



**Cihan University/ Sulaymaniya**

**College of Health Science**

**Medical Laboratory Analysis**

**4<sup>th</sup> Stage- 1<sup>st</sup> Semester**

**Pr. Clinical Immunology**

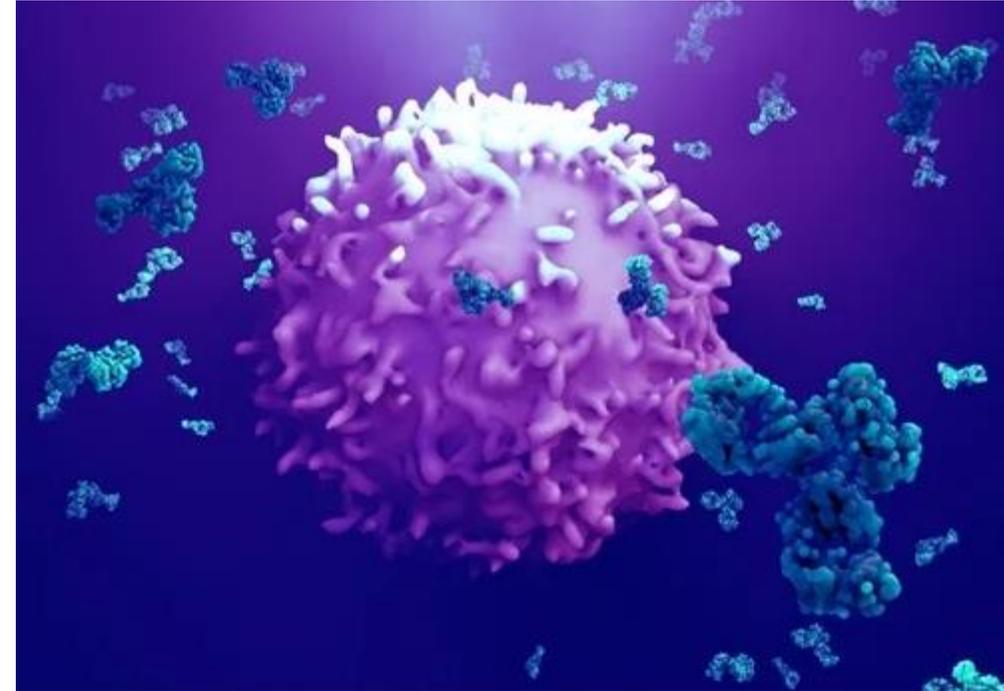
**Lab- 2: Antigen preparation**

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# Antigen

- Chemically complex, large-sized molecules that are recognized as non-self by an individual's immune system.
- They are substances usually **protein** in nature and sometimes **polysaccharide**, which are capable of stimulating an immune system and generate an immune response that induces the formation of a specific antibody or specially sensitized T cells or both.



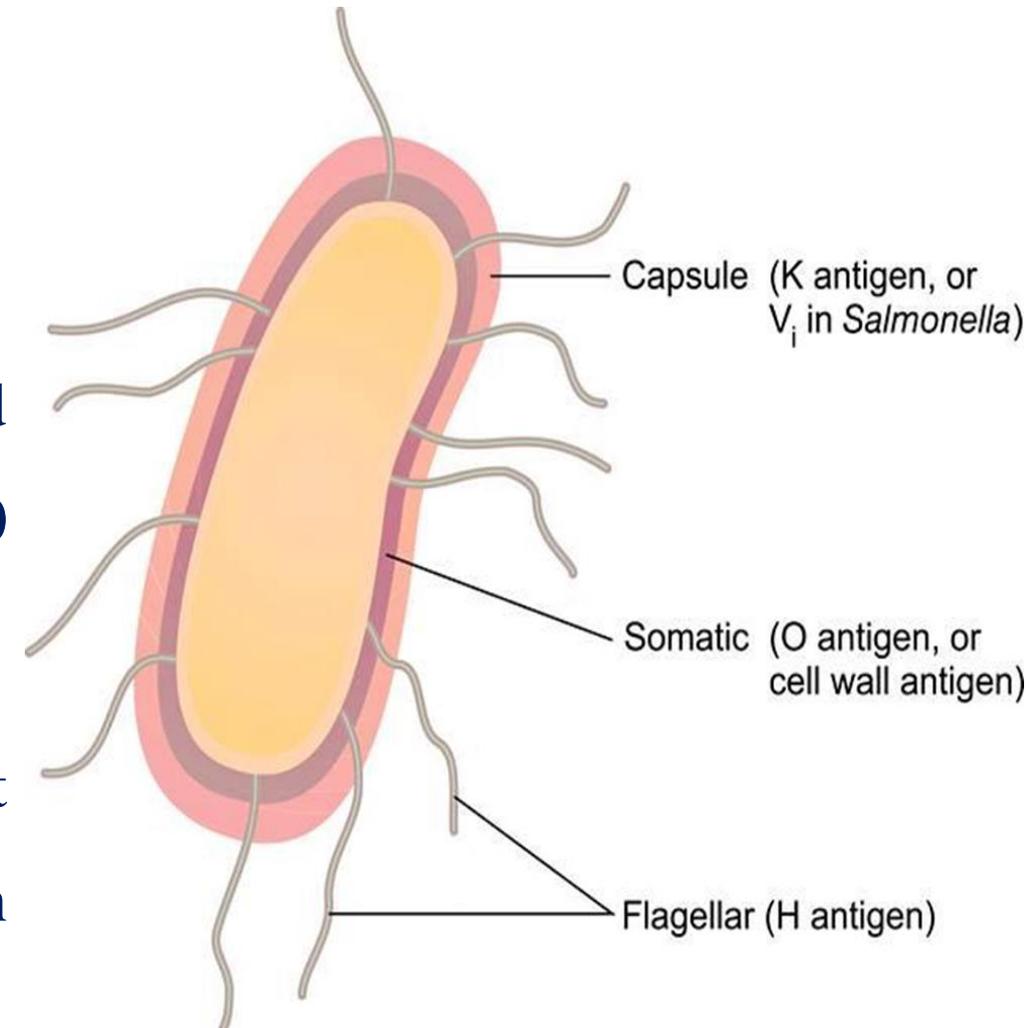
# According to the motility, bacteria can be classified into:

