# Complement Fixation Test

- It is a method that allows to investigate the presence of suspected Ag or Ab through the hemolytic system by using the complement's ability to attach the antigen-antibody complex.

# What we need to perform the CFT

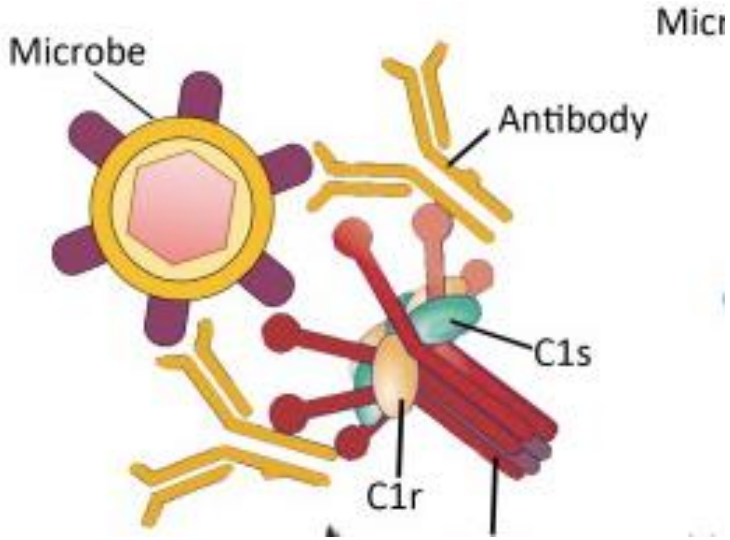
- Antigen (suspected or known)
- Antibody (suspected or known)
- Complement (Fresh guinea pig serum)
- Haemolytic system
    - 1. sheep red blood cells (SRBC)
    - 2. amboceptor
    - amboceptor: Hyperimmune serum obtained by administration of sheep erythrocytes into the rabbit ear vein. anti-SRBC antibody.

- The hemolytic system is the indicator medium required for the evaluation of the test.

- Since the amboseptor, used in this test, is the antibody specific for sheep erythrocytes, the amboseptor and sheep erythrocytes are specific antigen-antibodies for each other.

# What is Complement?

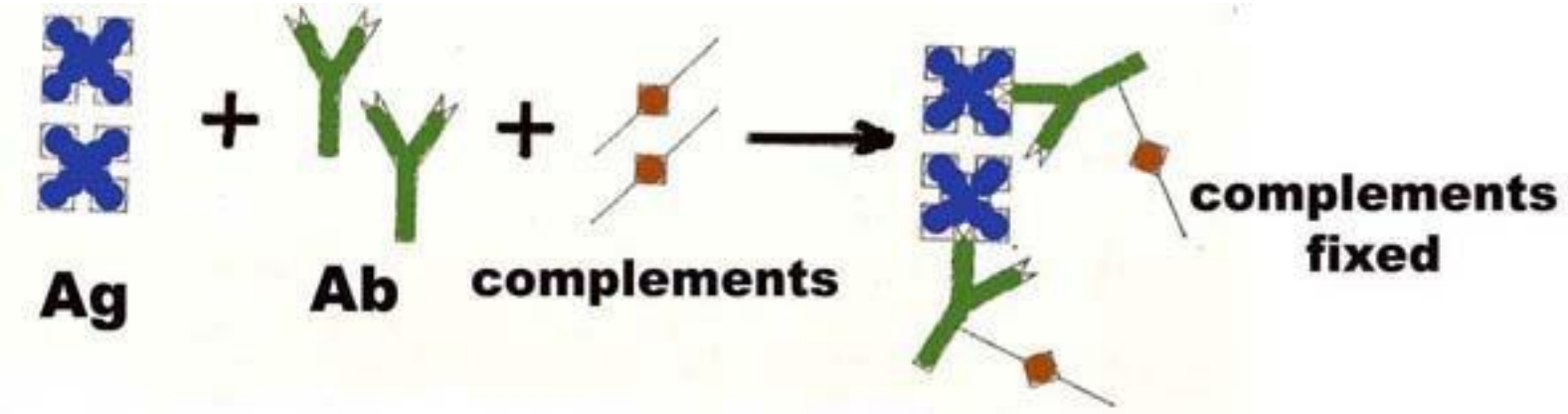
- It is a protein substance that
  - is found in the normal fresh serum of vertebrates,
  - is not heat resistant (can be inactivated at 56 ° C for 30 minutes),
  - is not associated with the antibody, however, it can be attached to the antigen-antibody complex under appropriate conditions and causes cytolysis in this way.




