
Detection Methods for Lipopolysaccharides: Past and Present

Loreen R. Stromberg, Heather M. Mendez and
Harshini Mukundan

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Abstract

Lipopolysaccharide (LPS) is the primary component of the outer membrane of Gram-negative bacteria. LPS aids in protecting bacterial cells, and also defines the unique serogroups used to classify bacteria. Additionally, LPS is an endotoxin and the primary stimulator of innate immune cells in mammals, making it an ideal candidate for early detection of pathogens. However, the majority of methods for detection of LPS focus on detection of the endotoxic component of the molecule, lipid A. Since lipid A is largely conserved among bacterial species and serogroups, these detection approaches are highly nonspecific. Thus, the importance of identifying the O-polysaccharide antigenic portion of LPS, which confers serogroup specificity, has received a great deal of attention in recent years. However, methods that are highly selective to the O-antigens are typically less sensitive than those that target the endotoxin. Here we present a history and comparison of the sensitivity of these methods and their value for detecting bacteria in a variety of different sample types.

Keywords: lipopolysaccharide, endotoxin, O-antigen, Serogroup, biodetection

1. Introduction

The increasing occurrence of infectious disease is a global issue. Emerging pathogens with increasing levels of drug resistance are a continuing danger to both public health and agriculture. Accurate and rapid detection of pathogens is critical to implement preventative measures to mitigate this problem. Despite this urgent need, conventional methods for bacterial detection require cell culture and serology, which can take several weeks. As new pathogens emerge, it is even more important that our detection technologies evolve to keep pace with the need to

discriminate pathogen from host flora. This requires an understanding of pathogen biology, the types of samples they occur in, and their mechanism of immune interaction within the hosts [1].

The innate immune system is able to discriminate pathogens from nonpathogens, and rapidly sense pathogen biomarkers in the complex milieu of the host. Exploiting this recognition via measurement of pathogen signatures, can provide an optimal strategy for discriminatory biodetection. A primary category of such biomarkers is virulence signatures termed pathogen-associated molecular patterns (PAMPs) [2]. PAMPs are evolutionarily conserved molecules that bind pattern-recognition receptors in the host, and activate the innate immune response [2, 3], providing a means for both early and specific pathogen detection. Biochemically, PAMPs are a diverse array of proteins, lipopeptides, lipoglycans, peptidoglycans, teichoic acids, and nucleic acids [4]. However, many detection methods have largely focused on proteins and nucleic acids [1, 5], ignoring other categories of PAMPs [2, 6–8]. Also, their small size, biochemistry, and low concentration in hosts make them difficult to target in detection assays [8, 9].

Classified as a lipoglycan, lipopolysaccharides (LPS) are small amphiphilic molecules that are associated with Gram-negative bacteria [7, 10]. LPS is an indicator of active infection, is serogroup-specific [11–13], more stable than its protein counterparts, and is released early in infection, making it an ideal candidate for detection and diagnostics. LPS serves as a biomarker that aids in serological discrimination of Gram-negative bacteria; this allows for identification and characterization of pathotypes that are essential for timely mitigation and treatment of infections. Since LPS is a pathogen-specific biomarker, it is an indicator of acute infection, which is an advantage over serological assays. In addition to medical diagnostics, LPS detection provides a method for detecting *Escherichia coli* in the food-industry, which is often associated with food-borne illnesses. Finally, LPS is also a virulence factor whose structure and function determines *E. coli* serogroup, a factor which has ramifications on vaccine design and therapeutic interventions. While many methods for LPS detection exist, most of them are not optimized for amphiphilic detection in physiological samples. An ideal measurement for LPS should be sensitive enough to detect low concentrations of the amphiphile in aqueous physiological milieu (e.g., blood), and use antibodies or ligands that provide serogroup selectivity [14]. Coupling sensitive detection platforms with surfaces designed to maximize the binding of amphiphilic PAMPs is a potential solution to achieve such an ideal.

2. Sources of lipopolysaccharides

Bacteria are classified into Gram-negative and Gram-positive [15], which release amphiphilic virulence factors such as LPS, lipoarabinomannan (LAM), and lipoteichoic acid (LTA) in the host. Species of pathogenic Gram-negative bacteria of concern to human health, include *Acinetobacter* [16], *Burkholderia* [17], *Bordetella* [18], *Campylobacter* [19–21], *Chlamydia* [22, 23], *E. coli* [20, 24], *Helicobacter* [25, 26], *Hemophilus* [27], *Klebsiella* [28], *Legionella* [20, 29], *Moraxella* [30], *Neisseria* [31], *Pseudomonas* [32], *Proteus* [33], *Salmonella* [20, 34], *Shigella* [35], *Yersinia* [36], and others, grouped into the Enterobacteriaceae family. These pathogens are contaminants in food, water, and soil, used as agents of bioterrorism, and can cause nosocomial infections [5]. Detection of these organisms, particularly *E. coli*, is an important aspect for epidemiology, disease control, and treatment.

