

Complement Fixation Test

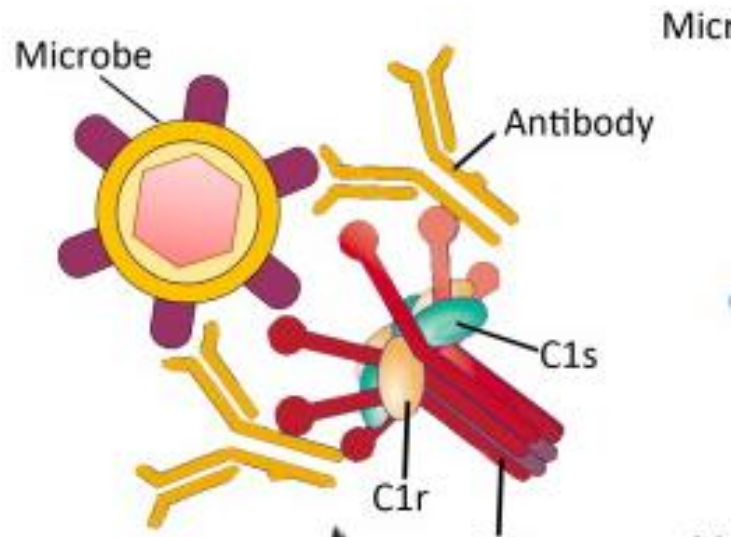
- It is a method that allows to investigate the presence of suspected Ag or Ab through the **hemolytic** system by using the complement's ability to attach the antigen-antibody complex.

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**.

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 does not bind to antigen or antibody alone. It can bind only to the antigen-antibody complex.

- The hemolytic system is used as the indicator system for the evaluation of the test results.
- The amboceptor used in this test is an antibody specific to sheep erythrocytes. In other words, the amboceptor and sheep erythrocytes form a specific antibody–antigen complex.

