Notes

61

COMPLEMENT FIXATION TEST

61.1 INTRODUCTION

Jules Bordet's pioneering research made clear the exact manner by which serums and antiserums act to destroy bacteria and foreign blood cells in the body, thus explaining how human and animal bodies defend themselves against the invasion of foreign elements. Bordet was also responsible for developing complement fixation tests, which made possible the early detection of many disease-causing bacteria in human and animal blood.

OBJECTIVES

After reading this chapter, the student will be able to:

- describe the term Complement
- explain the principle of Complement Fixation Test
- describe steps involved in the Complement Fixation Test
- enlist the uses of Complement Fixation Test
- describe the modifications of Complement Fixation Test

61.2 COMPLEMENT FIXATION TEST

In 1894, Richard Pfeiffer, a German scientist, had discovered that when cholera bacteria were injected into the peritoneum of a guinea pig immunized against the infection, the pig would rapidly die. This bacteriolysis, Bordet discovered, did not occur when the bacteria was injected into a non-immunized guinea pig, but did so when the same animal received the antiserum from an immunized animal. Moreover, the bacteriolysis did not take place when the bacteria and the antiserum were mixed in a test tube unless fresh antiserum was used. However,

when Bordet heated the antiserum to 55 degrees centigrade, it lost its power to kill bacteria. Finding that he could restore the bacteriolytic power of the antiserum if he added a little fresh serum from a non-immunized animal, Bordet concluded that the bacteria-killing phenomenon was due to the combined action of two distinct substances: an antibody in the antiserum, which specifically acted against a particular kind of bacterium; and a non-specific substance, sensitive to heat, found in all animal serums, which Bordet called "alexine" (later named "complement").

In a series of experiments conducted later, Bordet also learned that injecting red blood cells from one animal species (rabbit cells in the initial experiments) into another species (guinea pigs) caused the serum of the second species to quickly destroy the red cells of the first. And although the serum lost its power to kill the red cells when heated to 55degrees centigrade, its potency was restored when alexine (or complement) was added. It became apparent to Bordet that haemolytic (red cell destroying) serums acted exactly as bacteriolytic serums; thus, he had uncovered the basic mechanism by which animal bodies defend or immunize themselves against the invasion of foreign elements. Eventually, Bordet and his colleagues found a way to implement their discoveries. They determined that alexine was bound or fixed to red blood cells or to bacteria during the immunizing process. When red cells were added to a normal serum mixed with a specific form of bacteria in a test tube, the bacteria remained active while the red cells were destroyed through the fixation of alexine. However, when serum containing the antibody specific to the bacteria was destroyed, the alexine and the solution separated into a layer of clear serum overlaying the intact red cells. Hence, it was possible to visually determine the presence of bacteria in a patient's blood serum. This process became known as a complement fixation test. Bordet and his associates applied these findings to various other infections, like typhoid fever, carbuncle, and hog cholera. August VonWasserman eventually used a form of the test (later known as the Wasserman test) to determine the presence of syphilis bacteria in the human blood**.**

The complement fixation test (CFT) was extensively used in syphilis serology after being introduced by Wasserman in 1909. It took a number of decades before the CFT was adapted for routine use in virology.

CFT meet the following criteria

- it is convenient and rapid to perform
- the demand on equipment and reagents is small
- a large variety of test antigens are readily available.

However, there is now a trend to replace the CFT with more direct, sensitive and rapid techniques, such as RIAs and EIAs. Although CFT is considered to

be a relatively simple test, it is a very exacting procedure because variables are involved

Guinea pig is the commonest source of fresh complement. The serum should be collected from guinea pig just before the test because complement is easily destroyed by heat. However, complement can be preserved either by lipophilizing, freezing or by adding preservatives. Preserved complement is also obtained from commercial sources. Complement should be titrated for its haemolytic activity. One unit or minimum haemolytic dose (MHD) is the highest dilution of the guinea pig serum that lyses one unit volume of washed sheep red blood cells in the presence of excess of haemolysin (amboceptor) in either 30 or 60 minutes, at 37C. Physiological saline with added magnesium and calcium ions is used as the diluent for titration and CFT.

