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ENDOTOXINS AND PYROGEN

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ABSTRACT
Endotoxin, present in the outer membrane of all gram-negative bacteria, can pose serious risks to human health, from irreversible shock to death. Therefore, it is essential to develop sensitive, accurate, and rapid methods for its detection. The rabbit pyrogen test is the first standard technique for endotoxin detection and, nowadays, has been replaced by the Limulus *Amoebocyte Lysate* test, which is the most popular detection technique for endotoxin. With in-depth understanding of endotoxin, biosensors based on endotoxin-sensing components are promising alternatives to pursue in developing low-cost, easy-operation, and fast-response endotoxin detection techniques. This article summarizes the recent advances of endotoxin detection methods with a particular emphasis on optical and electrochemical biosensors based on various sensing elements ranging from nature biomolecules to artificial materials. As the research and technological revolution continues, the highly integrated and miniaturized commercial devices for sensitively and reliably detecting endotoxin will provide a wide range of applications in people's daily life.

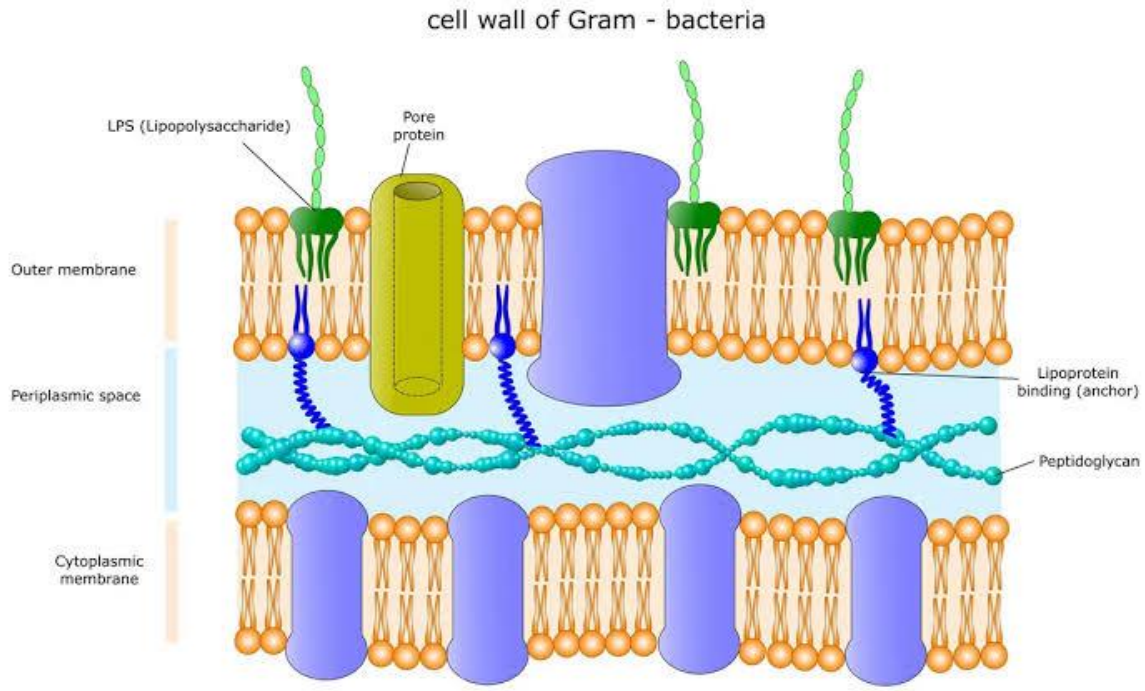
KEYWORDS: Therefore, it is essential to develop sensitive, accurate, and rapid methods for its detection.

INTRODUCTION
Pyrogens are fever-inducing substances, such as lipopolysaccharides (LPS) from Gram-negative bacteria (endotoxins), representing significant hazards as contaminants of health products (Montag-Lessing et al., 2010; Daneshian et al., 2008). Medical products often interface directly with blood and organs, providing the opportunity for pyrogens to elicit potent inflammatory responses from the systemic innate immunity, the early 20th century, the Rabbit Pyrogen Test (RPT) was developed to screen parenteral products (Hartung, 2015) based on measuring the rectal temperature in rabbits after intravenous injection. Monitoring the rabbit's febrile response has been used as a sample contamination marker despite several shortcomings An endotoxin is a complex lipopolysaccharide found in the outer cell wall of gram-negative bacteria, consisting of three main regions: O-specific antigen, core polysaccharide, and lipid A. It is responsible for toxic effects such as fever, multi-organ failure, septic shock, and seps.