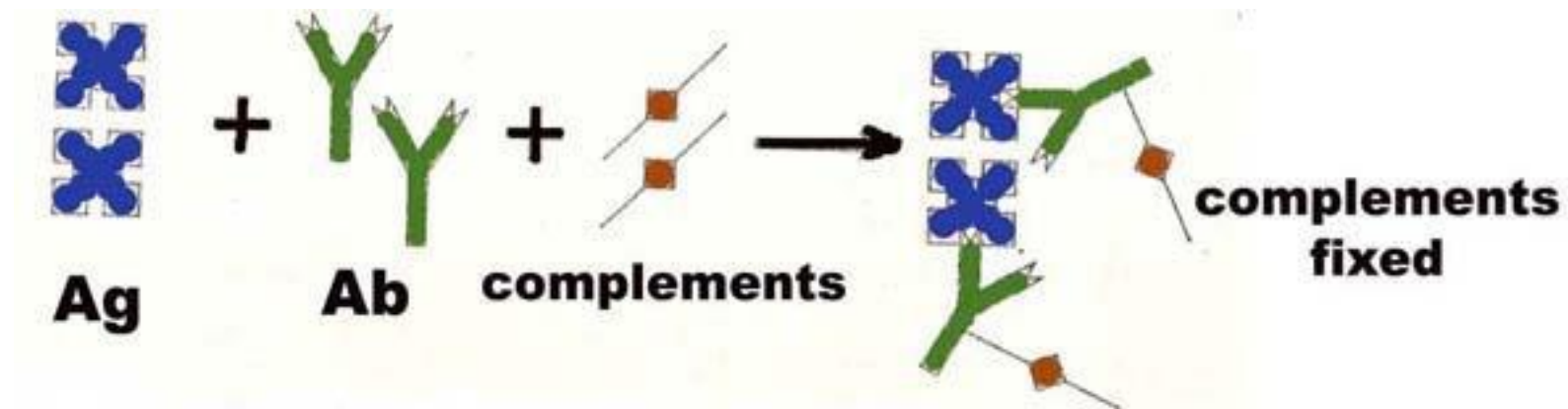
Purpose of the test

1. Antigen identification

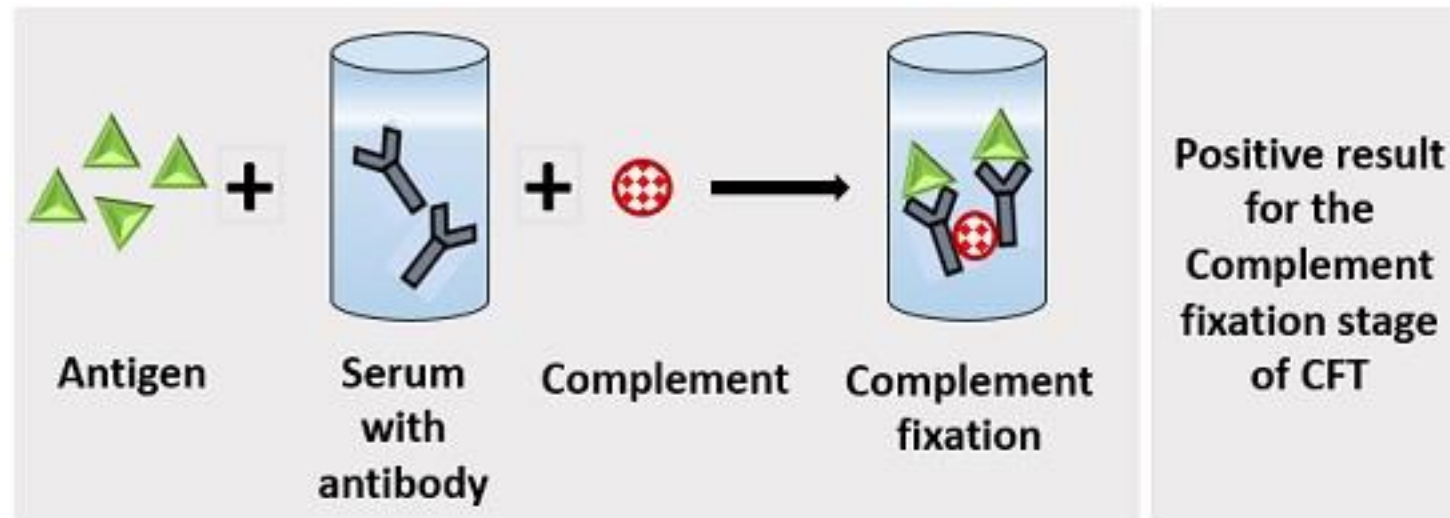
- (Suspected virus, known serum)

2. Detection of antibody presence / antibody titer

- (Known virus / suspected serum)