Complement cannot bind to antigen or antibody alone. İt is capable of binding to the antigen-antibody complex.

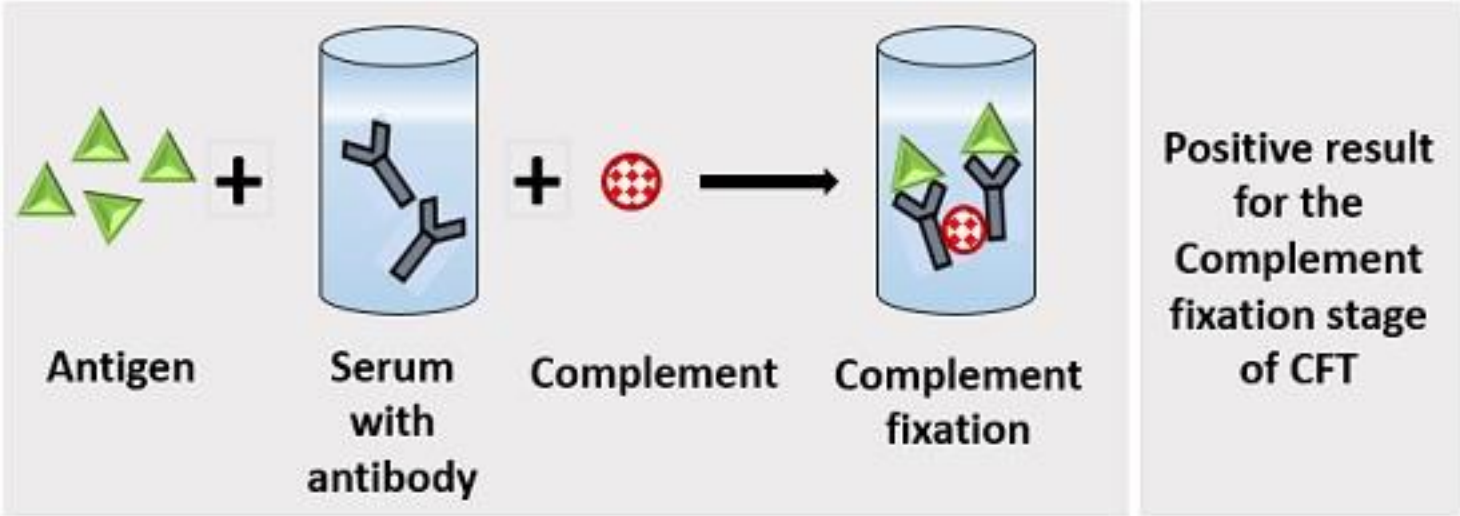
# Purpose of the test

1. Antigen identification
   - (Suspected virus, known serum)
2. Detection of antibody presence / antibody titer
   - (Known virus / suspected serum)




http://biosiva.50webs.org/compft.htm

- If the antibody is present in the serum, it binds to the antigen, and the complement reagent is completely consumed in the reaction. (The test can also be used to look for antigen in the serum by modifying the reagents used).