Pyrogens are fever-inducing substances that can be of microbial or non-microbial origin. Among them, bacterial endotoxins are the most potent and prevalent, particularly in healthcare and industrial settings. Their presence in injectable drugs, dialysis fluids, or medical devices can result in severe febrile reactions, septic shock, or death. Hence, understanding their structure, activity, and control is critical.

Endotoxins: structure and sources.

Structure



Endotoxins have a weight of around 10 kDa and their general structure consists of three parts: a lipid component containing fatty acids and disaccharide phosphates (Lipid A), O-specific polysaccharide side chains (O-antigen) and a core polysaccharide chain.

Lipid A
Responsible for toxic effect
Core polysaccharide Polycarbohydrates, are the most abundant carbohydrates found in food. They are long-chain polymeric carbohydrates composed of monosaccharide units bound together by glycosidic linkag.

O-Antigen Variable region contributing to antigenicity.
O-Antigens (also known as O-specific polysaccharides or O-side chains) are major component of the surface lipopolysaccharide (LPS) of Gram-negative bacteria and are highly variable in structure. There are three main biosynthesis pathways by which O-antigens can be synthesized, namely Wzx/Wzy, adenosine triphosphate-(ATP-)

Sources of Endotoxins and Pyrogens
1. Endotoxins
Endotoxins are a **subset of pyrogens** specifically derived from **Gram-negative bacteria**.

A. Microbial Sources (Endotoxins)
- **Gram-negative bacteria**: Most common source.
  - Examples: *Escherichia coli*, *Pseudomonas aeruginosa*, *Klebsiella*, *Salmonella*, *Enterobacter*.
- Found in.
  - Contaminated **water systems** (especially stagnant or biofilm-rich systems)
  - **Raw materials** used in drug manufacturing
  - **Biotechnological processes** involving bacterial expression systems (e.g., recombinant protein production)
  - **Medical equipment** or containers that were inadequately sterilized

B. Environmental Sources
- **Airborne contamination** in manufacturing areas
- **Biofilms** in piping, filters, and tanks
- **Reused or improperly cleaned labware/equipment**
- **Contaminated personnel contact** (human skin or respiratory flora)

2. Non-Endotoxin Pyrogens (Other Pyrogen Sources)
Not all pyrogens are endotoxins; some come from other microorganisms or even non-microbial origins.

A.Microbial (Non-Endotoxin)
Gram-positive bacteria:
Peptidoglycan, lipoteichoic acid.
Examples: Staphylococcus aureus, Streptococcus pyogenes.

Fungi
Cell wall components like β-glucans, mannans
Example: Candida albicans

Viruses
Viral proteins or RNA can stimulate immune responses indirectly.

Mycoplasma
Lack cell walls but can trigger cytokine release via membrane lipoproteins

B.Non-Microbial Pyrogens
Leachables from plastics or rubber (e.g., phthalates, bisphenols)
Chemicals or residual solvents from manufacturing
Metal particles or ions (e.g., iron, nickel)
Dust particles, fibers, or other physical contaminants
Degradation products from materials used in packaging or devices

Pyrogens: classification
Pyrogens have been classified as either 'endogenous' or 'exogenous'. Pyrogenic cytokines are endogenous immunoregulatory polypeptides, such as interleukin (IL)-1β, IL-6 and tumor necrosis factor (TNF).

1. Endogenous Pyrogen
Endogenous pyrogen is a low-molecular-weight protein that is produced by phagocytic leukocytes in response to stimulation by exogenous pyrogens and released into the circulation.lt induces fever by acting on the preoptic area of the hypothalamus to raise the set-point of the hypothalamic thermostat.
Endogenous pyrogens are produced by immune cells such as neutrophils, macrophages and lymphocytes as well as by endothelial cells, astrocytes and glial cells in response to exposure to exogenous pyrogens.
Ex:interleukins (IL) 6, IL-1, interferon gamma (INF-γ)

2. Exogenous pyrogen
Exogenous pyrogen is a fever-producing agents of external origin, e.g., bacterial endotoxins and other microbial products, antigen-antibody complexes, viruses and synthetic polynucleotides, incompatible blood and blood products, and androgen breakdown products such as etiocholanolone.

