

AGAR GEL IMMUNODIFFUSION
(AGID) TEST

or

AGAR GEL PRECIPITATION TEST
(AGPT)

Description

- The test is based on the diffusion of antigen and antibody through a semi-solid agar medium and formation precipitin lines at the sites where the antigen and antibody interact.
- The principle of the test is the passive diffusion of soluble antigens and antibodies toward each other in a gel, resulting in visible precipitation at the zone of equivalence.

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](#).
- **Precipitin line (Precipitate):** the visible line formed at the Ag + Ab junction in positive reactions

Sensitivity

- AGID test; it is generally less sensitive than neutralization and CF test. However, it is recommended as a reference method for detecting persistent infections such as retroviral infections, and for diagnosis of certain acute infections.

What for do we use AGID test?

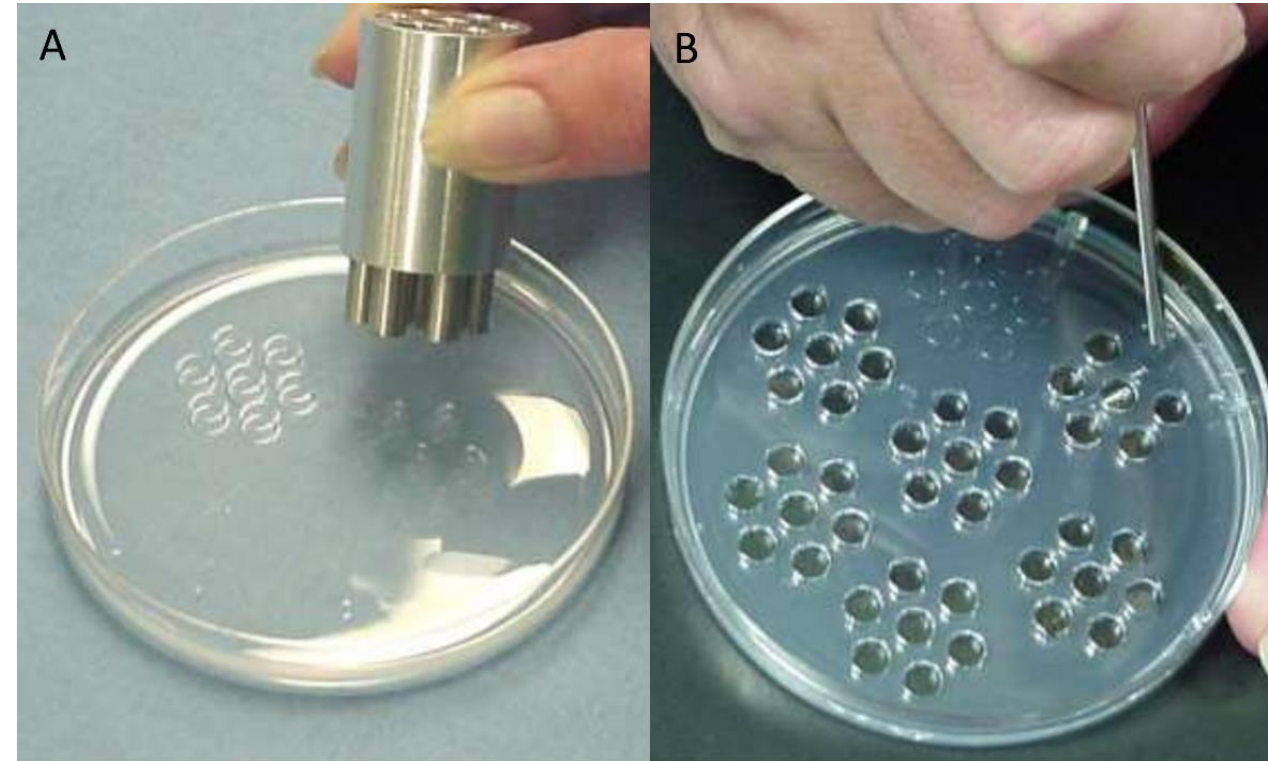
1. To detect Antibody or antibody titration
2. To detect viruses
 - Virus identification

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)