- ❑ Motile
- ❑ Non motile

Motile bacteria have two types of antigens:

**A. Somatic antigen (O Ag):** This antigen composed mainly of polysaccharide, it is heat stable, resists (100 °C) and not affected by diluted **alkaline** and **acids**.

**B. Flagellar antigen (H Ag):** it is protein in nature, heat labile (unstable), it is decomposed by heat more than (60 °C) and by diluted alkaline and acids.



# Example of motile bacteria (Escherichia coli)



## Procedure of preparation of Flagellar antigen (H Ag) of E. Coli

- Prepare a pure culture of E.coli, the purity of bacterial culture can be examined by Grams stain.
- Overgrowth of bacteria on suitable media by using large number of petri-dishes or using flask, then incubate these culture plate or flask at 37°C for 18-24 hrs.
- Harvest E.coli by using sterile **isotonic solution (normal saline)** or **sterile phosphate buffer saline (PBS)** with pH 7.2 and a glass harvester. Keep the bacterial suspension in a sterile container.
- Mix equal volume of bacterial suspension with formalinized saline (0.6%) to kill bacteria, the mixture should be incubated at room temperature for 24-48 hr. Formalinized saline (0.6%)=0.6ml formalin+99.4ml normal saline
- Sterility test is done by taking a **loop of bacterial suspension and culture** it on a suitable media, incubate at 37c for 24-48 hr.
- Wash bacterial suspension three time with **sterile normal saline**.
- Discard the supernatant of last washing then re-suspend the bacteria with **formalinized saline 0.3%** and incubate in the refrigerator until use as flagellar antigen.

## B. Procedure for the preparation of somatic antigen (O Ag)



**The same as in preparation of flagellar antigen.**

- The same as in preparation of flagellar antigen.
- Inactivate (kill) bacteria by heating in a water bath at 100 °C for 30-120 min
- Sterility test same as in the preparation of flagellar antigen.
- Washing process of the bacteria is the same as in preparation of flagellar antigen.
- Re-suspend bacteria with formalinized saline (0.3%) and incubate in refrigerator until use as somatic antigen.



# The Preservative materials for the antigens

- Formalinized saline (0.3 %).
- Phenol (0.1-0.5 %).
- Merthiolate (1:10,000).
- Sodium azide (0.1 %): It is consisting of distilled water and purest sodium azide, is used in medical diagnostics, histology and scientific laboratories. It is used as a preservative in specimen collection and storage to prevent bacterial and fungal growth.



# Determination of Bacterial Antigen Concentration in (1 ml) of Normal Saline

- There are two methods for determination of bacterial antigen concentration
  1. Using spectrophotometer.
  2. Using McFarland turbidity standards (McFarland tubes) or (opacity tubes) or (barium sulphate turbidity tubes).



# McFarland Standard

- It provides a reference to adjust the turbidity of bacterial suspensions so that the number of bacteria will be within a given range to standardize microbial testing.
- Original McFarland standards were prepared by adding **barium chloride** to **sulfuric acid** resulting in a **barium sulfate** precipitation.
- A no. (0.5) McFarland standard is prepared by mixing 0.05 ml of 1.175% barium chloride dihydrate, with 9.95 ml of 1% sulfuric acid.
- The cell density of a bacterial suspension is adjusted in a simple comparison with the turbidity of the standard. This can be done both in direct visual comparison and by measurement in a spectrophotometer.
- The turbidity value of the standard of 0.5 MFU (McFarland Unit) corresponds approximately to a culture density of  $1.5 \times 10^8$  cells/ml. This cell density is especially required for bacterial inoculum for the antibiotic sensitivity test.

# McFarland Standard





# References

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