



IMMUNOLOGY FIRST COURSE

Effect of complement against bacteria.

Sawa University

جامعة ساوة الاهلية

College of health and medical techniques

كلية التقنيات الصحية والطبية

Department of Medical Laboratories

قسم تقنيات المختبرات الطبية

Third Stage

المرحلة : الثالثة

محاضرة رقم 4

الجانب العملي

Lecture No. 4

Practical

تدريسي المادة : م.د عبد الرحمن سالم

Complement fixation

Items:-

- 1. Complement fixation**
- 2. Type of complement fixation**
- 3. Procedures**

Complement fixation :-

Complement fixation tests detect the presence of antigen-antibody complexes on cells or intracellular matrix or as "soluble" complexes in the serum.

There are two parts to this test :-

- 1. The indicator system:-**
- 2. To assay for the presence of antigen-antibody complexes :-**

Principle :-

Antigen-antibody complexes in the serum or tissue-bound antibody requires that the serum or tissue is incubated with complement. The presence of complement-fixing antigen-antibody complexes depletes the limited amount of complement so that antibody-coated erythrocytes, when added to the reaction mixture, are not lysed and hemoglobin is not released and determined spectrophotometrically.

Procedure :-

In the assay, serum is collected from an individual and heated to 56°C for 30 minutes to inactivate endogenous complement, and a predetermined amount of complement is added.

(1)-The mixture is incubated to allow complement to bind to antigen-antibody complexes (if present).

(2)-Sheep RBC and anti-Sheep RBC antibodies are added and allowed to incubate.

(3)-The presence of antigen-antibody complexes in the test serum will have depleted the added complement, and the SRBC will not be lysed.

(4)-Absence of antigen-antibody complexes in the test serum will result in a failure to deplete complement, and the indicator cells (SRBC) will be lysed, releasing hemoglobin into the supernatant that can be measured in a spectrophotometer.



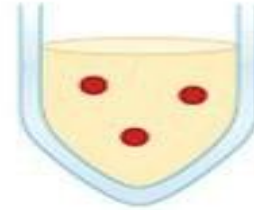
Serum with
antibodies



Serum without
antibodies



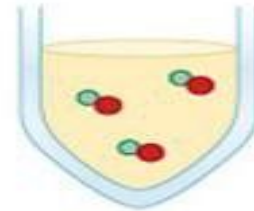
Antigen binds
with antibodies



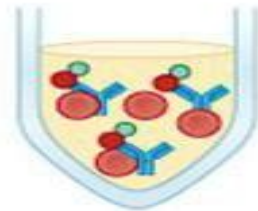
Unbound
antigen



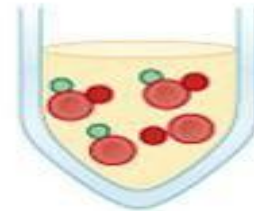
Complement
binds with
Ag/Ab complex



Unbound
complement



Hemolysin-
sensitized red
blood cells
serve as an
indicator



Hemolysin-
sensitized
RBCs serve
as an indicator



RBCs settle into
a pellet

No lysis

Reactive



RBCs lysed by
unbound
complement

Lysis

Nonreactive

Any questions?

*Thank
You*