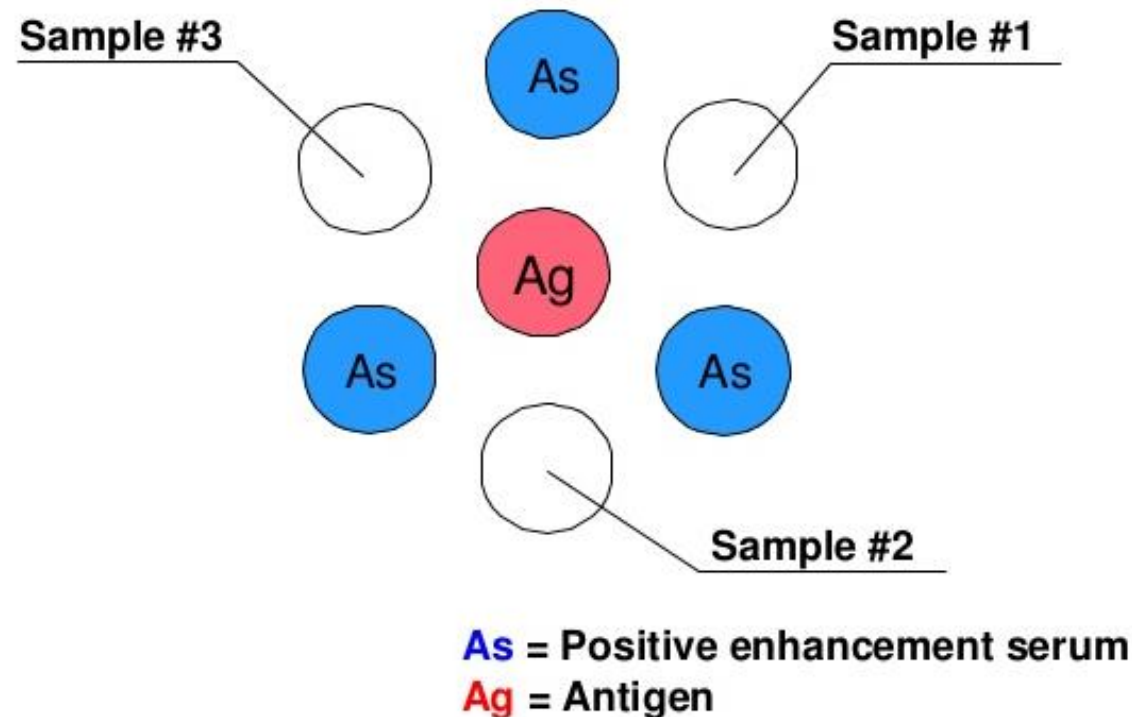
Protocol

- After autoclaving, the agar is poured into Petri dishes and allowed to solidify.
- Using a special well cutter, one central well and six surrounding wells are cut at equal distances from the center.



- Depending on the purpose;
Add the known material (serum or antigen) is added to the center well
And
Add the suspected materials to the peripheral wells.