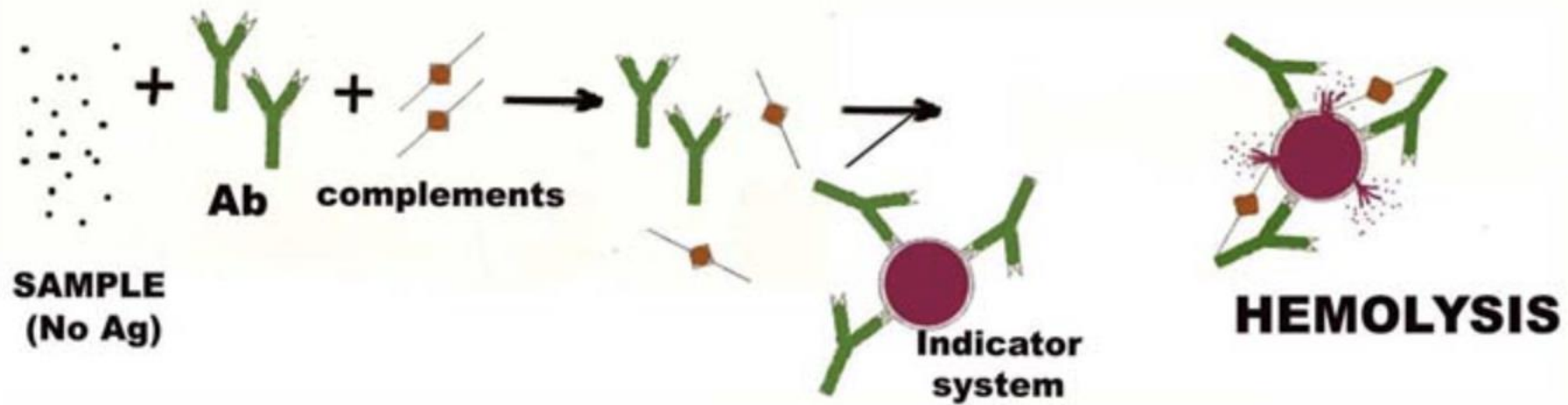
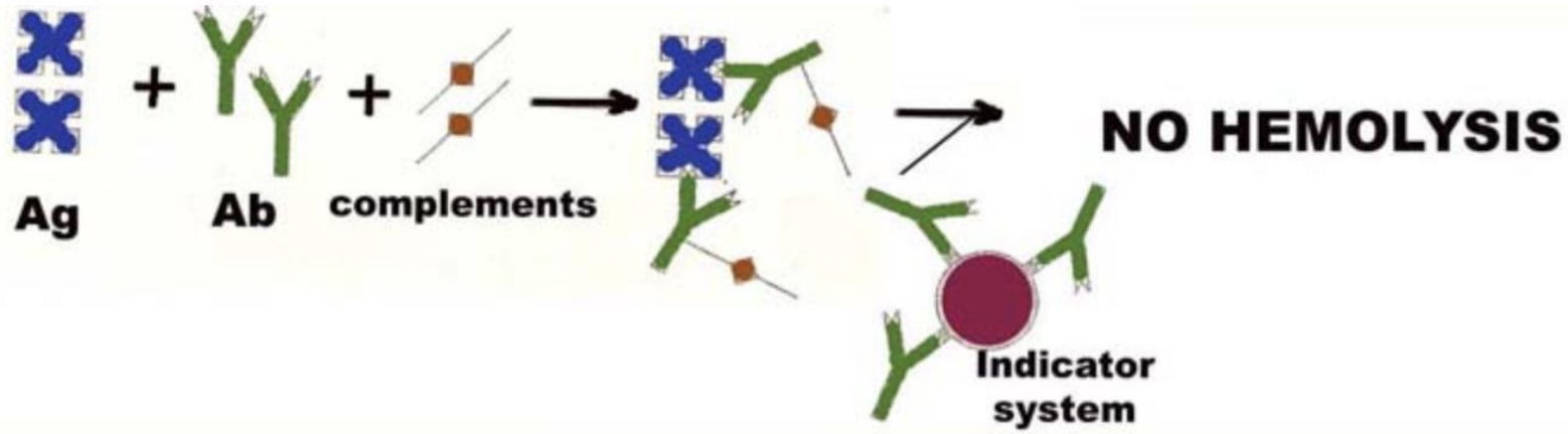


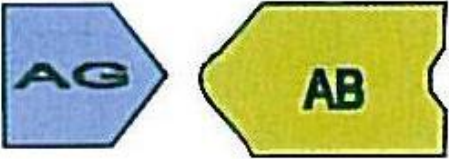
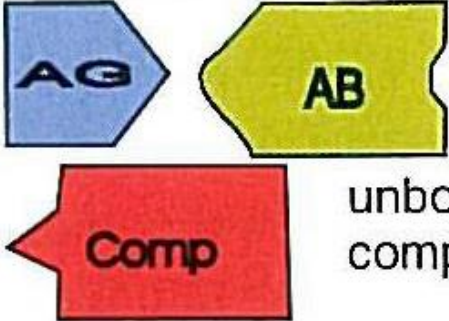
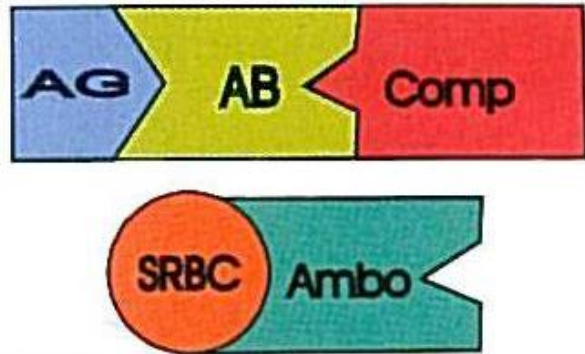
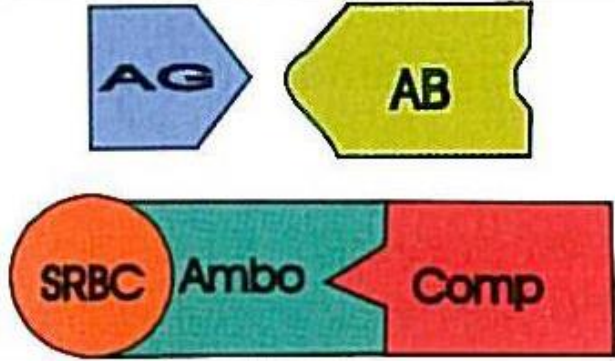
- If the antibodies are present in the serum, they bind to the antigen, and the complement is completely consumed in the reaction. (The test can also be used to detect antigen by modifying the reagents used).



