Cihan University/ Sulaymaniya

College of Health Science

Medical Laboratory Analysis

4th Stage- 1st Semester

Pr. Clinical Immunology

Lab- 2: Antigen preparation

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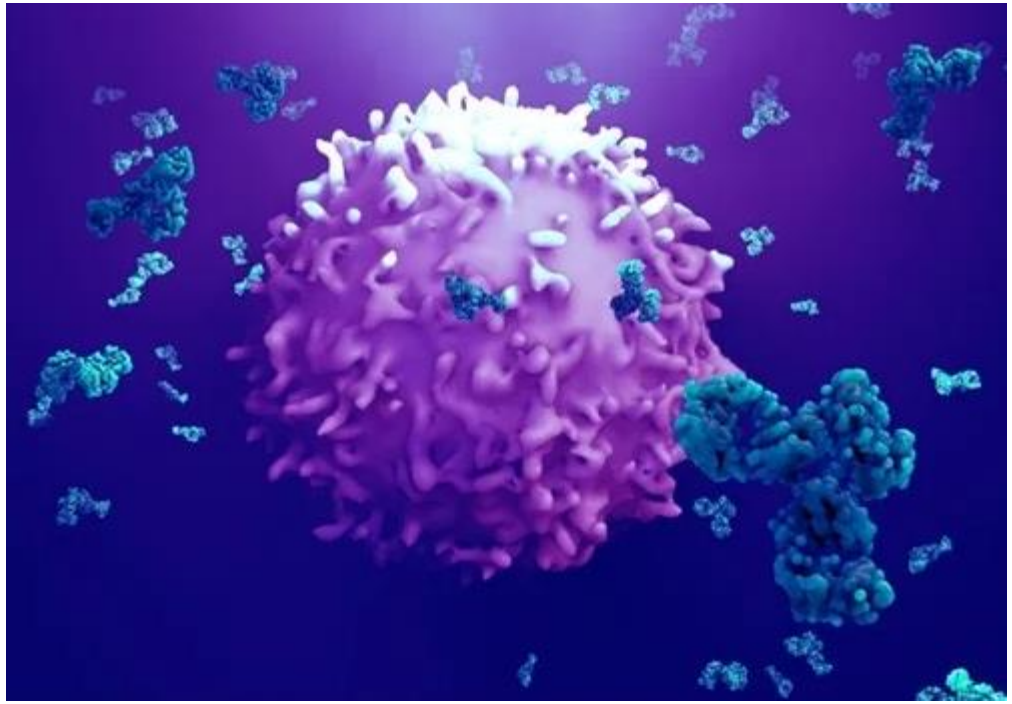
Antigen

- 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.



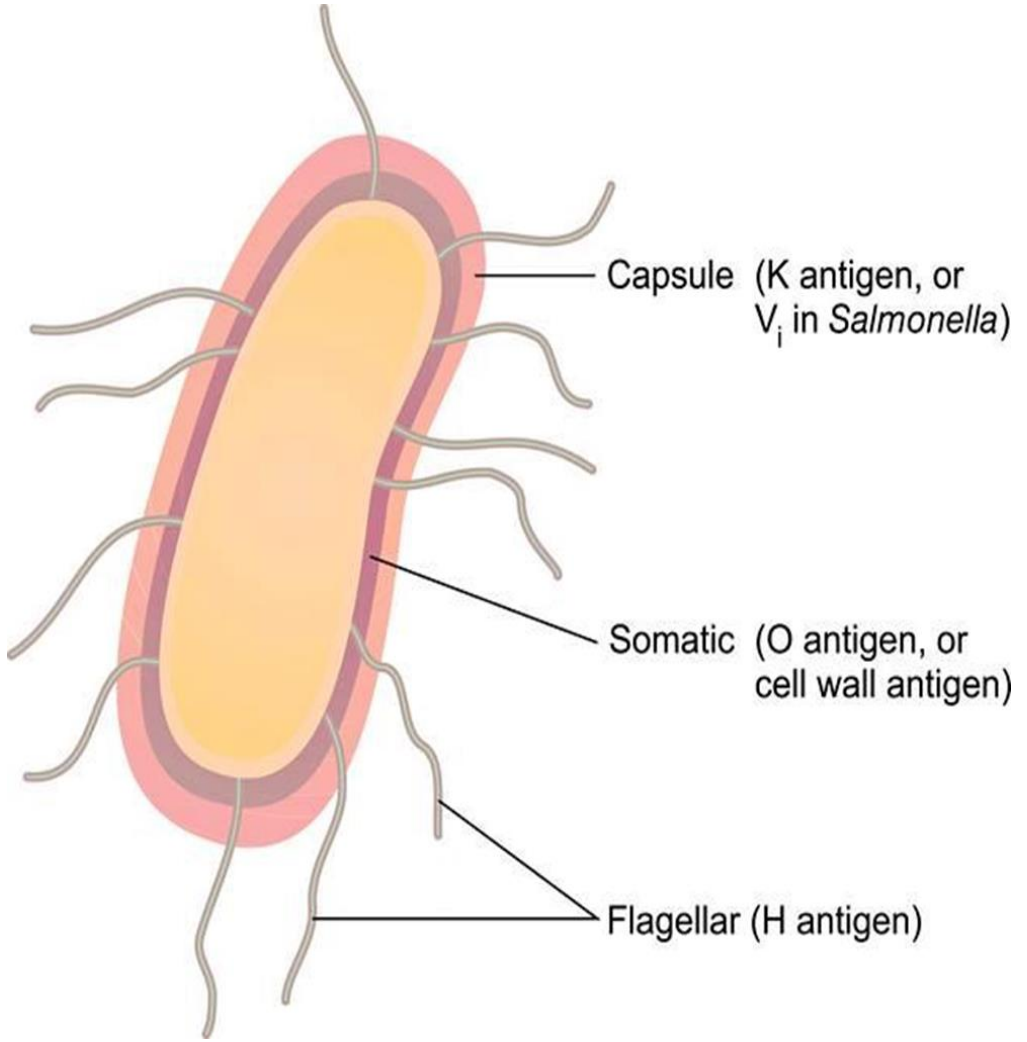
According to the motility, bacteria can be classified into:

- ❑ 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 ºC) 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 ºC) 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 x 108 cells/ml. This cell density is especially required for bacterial inoculum for the antibiotic sensitivity test.

McFarland Standard

References

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- Find a Test | MLabs (umich.edu).