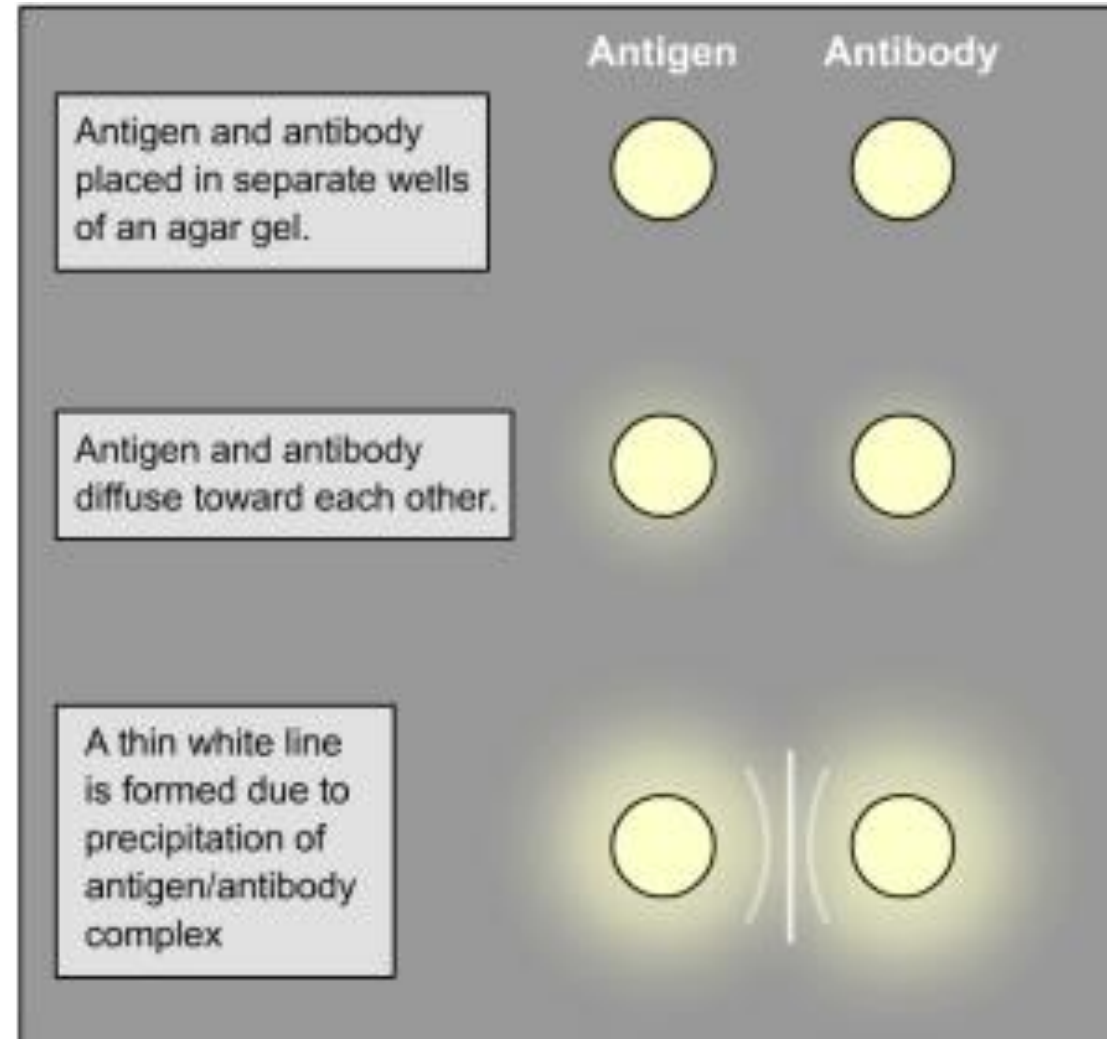
Placement of Reagents/Samples



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

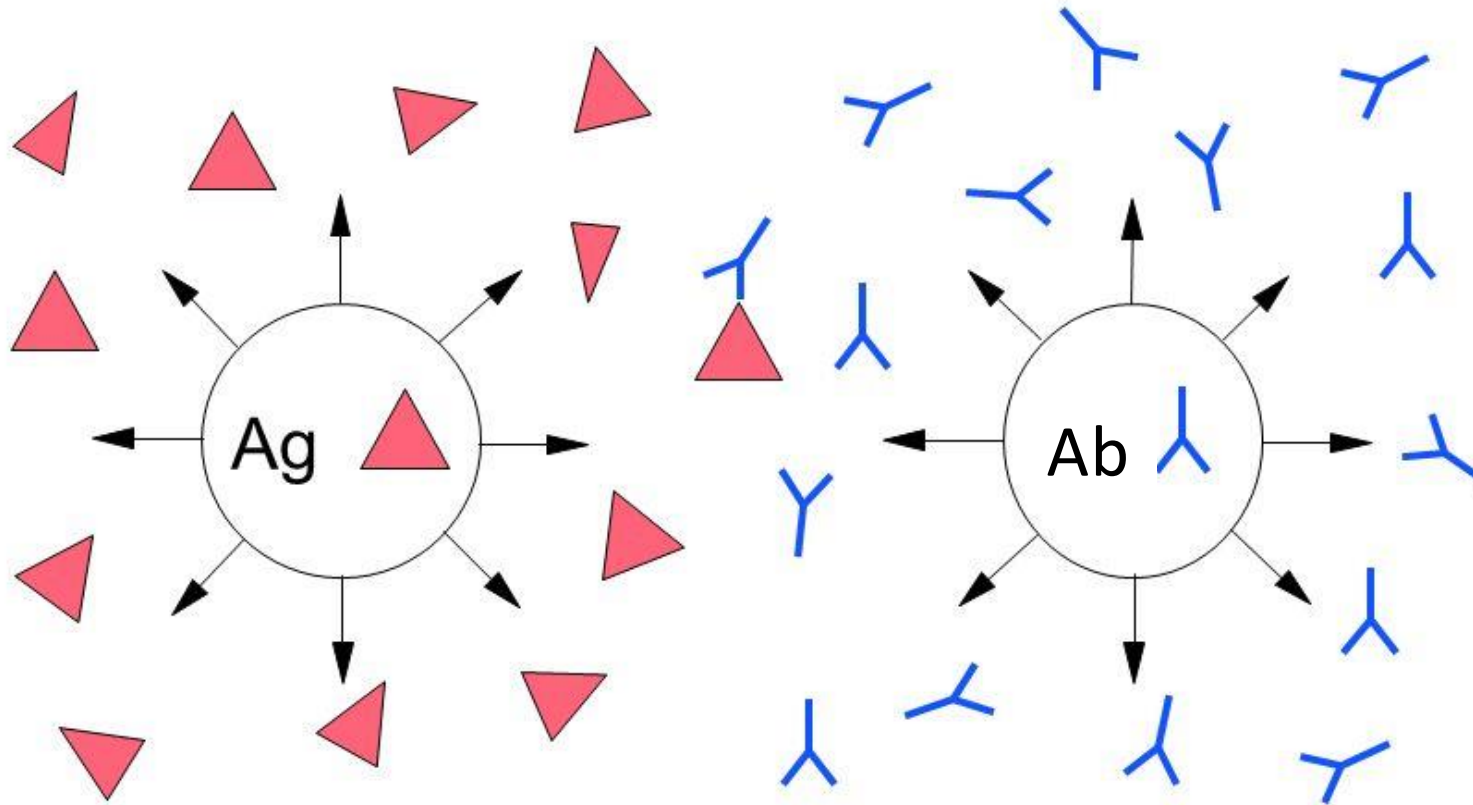


Mechanism

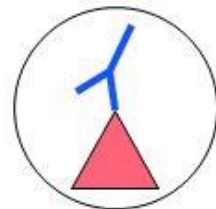


Diffusion of Reagents

Antigens diffuse in all directions within the gel.