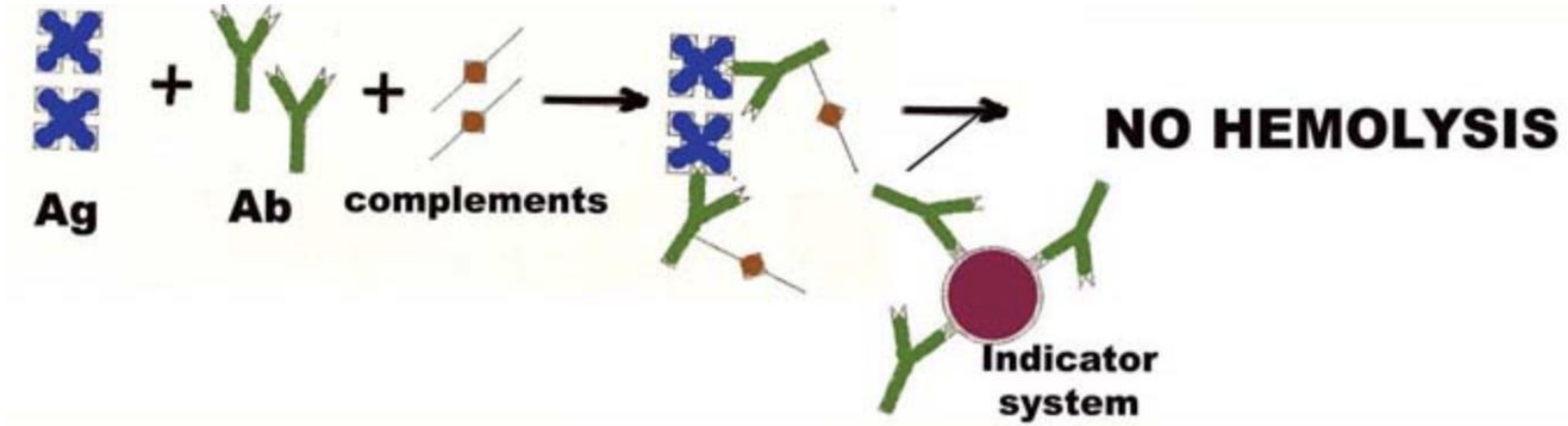

https://biologyreader.com/complement-fixation-test.html#Procedure

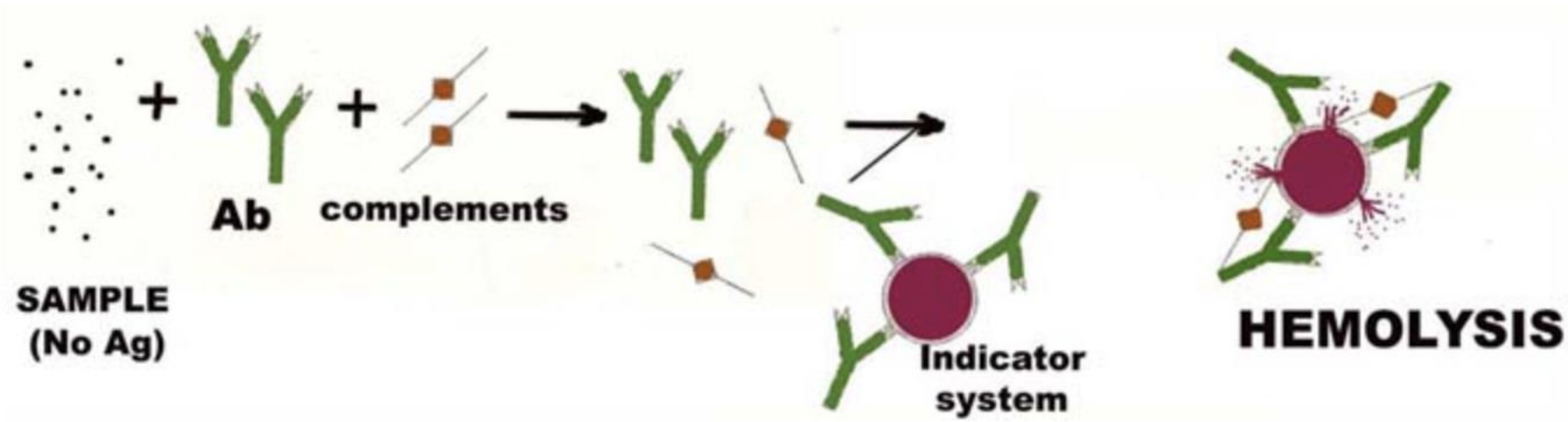
# Protocol

- incubate an antigen (suspected), inactivated serum (Ab) and a complement in a tube.

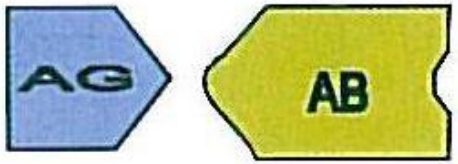
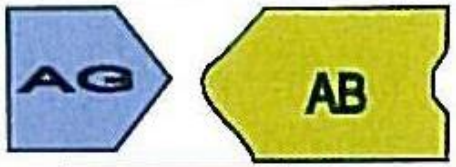
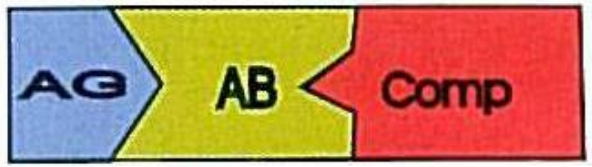
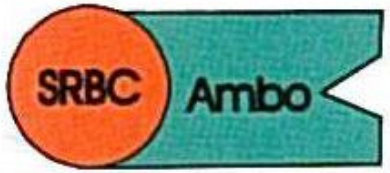
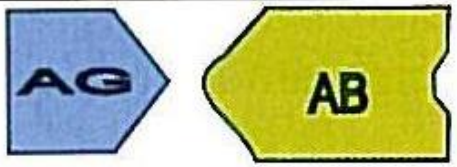
- At the end of the time hemolytic system, sheep red blood cells (SRBC) + amboseptor were added and incubated.