Protocol

- incubate an antigen (suspected), inactivated serum (Ab) and a complement in a tube.
- After incubation the hemolytic system, **sheep red blood cells** (SRBC) + **amboceptor**, is added and incubated again.
- Check the result.



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

Suspected virus

- Ab specific
- Not Ab specific

Hemolysis

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CFT

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Suspected Serum

- Ag specific
- Not Ag specific

Hemolysis

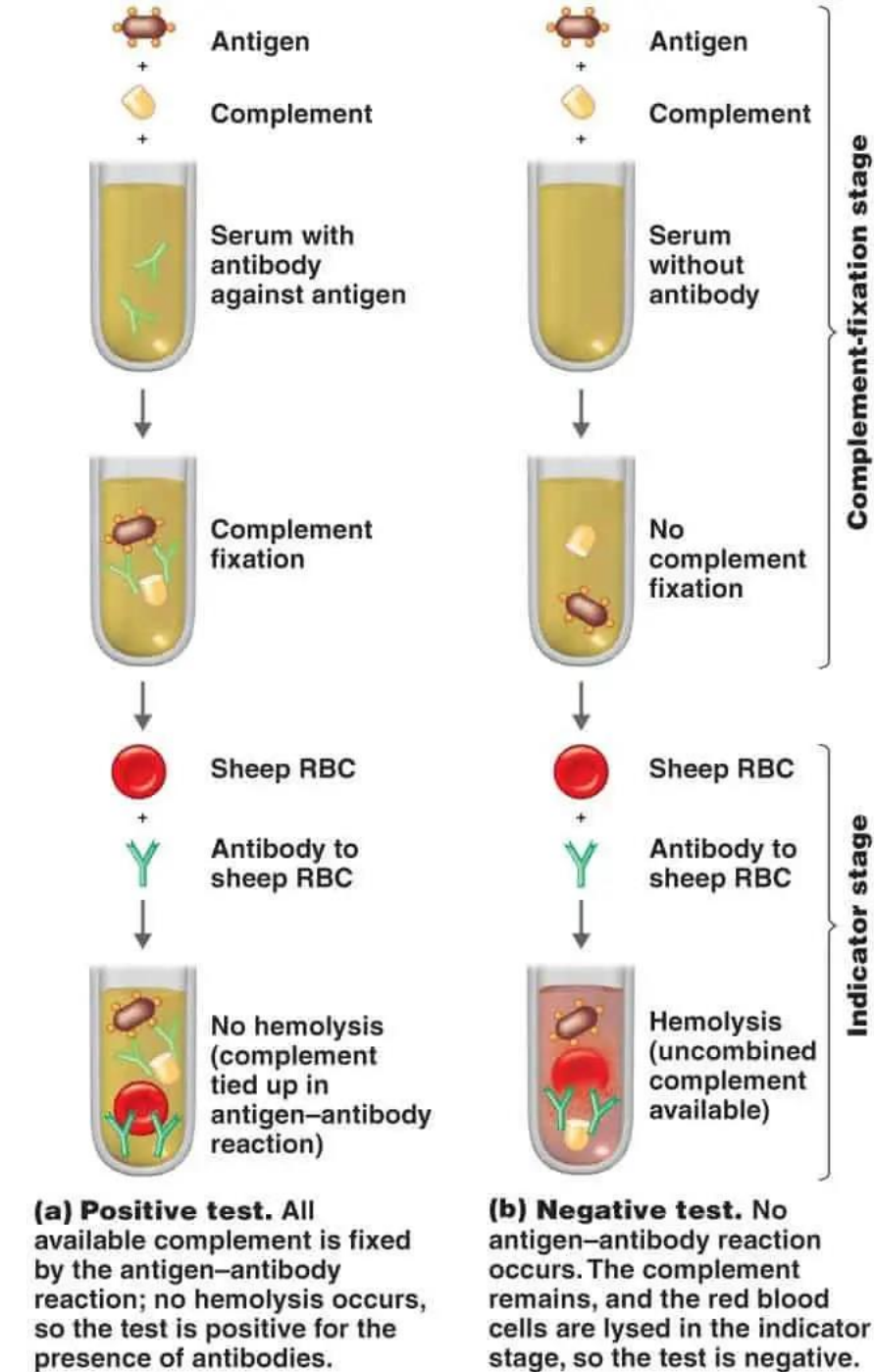
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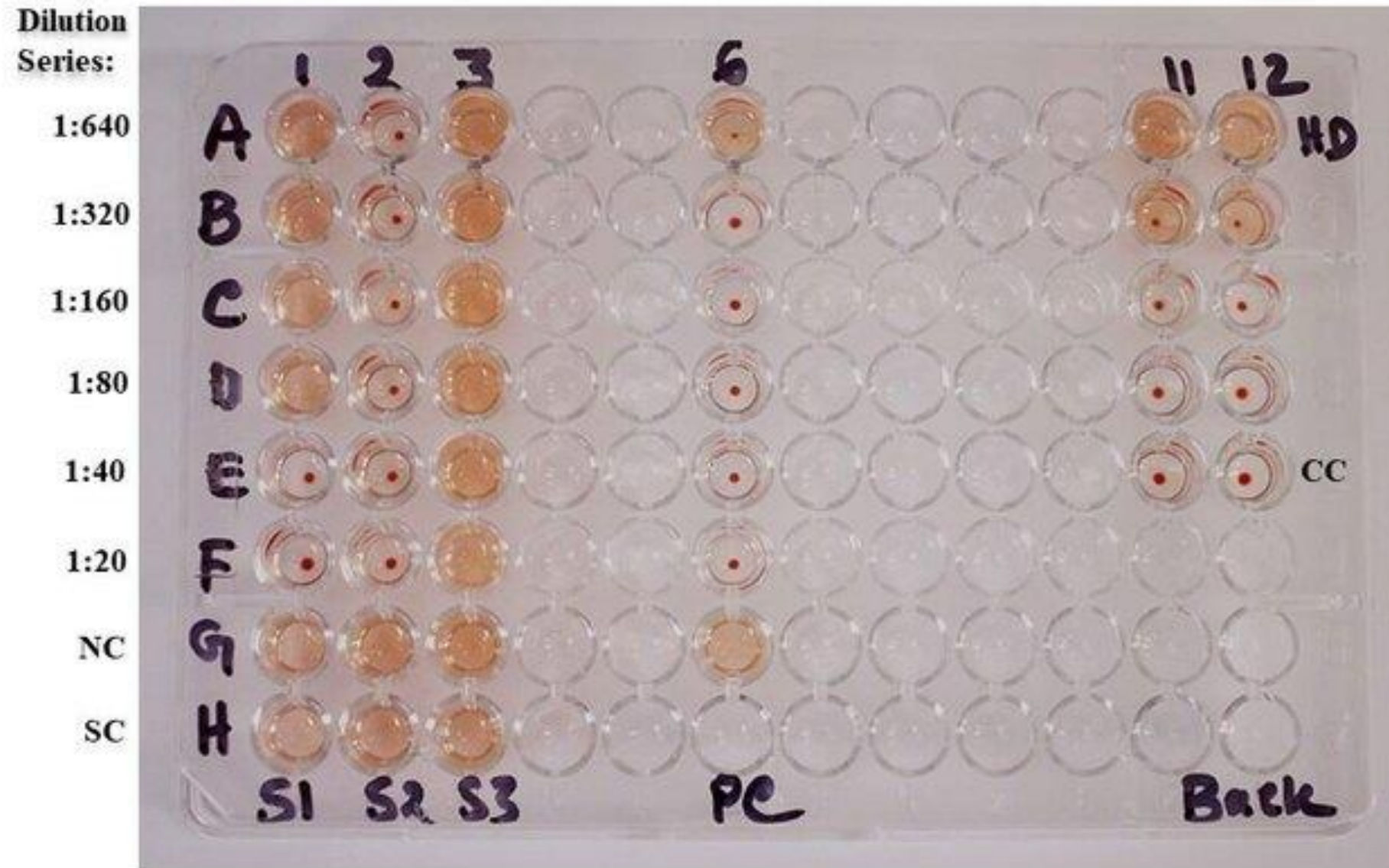
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CFT

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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.