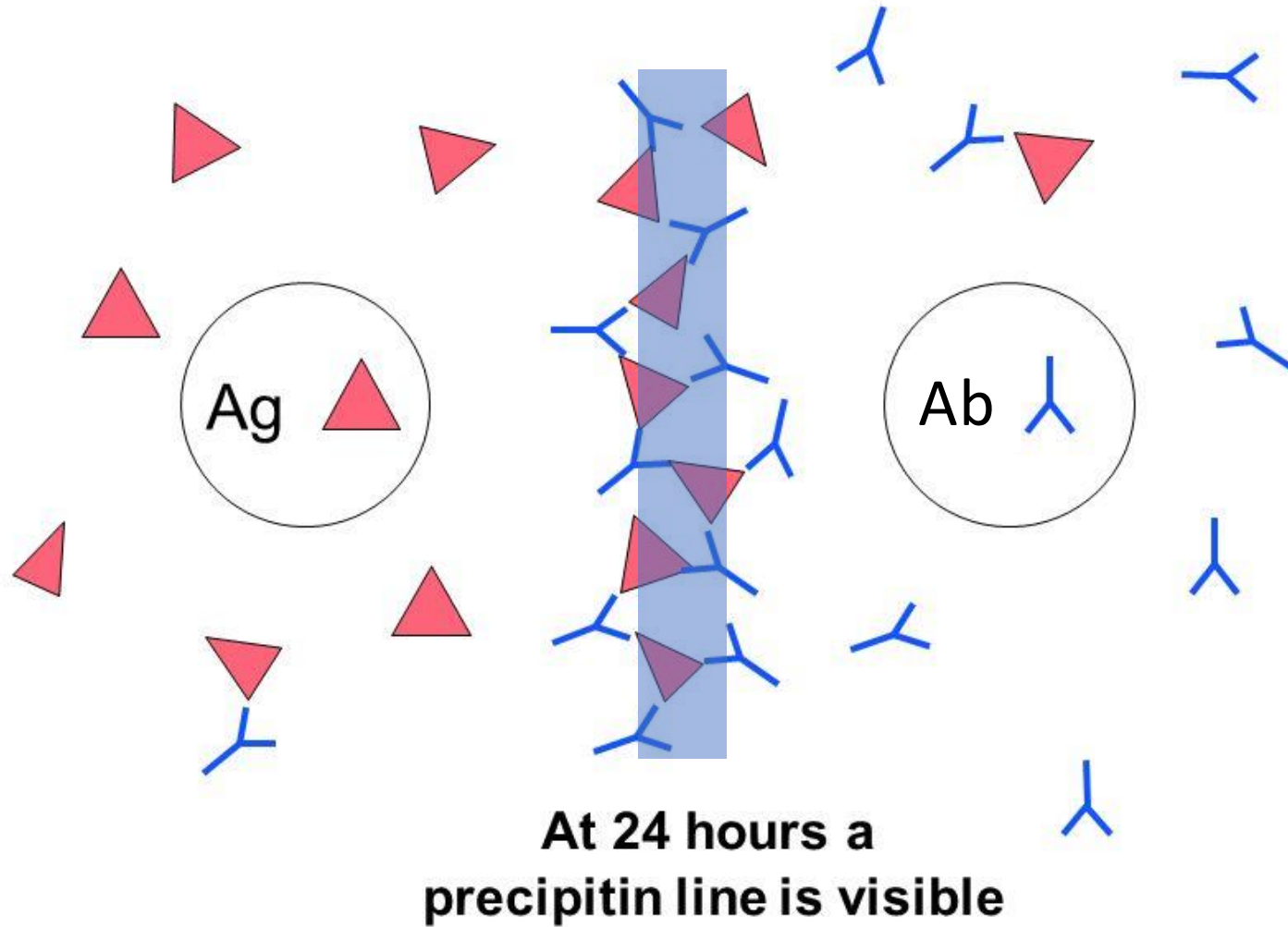


Antibodies diffuse in all directions within the gel.