- Read the result.



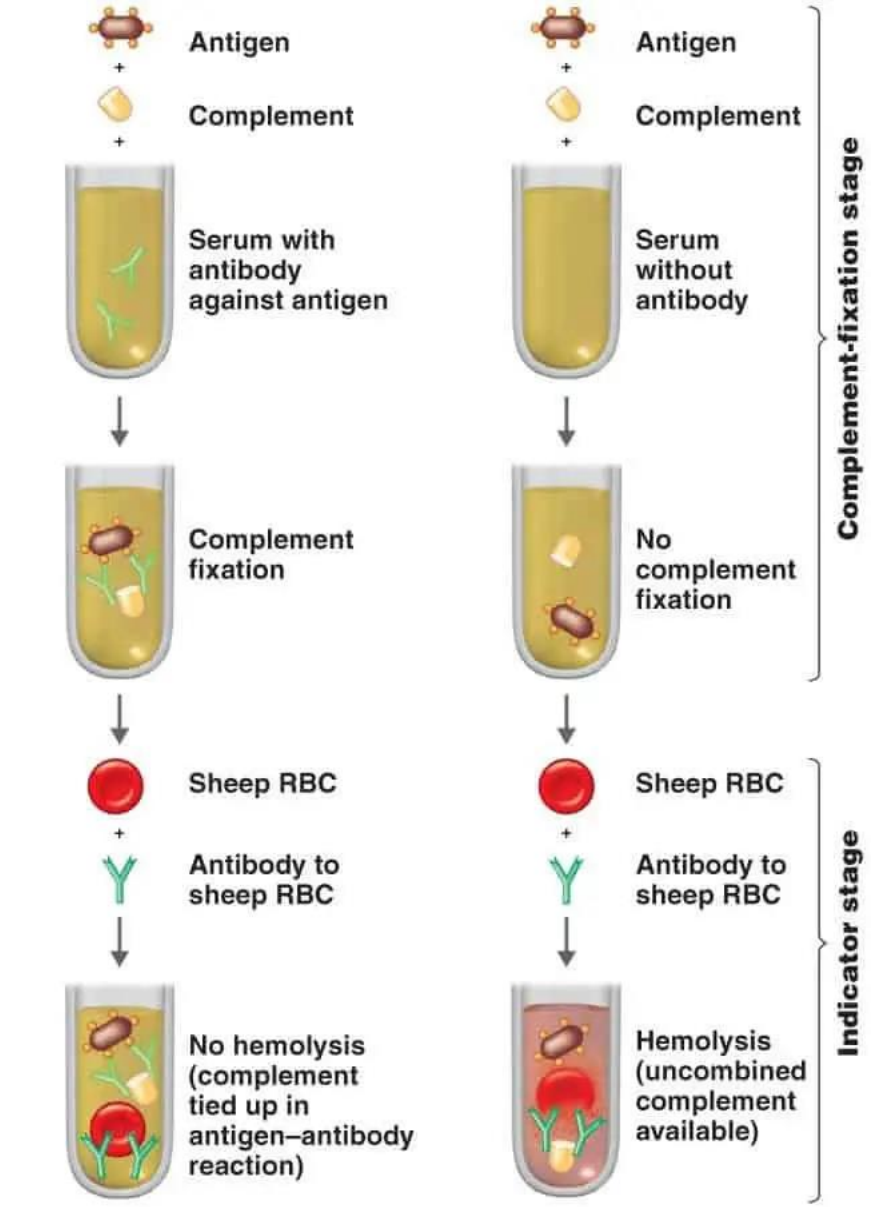

http://biosiva.50webs.org/compft.htm

| Positive sample | Reaction | Negative sample |
|---|---|---|
|  <br>Formation of immune complexes | Test reaction :<br><br>Addition of serum sample to test antigen |  <br>unbound antibodies |
|  <br>Fixation of complement by immune complexes | Addition of complement |  <br>  <br>unbound complement |
|  <br>  | Indicator reaction :<br><br>Addition of SRBC and Amboceptor |  <br>  |
| ⇒ Sedimentation of SRBC, no lysis | Result | ⇒ Lysis of SRBC by activated complement |

C.Staak, F.Salchow, N.Denzin : Practical Serology from the Basics to the testing, 2001.

| Suspected virus | Hemolysis | CFT |
|---|---|---|
| • Ab specific | (-) | (+) |
| • Not Ab specific | (+) | (-) |

