Immunology Lab 1

Bactericidal power of the normal serum (Ability of serum to kill bacteria)

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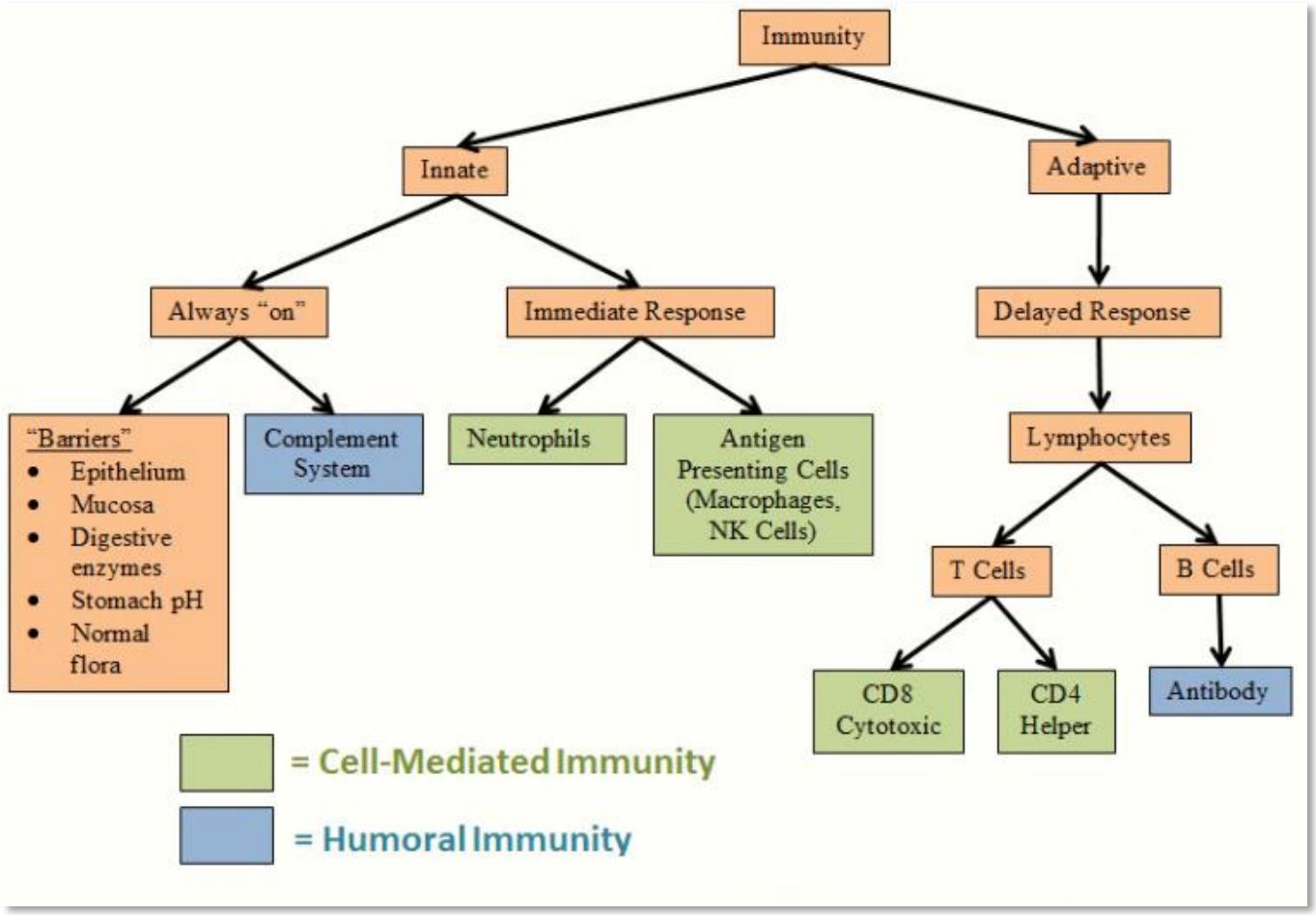
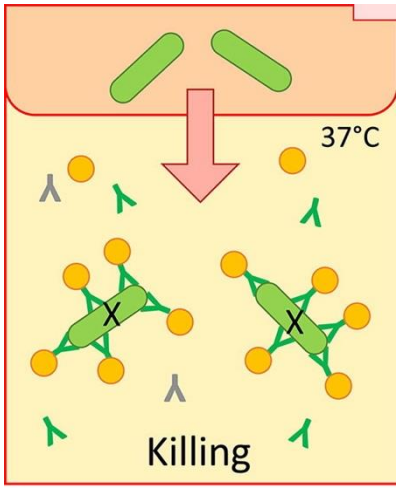
Type of Humoral Immunity