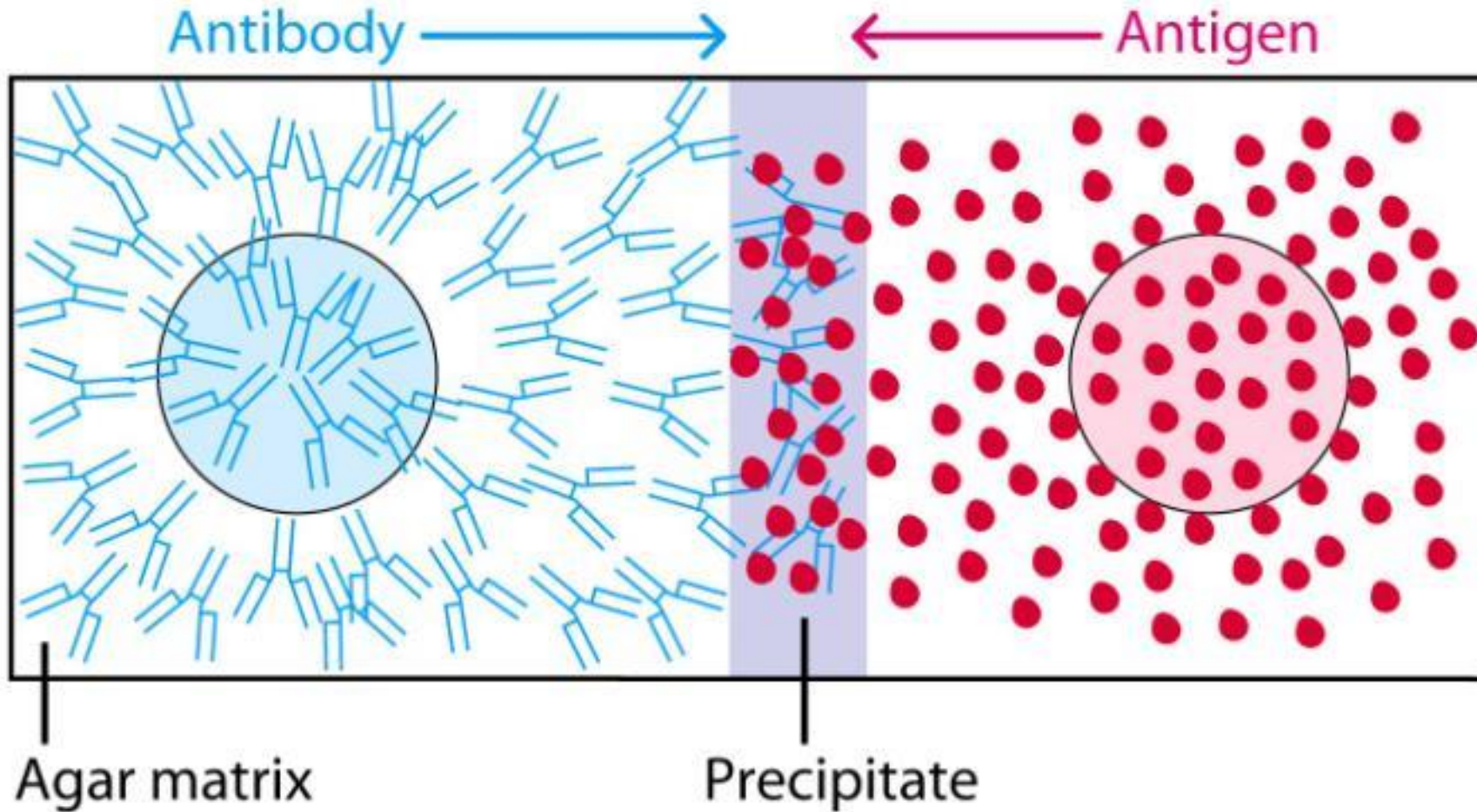


**Seen as a precipitin line
when concentrations are
optimal**

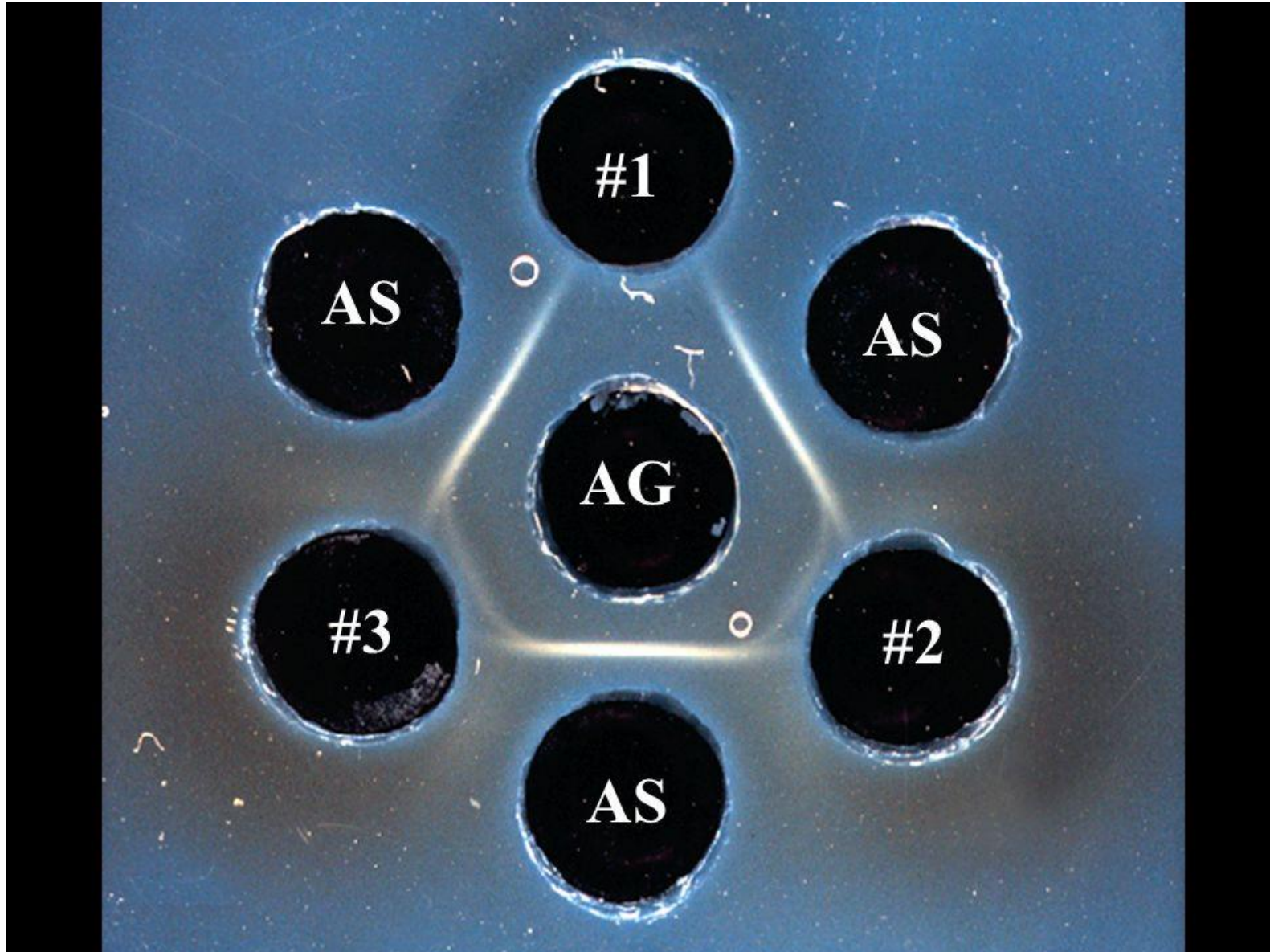
Diffusion of Reagents



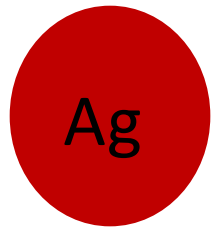
DOUBLE IMMUNODIFFUSION



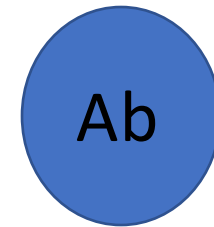
Both Ab and Ag diffuse radially from the wells
As equivalence is reached a visible ring of precipitation is formed

