**Lab 3 Preparation of Bacterial antigens**

**Serology and Vaccines**

Antigens which stimulate antibody production by the host in enterobacteriacae include:

* 1. K - polysaccharide [capsular](http://www.life.umd.edu/classroom/bsci424/Definitions.htm#Capsule) antigens
  2. O - somatic polysaccharide antigen (associated with [LPS](http://www.life.umd.edu/classroom/bsci424/Definitions.htm#LPS))
  3. H - [flagellar](http://www.life.umd.edu/classroom/bsci424/Definitions.htm#Flagellum) antigen

**Somatic antigen (O- Ag):**

O antigens are the most external part of the cell wall lipopolysaccharide (LPS), also known as lipoglycans and endotoxin, and consist of repeating units of polysaccharide. Some O-specific polysaccharides contain unique sugars. O antigens are resistance to heat (100° C/2.5hr) and alcohol (96% Ethanol /4 hrs) and usually are detected by bacterial agglutination. It stimulates earliest antibodies to O antigens which are predominantly **IgM.**

Lipopolysaccharides (LPS) are large [molecules](http://en.wikipedia.org/wiki/Molecule) consisting of a [lipid](http://en.wikipedia.org/wiki/Lipid) and   [polysaccharide](http://en.wikipedia.org/wiki/Polysaccharide) composed of O-antigen, outer core and inner core joined by a [covalent bond](http://en.wikipedia.org/wiki/Covalent_bond); they are found in the [outer membrane](http://en.wikipedia.org/wiki/Bacterial_outer_membrane) of [Gram-negative bacteria](http://en.wikipedia.org/wiki/Gram-negative_bacteria), and elicit strong [immune responses](http://en.wikipedia.org/wiki/Immune_response) in animals.

It comprises three parts:

1. O antigen (or O polysaccharide)
2. [Core oligosaccharide](http://en.wikipedia.org/wiki/Core_oligosaccharide)
3. [Lipid A](http://en.wikipedia.org/wiki/Lipid_A)
4. **O-antigen**

A repetitive [glycan](http://en.wikipedia.org/wiki/Glycan) [polymer](http://en.wikipedia.org/wiki/Polymer) contained within an LPS is referred to as the O [antigen](http://en.wikipedia.org/wiki/Antigen), O [polysaccharide](http://en.wikipedia.org/wiki/Polysaccharide), or O side-chain of the bacteria. The O antigen is attached to the core oligosaccharide, and comprises the outermost domain of the LPS molecule. The composition of the O chain varies from strain to strain. For example, there are over 160 different O antigen structures produced by different [*E. coli*](http://en.wikipedia.org/wiki/Escherichia_coli) strains. The presence or absence of O chains determines whether the LPS is considered rough or smooth. Full-length O-chains would render the LPS smooth, whereas the absence or reduction of O-chains would make the LPS rough. Bacteria with rough LPS usually have more penetrable cell membranes to hydrophobic antibiotics, since a rough LPS is more [hydrophobic](http://en.wikipedia.org/wiki/Hydrophobic). O antigen is exposed on the very outer surface of the bacterial cell, and, as a consequence, is a target for recognition by host [antibodies](http://en.wikipedia.org/wiki/Antibody). It is responsible for **SPECIFICITY.**

1. **Core**

The Core domain always contains an oligosaccharide component that attaches directly to [lipid A](http://en.wikipedia.org/wiki/Lipid_A) and commonly contains [sugars](http://en.wikipedia.org/wiki/Sugar) . The LPS Cores of many bacteria also contain non-carbohydrate components, such as phosphate, amino acids, and ethanolamine substituents.It is responsible for **ANTIGENICITY.**

1. **Lipid A**

The lipid A domain is responsible for much of the TOXICITY of Gram-negative bacteria. When bacterial cells are [lysed](http://en.wikipedia.org/wiki/Lysis) by the [immune system](http://en.wikipedia.org/wiki/Immune_system), fragments of membrane containing lipid A are released into the circulation, causing fever, diarrhea, and possible fatal endotoxic shock (also called [septic shock](http://en.wikipedia.org/wiki/Septic_shock)). The Lipid A moiety is a very conserved component of the LPS.

**Store Transformation:** it is a phenomenon where pathogenic (Smooth) bacteria turns in Non-pathogenic (Rough) bacteria due to losing the ability of manufacturing Somatic-O-Antigen when it cultured on artificial media in the lab.

**Preparation of somatic O- Ag:**

Centrifugation

By Spreading

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***E.coli***

3000rpm/30min

Water bath

100°C/2.5hr

Brain Heart Infusion A.

37°/24hr.

Brain Heart Infusion B.

37°/18hr.

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Thyoglycolat 37°/48hr

To detect sterility

Suspend the supernatant with 0.03 Formaldehyde

Wash several times with Normal Saline

Keep at 4°C until use

**H (flagellar) antigens**:

Flagellar proteins of motile genera and species it is used for (serotype) and it is absent in non-motile genera (*[Shigella](http://www.life.umd.edu/classroom/bsci424/PathogenDescriptions/Shigella.htm)* and *[Klebsiella](http://www.life.umd.edu/classroom/bsci424/PathogenDescriptions/Klebsiella.htm)*). Flagellar Ag is heat labile {Break down at 60°C but keep its immunogenicity and denaturate at 100°C) H flagellar antigen is used to specific antisera to identify organisms beyond “species” level, Example: Escherichia coli O157:H7. Flagelar antigen cn be detected by agglutinatin with H-antiserum. The immunoglobulin formed against H –Ag is **IgG.**

Centrifugation

Centrifugation

***E.coli***

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With chlorophorm

To kill the bacteria

P.B.S

10 000 rpm/10min

To get rid of somatic O AG

Nutrient broth 37°C/24hrs

To enhance flagella formation

Nutrient agar

37°C/24hrs



Suspend with P.B.S and keep at 4°C

**HOMEWORK:**

**In your opinion what is the benefit of preparing these above antigens from bacteria?**