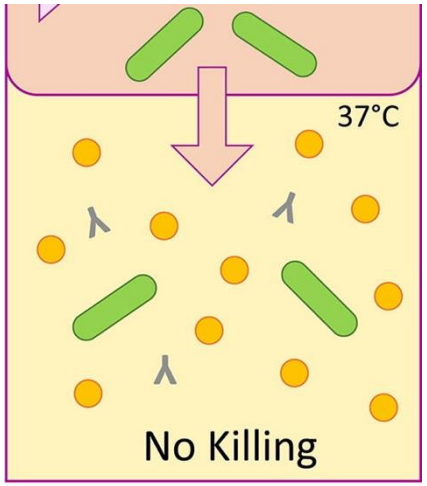


Principle:

The study of humoral factors (such as **Antibody (Ab)** and complement proteins) found in normal serum and their effect on the common pathogenic bacteria (G^{-ve} and G^{+ve}) by mixing them with heated and unheated sera.



Heated Serum



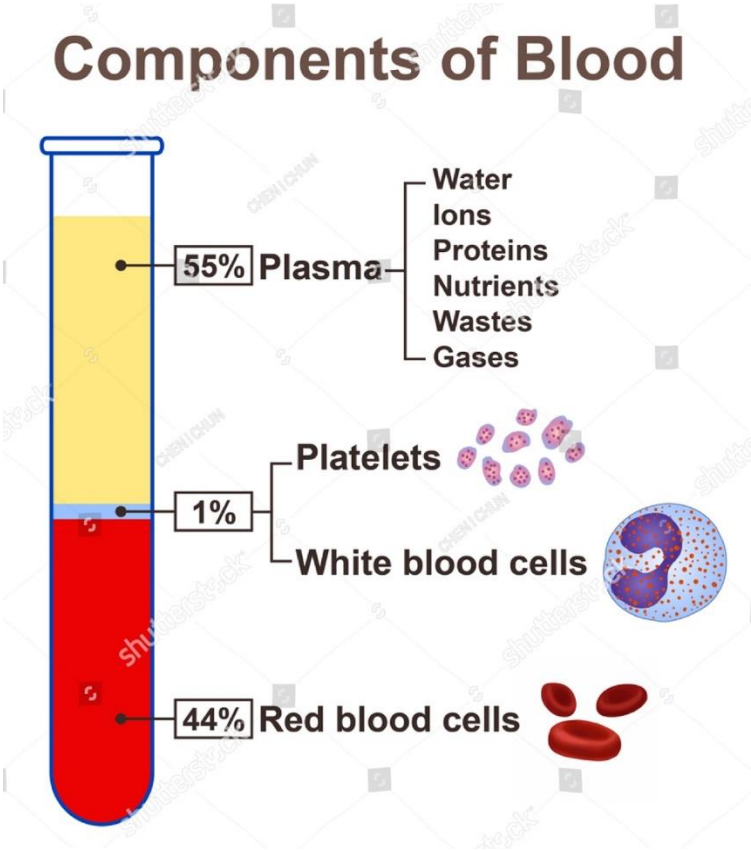
Unheated Serum

Plasma:

- It is the liquid portion of blood.
- 92% of plasma is water.
- 8% of plasma consists of various salts (ions) and organic molecules.
- **Small organic molecules** like glucose, amino acids, and urea, etc.
- **Large organic molecules** in plasma include hormones, complement, etc.
- The three major types of plasma proteins are:

  1- Albumin 2- Immunoglobulins

  3- Fibrinogen
- Most plasma proteins are made in the liver. An exception is the antibodies produced by B lymphocytes.