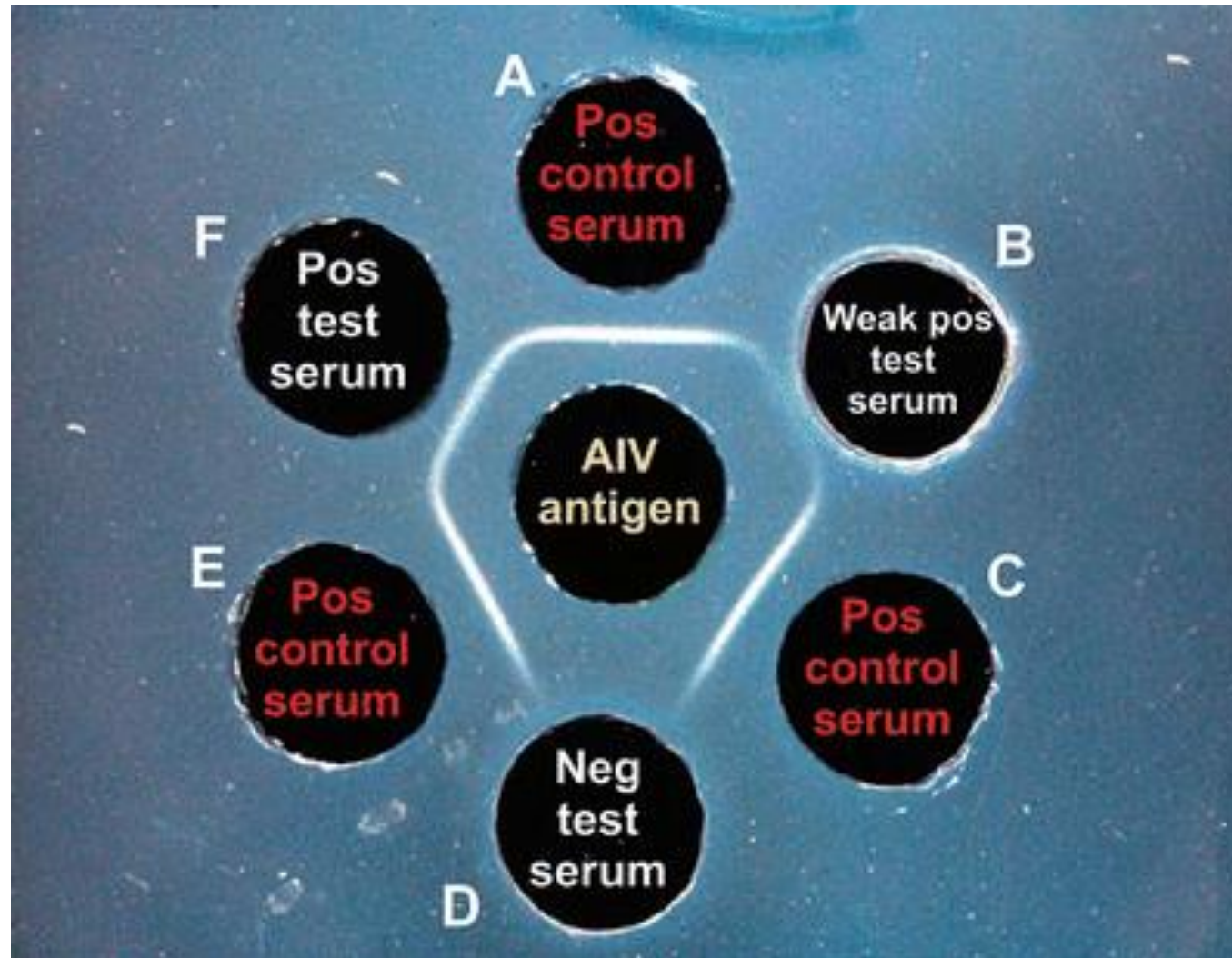


If **Ab** concentration is high, precipitate forms close to **Ag**



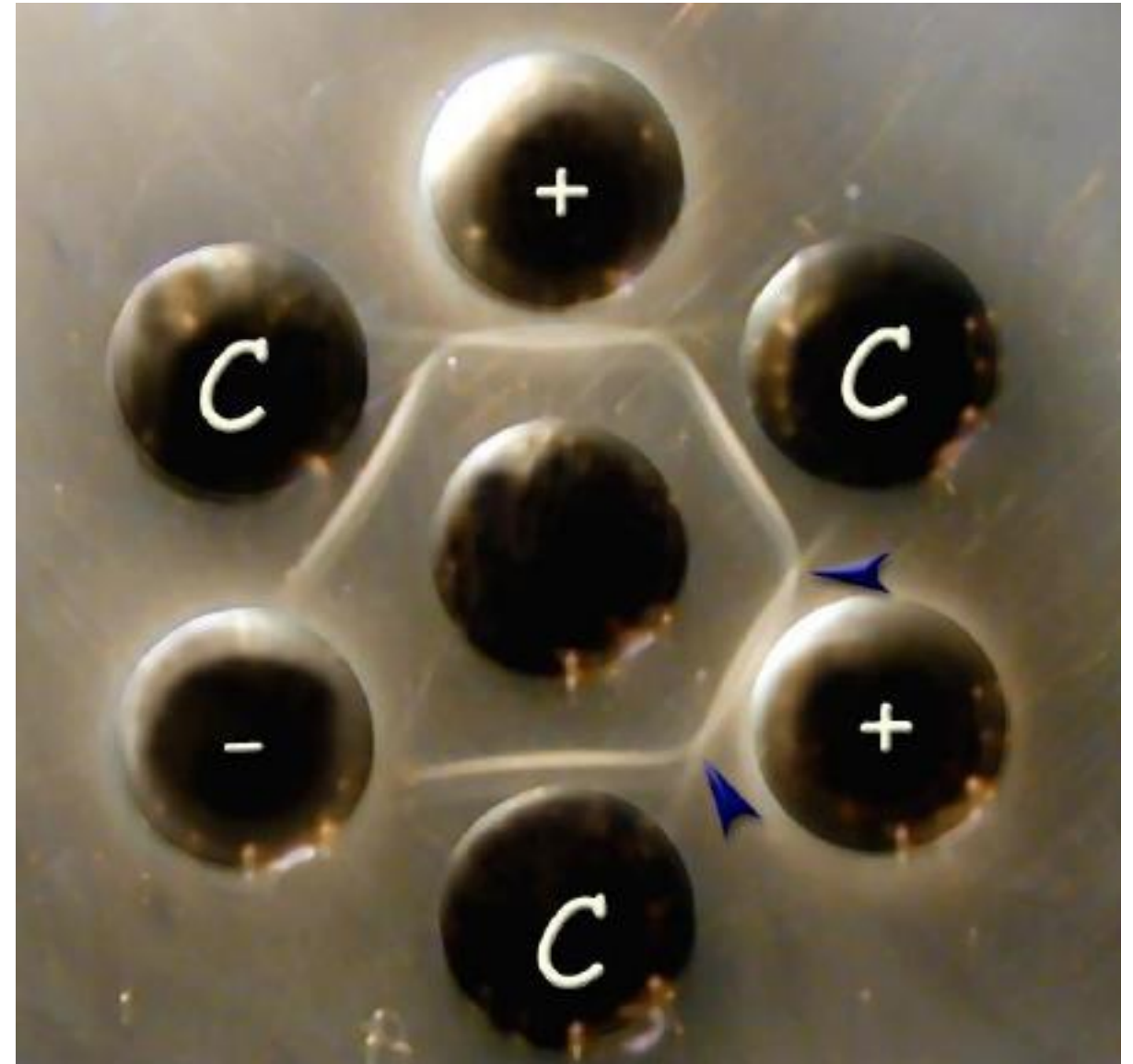
If **Ag** concentration is high, precipitate forms close to **Ab**





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

- 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 serial ($1/2$, $1/4$, $1/8$, ...) dilutions of the serum are prepared and the test is performed.
- Antibody titer is defined as the highest serum dilution at which precipitate formation is observed.