Ex: lipopolysaccharides

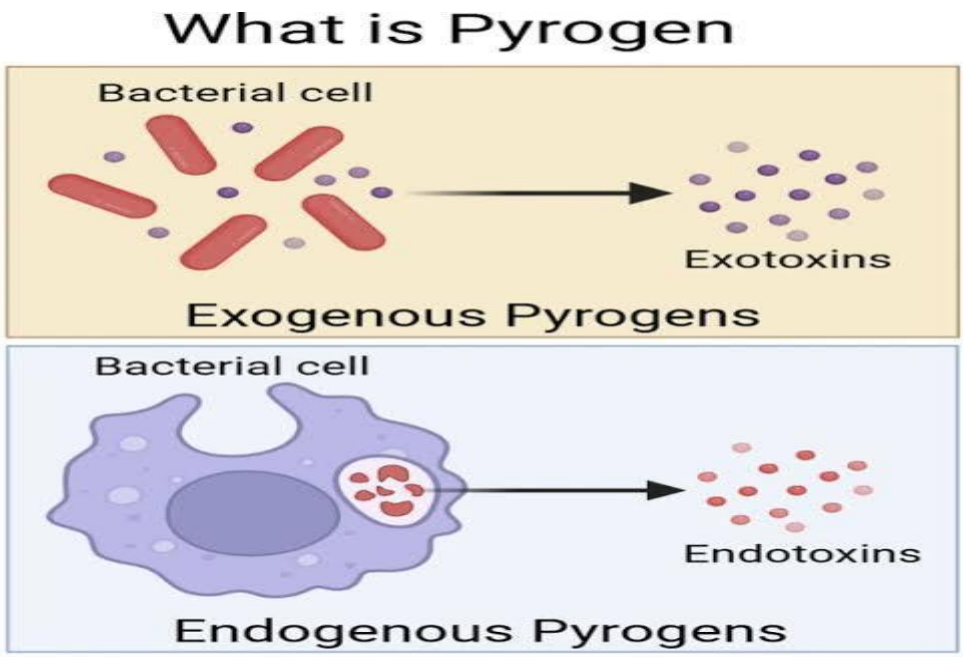


Fig.

Mechanism of Action
The mechanism of action of pyrogen involves its ability to stimulate the hypothalamus, the part of the brain that regulates body temperature. When pyrogen binds to its receptors in the hypothalamus, it triggers a series of signaling cascades that ultimately lead to an increase in the production of prostaglandins. Prostaglandins, in turn, raise the body's temperature set point, resulting in fever. This increase in body temperature is thought to be beneficial in fighting off infections, as many pathogens are sensitive to high temperatures.

Methods
1. Rabbit pyrogen Test

For a long time, the RPT was the only established and validated test method for pyrogen control. The technique is relatively easy; one puts a rabbit in a cage so it cannot move, injects whatever product one wants to test for pyrogens into his ear vein, and measures the body temperature. If the rabbit gets a fever, the product contains a significant amount of pyrogens; if there is no rise in body temperature, the product does not contain a substantial amount (for a rabbit) of pyrogens and is certified.

Advantages
Firstly, this method has a few quite intuitive advantages. The test is easy to perform, you do not need highly

trained personnel, and after approximately two hours, you get a result.

Dis advantages

The most prominent disadvantage is the use of approximately 400.000 rabbits per year. It is mandatory to use three animals which all have to show the same result; if one gets a fever and the other two do not, the test is invalid and has to be repeated.

2. Limulus Amebocyte Lysat Test (LAL)

The LAL test is an enzymatic-based in vitro test using the horseshoe crab's blood. The horseshoe crab's blood contains a protein called Factor C (FC) which interacts with endotoxins. An addition of endotoxin to the blood leads to a reaction cascade, starting by FC, resulting in an enzymatic reaction in which a proclottting enzyme is activated and builds a gel clot.

This gel clot is the marker for a positive test result and the presence of endotoxins in the tested substance.

Fig.