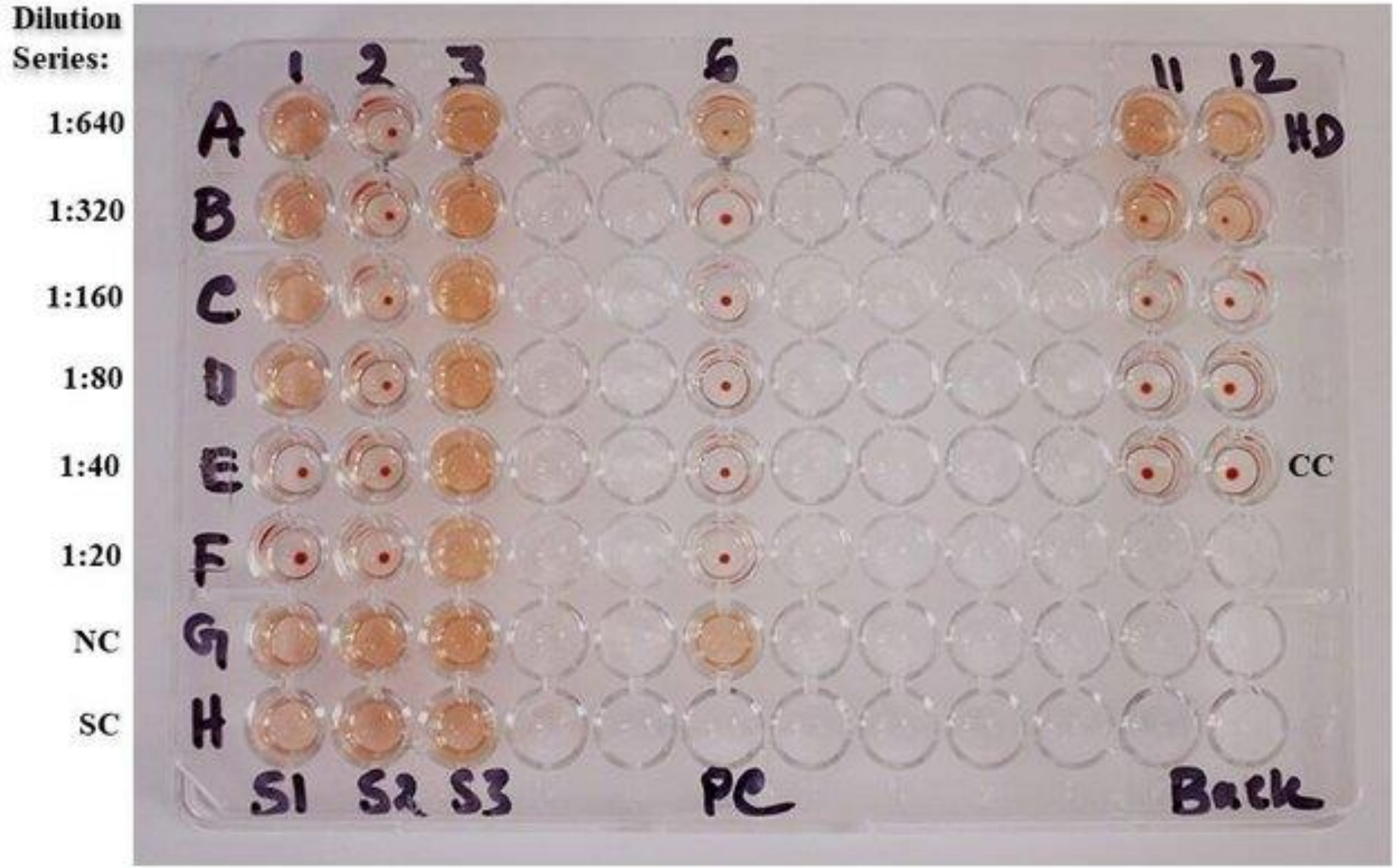
| Suspected Serum | Hemolysis | CFT |
|---|---|---|
| • Ag specific | (-) | (+) |
| • Not Ag specific | (+) | (-) |




**(a) Positive test.** All available complement is fixed by the antigen–antibody reaction; no hemolysis occurs, so the test is positive for the presence of antibodies.

**(b) Negative test.** No antigen–antibody reaction occurs. The complement remains, and the red blood cells are lysed in the indicator stage, so the test is negative.

https://laboratoryinfo.com/complement-fixation-test/




Khan, L., & Tsai, J. Y. (2019). A simple, cost-effective undergraduate workshop based on simulated complement fixation test to teach the concept of complement system.