate forms close to **Ab**



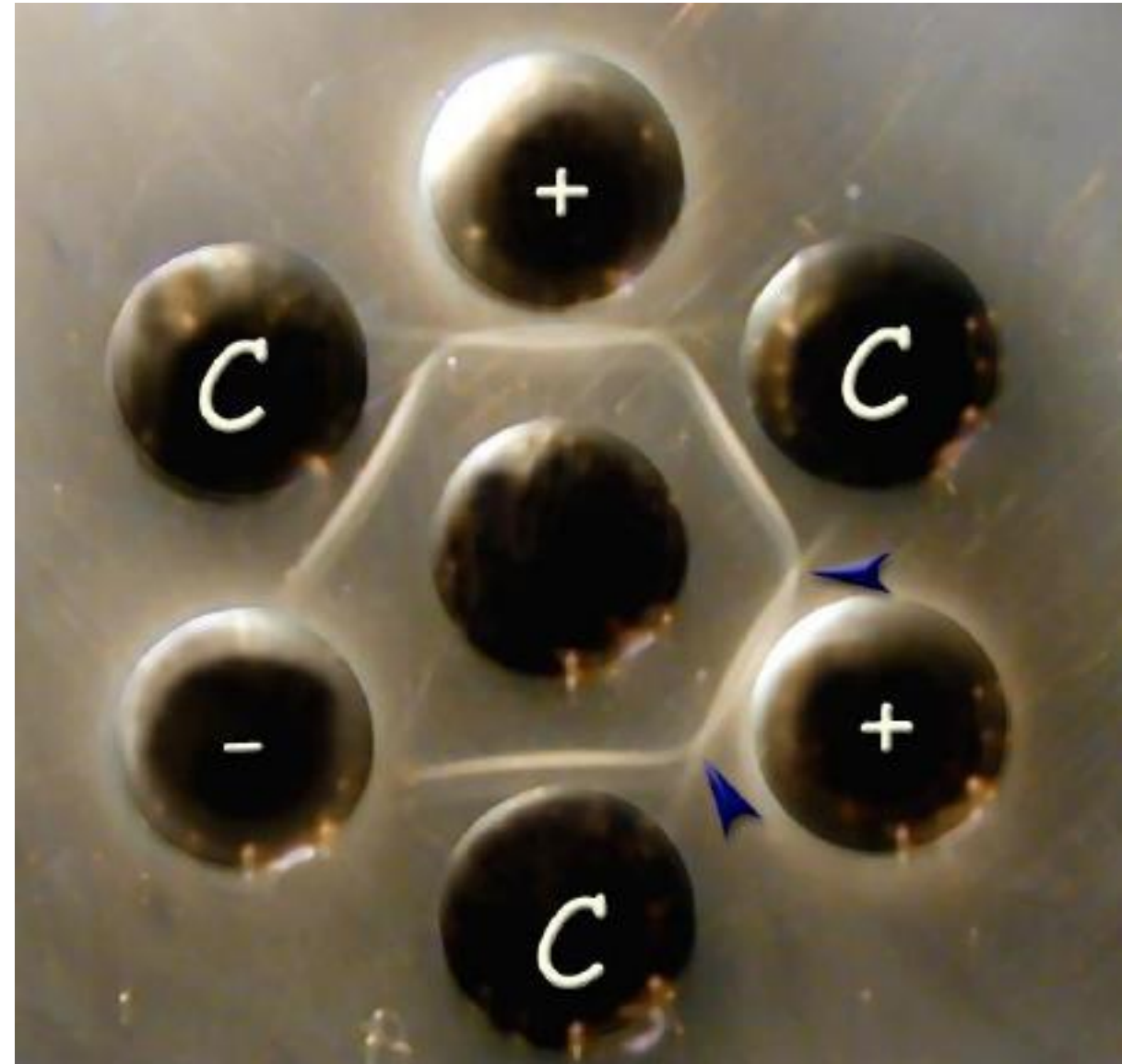






Jenson, T.A. (2014). Agar Gel Immunodiffusion Assay to Detect Antibodies to Type A Influenza Virus. In: Spackman, E. (eds) Animal Influenza Virus. Methods in Molecular Biology, vol 1161. Humana Press, New York, NY. [https://doi.org/10.1007/978-1-4939-0758-8\\_13](https://doi.org/10.1007/978-1-4939-0758-8_13)

- In this test, two samples showed lines of identity with the positive control sera and were positive (+).
- In positive samples, in addition to the specific reaction, the presence of another nonspecific precipitation is observed. (Between arrows)



# To calculate Ab titer

- If the test will be used to determine the antibody titer of the serum, a series ( $1/2$ ,  $1/4$ ,  $1/8$ , ...) dilution of the serum is prepared and the test is performed.
- Antibody titer is evaluated as the final dilution step where precipitate formation is seen.