3.Recombinant Factor C Test (rFC)

This pyrogen test is similar to the LAL test because it uses an identical biological mechanism. The huge advantage of this method is that the recombinant Factor C is synthesized instead of utilizing the crab's blood. Despite the animal use, the advantages and disadvantages are mostly the same as for the LAL test.

4.Monocyte Activation Test (MAT)

The Monocyte Activation Test (MAT) is a cutting-edge in-vitro assay that employs human blood to replicate the early stages of the human immune system. This innovative test is designed to detect all types of pyrogens in parenteral drugs, biologics, and medical devices.

Other Pyrogen Tests

Invitro Pyrogen Test (Ipt)

This test exploits the reaction monocytes/macrophages detection of pyrogens.

Human whole blood taken from healthy volunteers is incubated in presence of tes. Sample, pyrogenic contamination initiate the release of "the endogenous pyrogen.

Interlukin 1-ß determined by ELISA after incubation.

Regulatory Standards

Pharmacopeias (USP, EP, JP) define limits for endotoxins in injectable drugs and devices.

USP <85>: Bacterial Endotoxins Test.

USP <151>: Pyrogen Test (Rabbit).

USP <1085>: Guidance on endotoxin and pyrogen testing.

Acceptance criteria: Expressed in EU/mL (Endotoxin Units).

METHODS TO DETECT PYROGENS

	COMPLETE PYROGEN TESTS		BACTERIAL ENDOTOXIN TESTS	
Test type	RPT Rabbit Pyrogen Test	MAT Monocyte Activation Test	LAL Limulus Amebocyte Lysate Test	rFc Recombinant Factor C Test
Detects endotoxins	✓	✓	✓	✓
Detects non-endotoxin pyrogens	✓	✓	✗	✗
Animal free	✗	✓	✗	✓
Regulatory compliance	Ph. Eur. 2.6.8 USP<151>	Ph. Eur. 2.6.30	Ph. Eur. 2.6.14 <USP 85>	Ph. Eur. 2.6.32 USP <86>
Measuring mechanism	Raise of body temperature after injection of drug *(in vivo)*	Inflammatory cytokines released from human monocytes *(in vitro)*	Clotting cascade in blood amebocytes *(in vitro)*	Recombinant Factor C (first step in LAL cascade) *(in vitro)*

Physiological effects of pyrogen
Pyrogens elevate the circulating levels of inflammatory cytokines. They produces fever, blood coagulation, hypotension, lymphopenia, neutrophilia, elevated levels of plasma, acute phase proteins
Low doses of pyrogens induce asymptomatic inflammatory reactions and moderate doses induce fever and changes in plasma composition.

High pyrogenic doses results in
•Shock
•Characterized by cardiovascular dysfunction
•Vasodilation
•Vasoconstriction
•Endothelium dysfunction
•Multiple organ dysfunction

Control and Prevention
1.Water System Management
Water is a major source of endotoxin contamination in pharmaceutical production.

°*Use of Water for Injection (WFI)*
Must meet pharmacopeial standards (USP, Ph. Eur.)
Produced by distillation or reverse osmosis + ultrafiltration

°*Regular monitoring of*
Total organic carbon (TOC)

Conductivity
Bacterial endotoxin levels (using LAL test).

°*Design considerations*
Avoid dead legs, ensure constant circulation.
Use sanitary design (stainless steel, smooth surfaces).

2. Raw Material and Component Control
Use only certified endotoxin-free raw materials.
Test raw materials for pyrogens when applicable.
Supplier qualification and periodic audits.
Components such as stoppers, filters, and tubing should be depyrogenated or certified as low endotoxin.

CONCLUSION

Those exogenous pyrogens can be categorized either as endotoxin, deriving from the cell membrane of gram-negative bacteria, or as non-endotoxin pyrogens.

Endotoxins and pyrogens pose significant risks to patient safety, especially in parenteral drugs, biologics, and medical devices. While endotoxins-primarily derived from Gram-negative bacteria-are the most studied and regulated, non-endotoxin pyrogens also demand attention due to their potential to trigger adverse immune responses.

REFERENCE

1. Microbiology by J. Pelczar, JR. E.C.S. Chan and Noel.
2. Guy, D. (2003): "Endotoxins and Depyrogenation" in Hodges, N. and Hanlon, G. (Eds.). Industrial Pharmaceutical Microbiology.
3. Slide share.com
4. Dr. Barry whyte/BMG LAB TECH.