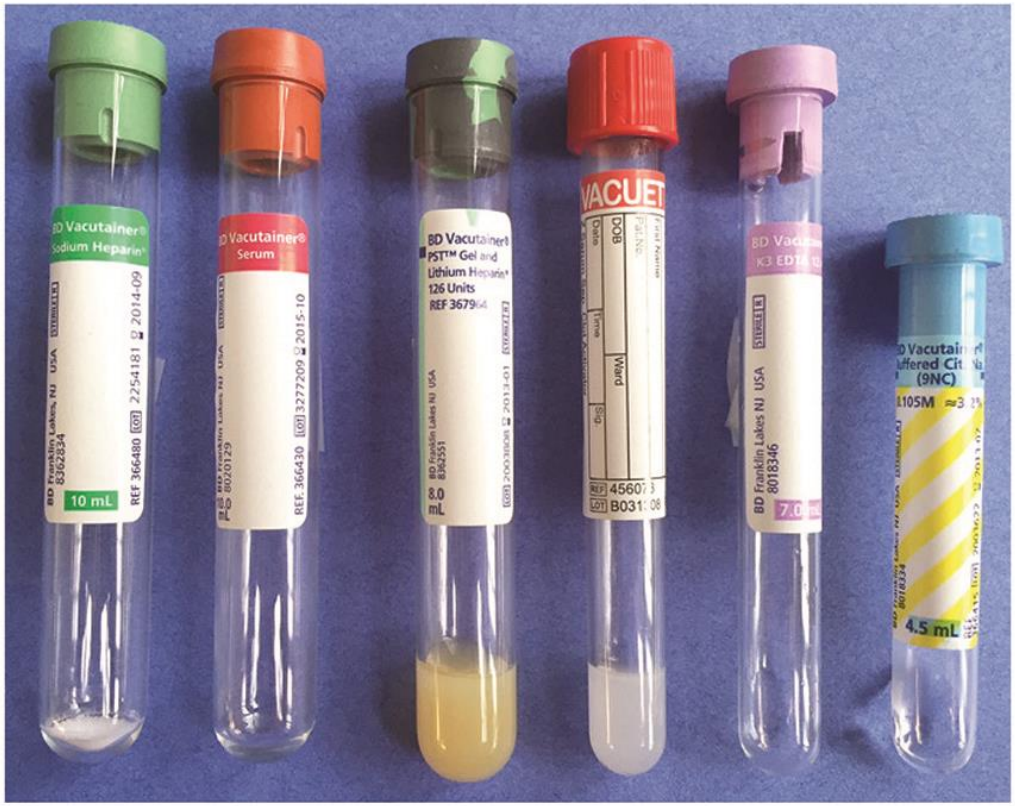


Serum:

- **Serum = Plasma- Fibrinogen**

The normal human serum contains substances capable of killing microorganisms. This ability varies according to the species and 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 plays an important role in the destruction of microorganisms under certain conditions, this substance (complement) can be destroyed if the serum heated at ***56 ºC*** *for* ***30 min****., this is due to denaturation of complement proteins, and also other humoral factors can be destroyed such as properdin, β-Lysin…., that contributes to the natural (innate) immunity.*

Tube type:	Glass	Glass	Glass + gel[1]	Plastic + gel[2]	Glass	Glass
						
Anticoagulant:	Heparin	None	Heparin	None	EDTA	Citrate
Yields:[3]	Plasma[4]	Serum	Plasma[4]	Serum	Whole blood[5]	Plasma
Uses:	Chemistry	Chemistry Electrophoresis (ELP)	Chemistry	Chemistry ELP	Hematology Cytology Flow cytometry Clonality (PARR) Coombs test	Coagulation

Method for serum preservation:

- For a short period, the freezing method is followed (-20^{o}C) or (-80^{o}C).
- For a long period, like the addition of chemicals such as 1% of sodium azide & 3.2% of sodium citrate.