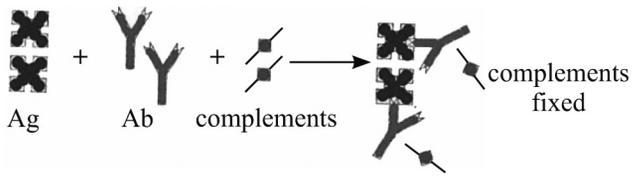


Fig. 61.1: Complement fixation

Complement fixation test consists of a test system and an indicator system, both of which can activate complement. When used to detect the presence of an antibody the test system is formed by the patient's serum and a known antigen. The indicator system is formed by sheep red blood cells coated with rabbit antibody to sheep red cells (amboceptors). The sheep red blood cells will lyse in the presence of complement.

- Sheep red cells: 5% suspension of washed sheep red blood cells should be used.
- Haemolysin (amboceptors): it is an antibody to sheep red cells which raised in rabbit. It should also be titrated for haemolytic activity. The MHD of the amboceptor is the highest dilution of the inactivated an amboceptor, which lyses one unit volume of sheep red blood cells in the presence of excess complement in 30 or 60 minutes at 37°C.

CFT consists of two steps:

Step 1: a known antigen and inactivated patient's serum are incubated with a standardized, limited amount of complement. If the serum contains specific, complement activating antibody the complement will be activated or fixed by the antigen-antibody complex. However, if there is no antibody in the patient's serum, there will be no formation of antigen-antibody complex, and therefore complement will not be fixed. But will remain free.

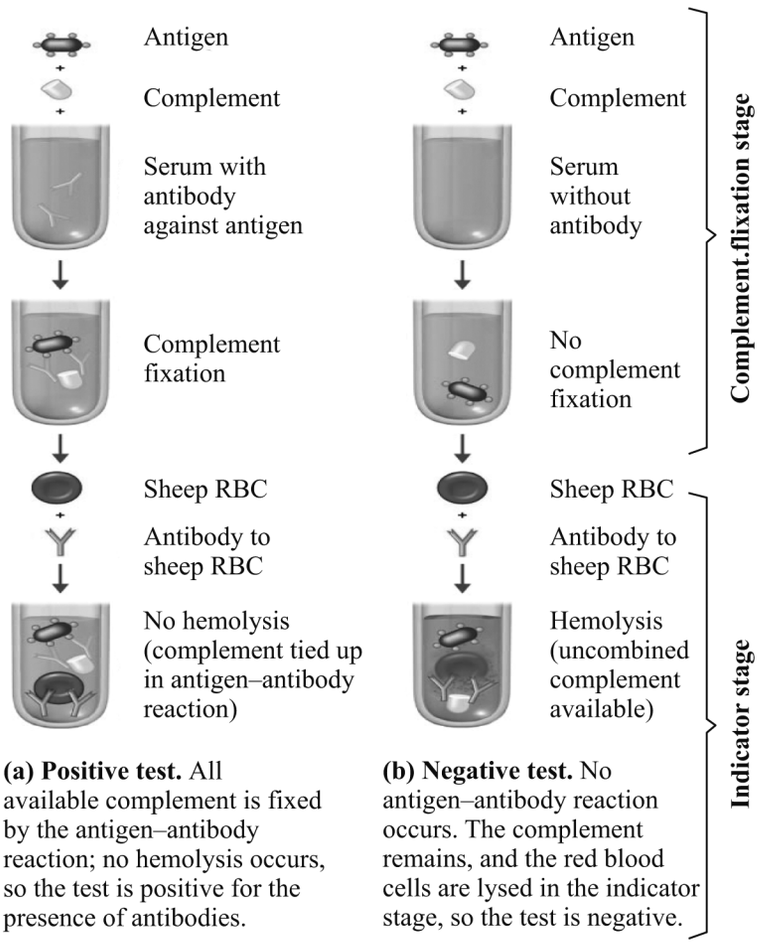


Fig. 61.2: Complement Fixation Test

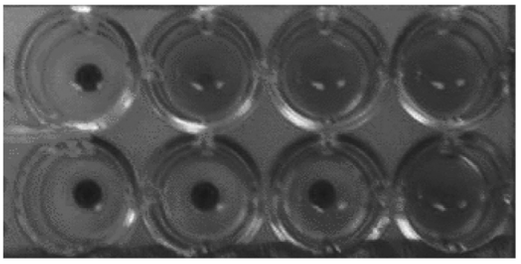
Step 2: The second step detects whether complement has been utilized in the first step or not. This is done by adding the indicator system. If the complement is fixed in the first step owing to the presence of antibody there will be no complement left to fix to the indicator system. However, if there is antibody in the patient's serum, there will be no antigen-antibody complex, and therefore, complement will be present free or unfixed in the mixture. This unfixed complement will now react with the antibody- coated sheep red blood cells to bring about their lysis. Thus, no lysis of sheep red blood cells (positive CFT) indicates the presence of antibody in the presence of antibody in the test serum, while lysis of sheep red blood cells (Negative CFT) indicates the absence of antibody in the serum.

