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Bactericidal power of serum **(Ability of serum to kill bacteria)**

Lab-I

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Immune System

- The immune system is a collection of **organs**, **cells**, and **tissues** that work together to protect your body from disease caused mostly by pathogens and detects them as foreign substances which include (bacteria, viruses, fungi, and parasites) and their toxin products. The immune system is divided into:-

Innate immunity (non-specific or natural)

- Innate immunity is a primary defense mechanism against invading organisms, that a host uses immediately after exposure to an antigen and does not depend on the host's prior exposure or memory of the pathogen. Consist of:

First line of defense:

- **Anatomical barriers**
- Mechanical factors; Skin, mucous membranes, and their secretions prevent entry of microorganisms.
- Chemical factors; such as Lysozyme; breakdown the cell wall of bacteria and, the acid pH of the stomach and vagina.
- Biological factors; the normal flora can prevent the colonization of pathogenic bacteria.

Second line of defense

- **Cellular factors** The body uses nonspecific cellular (phagocytes, Natural killer (NK) cells) and
- **Humoral factors** –antimicrobial proteins (complement and antibody) to protect themselves.



Adaptive immunity (specific or acquired)

- The third line of defense starts to act after innate immunity fails. It is more powerful, highly specific for pathogens, and long-lasting memory for a specific pathogen. It is mediated by either **antibodies** (Humeral immunity) or **T-cells** (Cellular immunity) depending on specific Antigen. It can be:
 - A- Passive,
 - B- Active.

Plasma

- Is the liquid portion of blood, and about 92% of plasma is water. The remaining 8% of plasma consists of various salts (ions) and organic molecules. **Small** organic molecules like glucose, amino acids, and urea can also dissolve in plasma. The **large** organic molecules in plasma include hormones and plasma proteins.
- The three major types of plasma proteins are **albumins, globulins, and fibrinogen**. Most plasma proteins are made in the liver. An exception is the antibodies produced by B lymphocytes.

Serum

The fresh serum contains substances capable of killing microorganisms, this ability varies according to the type of microorganisms. To be sure some of these activities are due to the normal antibodies, but there are other factors involved also, for example complement play an important role in the destruction of microorganism under certain conditions, this substance (complement) can be destroyed if the serum is heated at **56 °C** for **30 min.**, this is due to denaturation of complement proteins, and also other humoral factors can be destroyed such as properdine, β -Lysin...., that contributes to the natural (innate) immunity.

Preparation of Plasma and Serum

- If blood is transferred from a person's vein to a test tube and prevented from clotting (**adding anticoagulant** e.g. Heparin, EDTA, Sodium Citrate...), and centrifuged at **2500-3000 rpm** for **10 min.** it separates into two layers. The upper liquid layer, called **plasma**, represents about 55% of the volume of whole blood. The lower layer consists of red blood cells, white blood cells, and blood platelets. Collectively, these are called the formed elements and represent about 45% of the volume of whole blood.

If blood is transferred from a person's vein to a test tube **without adding anticoagulant**, and centrifuged at **2500-3000 rpm** for **10 min.** it separates into two layers, the upper liquid layer is called **serum**, and the lower layer consists of formed elements

Serum = Plasma – Fibrinogen

Method for serum preservation

1. For a short time, the freezing method is followed (-4 °C) or (-20 °C).
2. For a long time, like the addition of chemicals such as 1% sodium azide & and 3.2% sodium citrate.

Common Plasma and Serum Preparation Errors

- 1- Failure to separate plasma or serum from red cells within 30 to 45 minutes of vein puncture
- 2- Hemolysis (red blood cells damaged and intracellular components spilled into plasma or serum).

Bactericidal power of normal serum

Principle:


The study of humoral factors (such as complement proteins and Ab.), which were found in normal serum, and their effect on the common pathogenic bacteria (G - and G +) by mixing both of them with heated and unheated sera.

Procedure

- Take 5 *ml* of *blood* and prepare a *serum*.
- Prepare the suspension of G⁺ bacteria (*Staphylococcus aureus*) and G⁻ (*E. coli*).
- Add 1 *ml* of *serum* in to a *tube number* (1) and 1 *ml* of *serum* in to a *tube number* (2).
- Heat the *tube number* (2) at 55 – 60 °C for 30 *minutes*.
- Add 1 *ml* of bacterial suspension into each tube.
- Prepare the *nutrient agar* and labeled (0, 15, 30, and 45) *minutes*.
- From *tube number* (1) adds 0.1 *ml* into a plate agar after each 15 *minutes* (0, 15, 30, and 45 *minutes* for detect the *action* of *complement* in the *serum* against the *growth* of both types of bacteria.
- From *tube number* (2) adds 0.1 *ml* into a plate agar.
- After adding, *incubate* the all plates for 18 – 24 *hours*.
- Then record the *growth* of bacteria in each plate, and discuss your results.

Complement system

The complement system is an essential and effective part of the innate immune system. It consists of about 30 blood proteins include **C1** through **C9**, **factors B and D**. These proteins secreted by **liver cells** and **monocytes**, they are normally circulating as **inactive state** (pro-proteins) that form a biochemical reaction cascade which causes neutralization and destruction of foreign pathogens. When complement molecules bind to certain kinds of bacteria, they help eliminate the bacteria through lysis or opsonization.



Lysis: is the process whereby complement ruptures the bacterial membrane, which results in the destruction of the bacterium.

Opsonization: refers to the coating of bacteria with complement or antibodies (Opsonin), that increase the process of phagocytosis.

Opsonin: A substance (protein) such as an antibody and complement that attaches to foreign materials (bacteria), making them more susceptible to phagocytosis.

Activation of the complement system

There are three main pathways of complement activation.

The **alternative pathway** is activated by polysaccharides from yeasts and gram-negative bacteria,

the **classical pathway** by antigen-antibody complex, and

The **lectin pathway** starts with mannose-binding lectin to certain sugars.