Common Serum Preparation Errors:

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

Complements

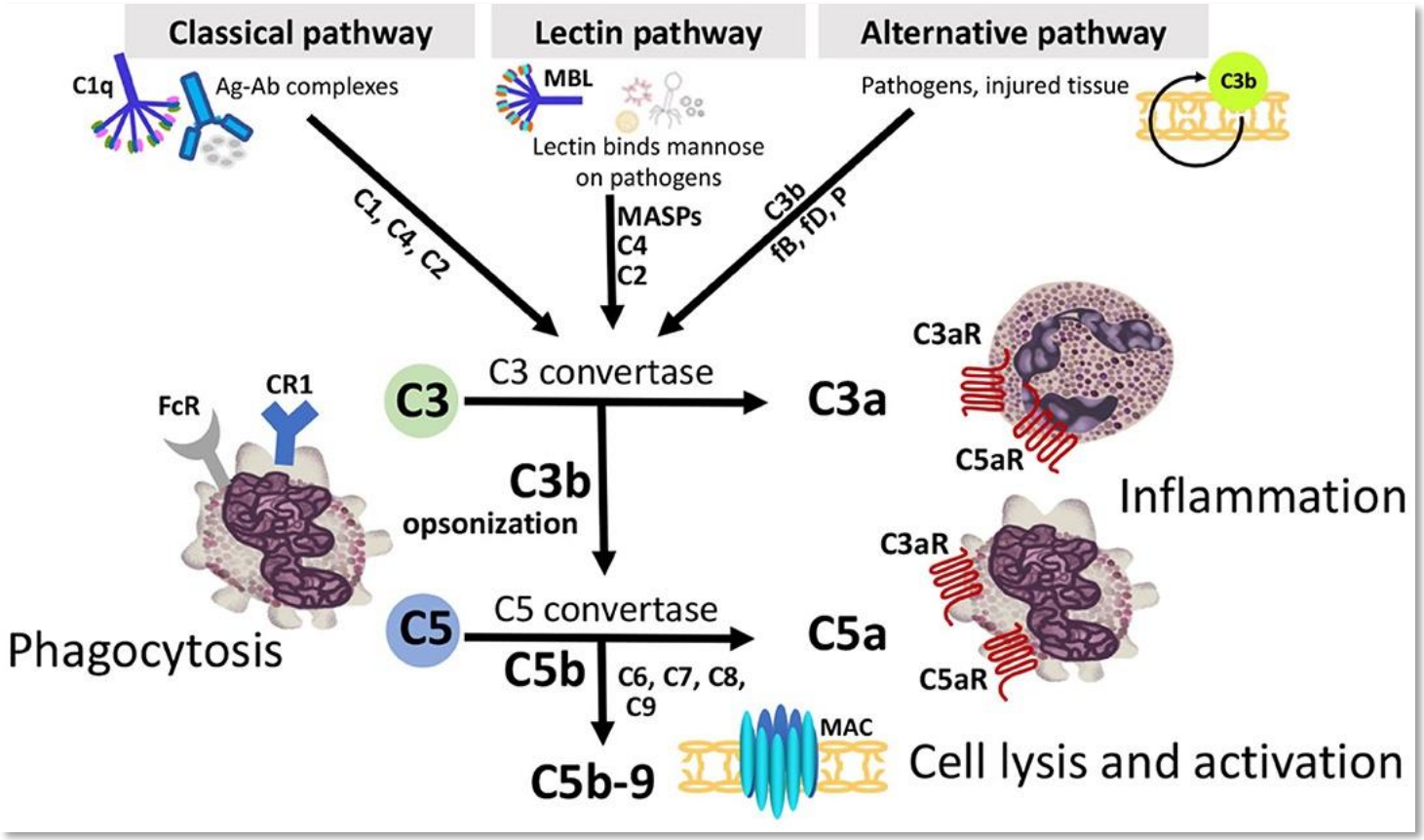
- The complement system refers to a series of >20 proteins.
- Most of the proteins are normally inactive, but in response to the microorganisms (Antigen) they become sequentially activated in an enzyme cascade.
- Complement activation results in opsonization of pathogens and their removal by phagocytes, as well as cell lysis

Inactivation of Complement:

The complement can be destroyed if the serum is heated at **56^{o}C** for **30 min**. This is due to the denaturation of complement proteins.