Controls should be used along with the test to ensure that

(a) Antigen and serum are not anti complimentary

(b) The appropriate amount of complement is used and

(c) The sheep red blood cells do not undergo autolysis.



Complement Fixation Test in Microtiter Plate, rows 1 and 2 exhibit complement fixation obtained with acute and convalescent phase serum specimens, respectively. (2-fold serum dilutions were used) The observed 4-fold increase is significant and indicates infection.

Advantages of CFT

1. Ability to screen against a large number of viral and bacterial infections at the same time.
2. Economical.

Disadvantages of CFT

1. Not sensitive - cannot be used for immunity screening
2. Time consuming and labor intensive
3. Often non-specific e.g. cross-reactivity between HSV and VZV

Modifications of complement fixation test

(a) **Indirect complement fixation test:** This modification is used when serums which don't fix guinea pig complement is to be tested. Here, the test is set up in duplicate. After step 1, standard antiserum to antigen which is known to fix complement is added to one set. If antibodies were not present in the test serum then the antigen would react with the standard antiserum fixing the complement. On the other hand if antibodies are present in the test serum the antigen would be utilized in the first step. So, no reaction would occur between the standard antiserum and the antigen and therefore no fixation of complement would cause lysis of sheep red blood cells. Thus in this case haemolysis indicates a positive result.

(b) **Congulatinating complement absorption test**: Here horse complement which is non-haemolytic is used. The indicator system used is sensitized sheep red blood cells mixed with bovine serum. Bovine serum contains

a beta globulin called conglutinin would also combine with this complement causing agglutination (conglutination) of the sheep red blood cells, indicating a negative result.

(c) **Immune adherence**: When some bacteria (such as vibrio cholera or treponemapallidum) combine with their specific antibody in the presence of complement and some particles such as erythrocytes or platelets, they adhere to the erythrocytes or platelets. This is called immune adherence.

(d) **Immobilisation test:** Here antigen is incubated with patient's serum in presence of complement. If specific antibody is present it would immobilize the antigen. Eg.Treponema palladium immobilization test, considered gold standard for the serodiagnosis of syphilis.

(e) **Cytolytic tests:** The incubation of a live bacterium with its specific antibody in the presence of complement leads to the lysis of the bacteria cells. This is the basis of vibriocidal antibody test used to measure anti-cholera antibodies.

INTEXT QUESTION 61.1

1. The complement fixation test was initially used in ..... serology
2. The commonest source of fresh complement is .....
3. The highest dilution of guinea pig serum that lyses red blood cells is called .....
4. ..... is the antibody to sheep red cells used in complement fixation test
5. ..... is used in coagulatinating complement absorption test
6. When bacilli combine with specific antibody in the presence of complement is called .....
7. Gold standard serodiagnosis of syphilis is by ..... test
8. ..... test is commonly used in diagnosis of cholera

WHAT HAVE YOU LEARNT

- CFT was developed by Jules Bordet. Complement fixation test consists of a test system and an indicator system, both of which can activate complement. When used to detect the presence of an antibody the test system is formed by the patient's serum and a known antigen. The indicator system is formed

by sheep red blood cells coated with rabbit antibody to sheep red cells (amboceptors). The sheep red blood cells will lyse in the presence of complement. There exits modification of complement fixation test- Indirect complement fixation test, Congulatinating complement absorption test, Immune adherence, Immobilisation test, Cytolytic tests.

TERMINAL QUESTIONS

1. What do you understand by the term complement?
2. Describe in brief CFT.
3. Give the advantages anf the limitations of CFT
4. Explain in brief various modifications of complement fixation test with proper examples.
5. Explain the term amboceptor.
6. Mention the advantages and disadvantges of CFT.
7. Enlist the modifications of complement fixation test

ANSWERS TO INTEXT QUESTIONS

61.1

1. Syphillis
2. Guinea Pig
3. Minimum Haemolytic Dose
4. Amboceptors
5. Horse Complement
6. Immune Adherences
7. Immobilization
8. Cytolytic