Neutralization Tests:

- The neutralization test is an assay based on the ability of antibody to inactivate the biological effects of an antigen or of a microorganism expressing it. Neutralization applies especially to inactivation of virus infectivity or of the biological activity of a microbial toxin.
- Neutralaization tests can be applied:
  - *in vitro = (Lab tests) e.g inoculation in cell culture , Haemolysis inhibition tests (ASOT Anti Streptolysine O test)*
  - *in vivo* = (Living body /Protection tests) e.g *inoculation in lab animals , egg inoculation , Schick test*

- **The protection tests**:

  Measure the Antibodies neutralization ability toward toxins or viruses and results are measured by intensity of damage on cells or the body of the lab animals which may die in the doses where the Ab can not tolerate the effects of the Ag (Virus or Toxins).

Uses of the neutralization tests :

1. Recognition of the pathogenic causative agents (Toxins / Viruses) and differentiation between their pathogenicity.
2. Study the antigenic relation for various toxins and viruses
3. Detection the biological protection ability of the neutralizing antitoxins and antviruses against toxins and Viruses .
4. Measure the potency of vaccines and the ability to induce immunity.

Toxin - Antitoxin Neutralization

1. **In Vivo Neutralization**

Schick test:

- Schick test - used to determine whether or not a person is susceptible to diphtheria *(Corynebacterium diphtheriae)*.
- It is simple procedure.: A small amount (0.1 ml) of diluted (1/50 MLD) diphtheria toxin is injected intra dermally into the arm of the person.
- the skin around the injection will become red and swollen, indicating a positive result.

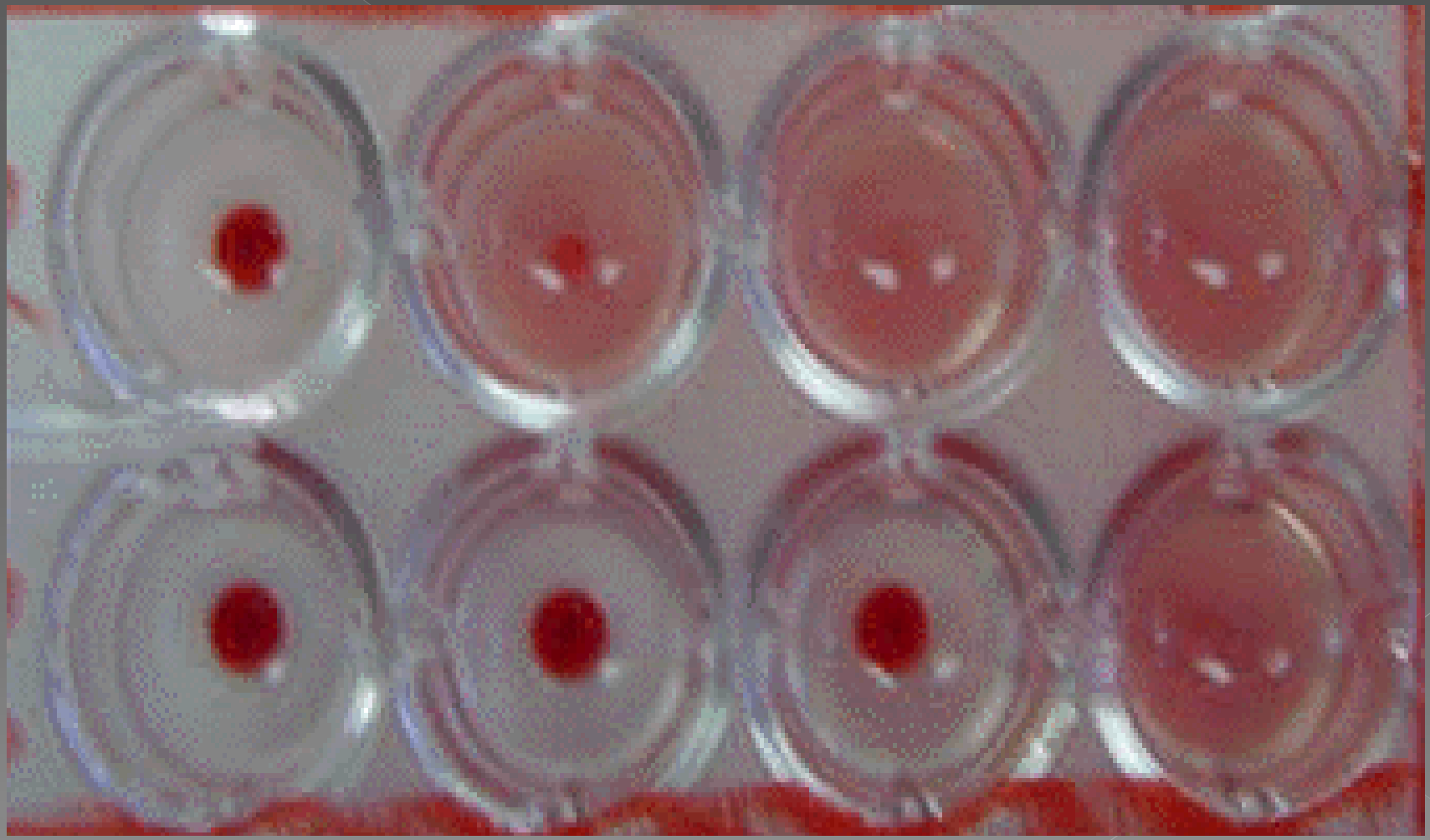
Results can be interpreted as:

- **Positive**: when the test results in a red necrotic area of 5-10 mm diameter
- **Pseudo-positive**: when there is only a red colored inflammation and it disappears rapidly
- **Negative reaction**: No Wheel and erythema

Neutralization test - Haemolysine

- Methodology

- 0.5 ml Haemolysine + 0.5 ml diluted SERUM --- Incubation at 37 ^{o}C for 15 Minutes
- Addition of O^{-} RBC ---Incubation at 37 ^{o}C for 45 Minutes
- Centrifuge 3000 RBM for one minute



- **Reversible neutralization** -
- The neutralization process can be reversed by diluting the Ab-Ag mixture within a short time of the formation of the Ag-Ab complexes (30 mins). It is thought that reversible neutralization is due to the interference with attachment of virions to the cellular receptors eg. the attachment of the HA protein of influenza viruses .
- **Stable neutralization** - with time, Ag-Ab complexes usually become more stable (several hours) and the process cannot be reversed by dilution. . An example of stable neutralization is the neutralization of polioviruses, whereby, the attachment of the antibody to the viral capsid stabilizes the capsid and inhibits the uncoating and release of viral nucleic acid.