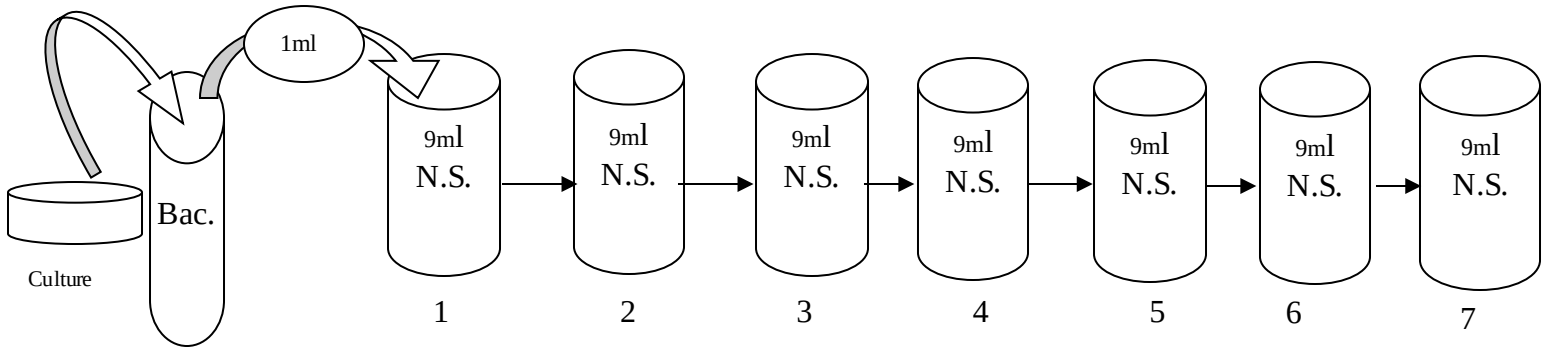
Three pathways of complement activation

- **The classical pathway** is activated through the antigen-antibody complex
- **The alternative pathway** is activated by lipopolysaccharide in G^{-ve} bacteria, teichoic acids of G^{+ve} bacteria
- The **Lectin pathway** is activated when either mannose-binding lectin (MBL) binds to the surface of microbes bearing mannan.



Procedure

1. Prepare three dilutions of 10^{-5}, 10^{-6}, and 10^{-7} of the organisms in normal saline.



2. Take 5-10 ml of blood and prepare a serum as mentioned before.
3. Set up three tubes for each dilution as follows:
    a. Normal unheated serum (active) 0.5 ml + culture dilution 0.5 ml (10^{-5}, $10^{-6,}$ and 10^{-7}).
    b. Normal heated serum (inactive at 56^{0}C for 30 min.) 0.5 ml + culture dilution 0.5 ml (10^{-5}, 10^{-6} and 10^{-7}).
    c. Normal saline 0.5 ml + culture dilution 0.5 ml (10^{-5}, 10^{-6} and 10^{-7}).

4. Mix the content of each tube and incubate at 37^0C for 30-60 min.

5. Place the content of each tube into a sterile plate and label.

6. Pour 10 ml of melted nutrient agar media (45^0C) into each plate and mix well.

7. Incubate all plates at 37 ^{0}C for 24 hrs.

8. After incubation, carefully make a plate count of all countable plates and record the results in the Table.

9. Make a comparison and explain the results

Organism	Dilution	Number of organism		
		Heated S.	Unheated S.	Normal saline
	10^{-5}			
	10^{-6}			
	10^{-7}			