Herein, we present a comprehensive description of the structural and biochemical properties of LPS, current methods for its detection, and potential approaches to overcome the current limitations for direct detection of the molecule in physiological matrices.

3. Lipopolysaccharide structures and conformations

Lipopolysaccharides have been the subject of intense study for over half a century [37–39]. LPS is the prototypical lipoglycan with an overall net negative charge [40–42], and is the primary component of the outer membrane of nearly all Gram-negative bacteria [11]. The bacterial membrane of each *E. coli* cell is composed of approximately 10^6 lipid A moieties and 10^7 glycerophospholipid molecules, comprising approximately three-quarters of the outer membrane [43–45]. Thus, there are approximately 62 pg of LPS per cell (for *E. coli* in log phase growth) [46]. LPS has an amphipathic tripartite structure (**Figure 1**). Lipid A is the most conserved portion of the LPS molecule, and consists of six, sometimes seven, fatty acid tails (*E. coli* and *Salmonella*, respectively), which gives the molecule its hydrophobic properties [10, 43, 45]. Lipid A is also called endotoxin [43], and is responsible for the biological effects of LPS

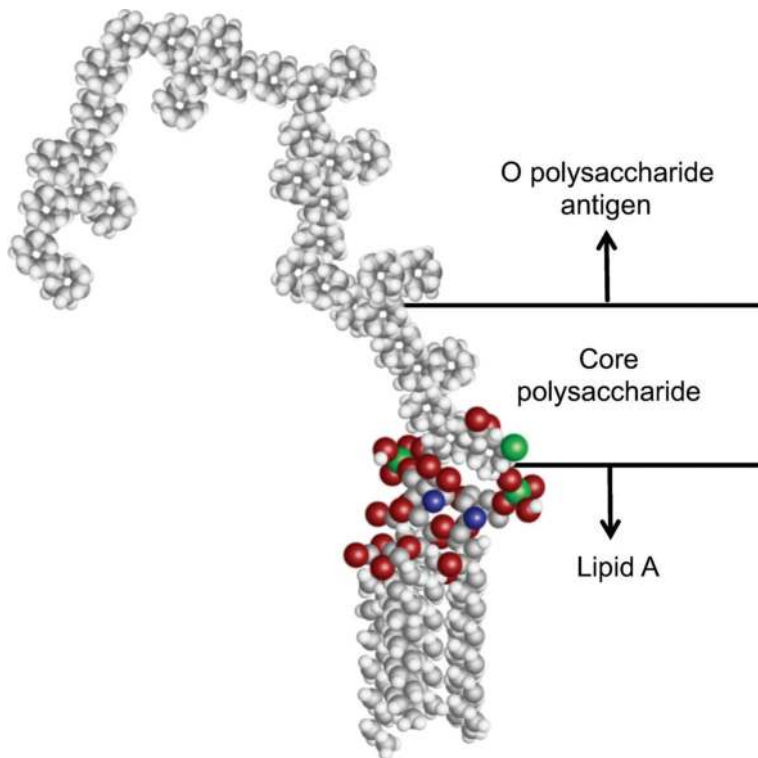


Figure 1. Representative structure of the molecular components of smooth LPS. The hypervariable O-polysaccharide antigen, core polysaccharide, and the hydrophobic lipid A group. Reprinted with permission from Ref. [74].

caused by its binding to the mammalian innate immune receptor, toll-like receptor 4 (TLR4) [11, 44, 47, 48]. Structurally, lipid A is covalently bound to the core polysaccharide, which is further divided into the inner and outer core polysaccharides, with the outer core being less conserved in both sugar moieties and location of glycosidic linkages [45, 49, 50].

There are two main forms of LPS—smooth (S-form) and rough (R-form) [42, 45, 46]. The distal end of LPS extends to a long chain O-polysaccharide antigen (O-ag(s)) in organisms possessing S-form, which is an indicator of virulence [51, 52]. R-form LPS is devoid of the O-ag [45], but can still induce an immunogenic response [53]. The O-ag is hyper-variable, and made up of repeating subunits, each composed of 1–7 glycosyl residues [45, 54]. As many as 40 size variations in subunit repeats of the O-ag have been reported just for *E. coli* O111:B4 [55], and 180 O-ag have been identified overall for *E. coli* species [47, 54]. The sugars (colitose, paratose, tyvelose, and abequose) that make the O-ag unique are seldom found elsewhere [54]. Other variations to the polysaccharide are implemented through addition of noncarbohydrate entities, such as acetyl or methyl groups [54]. These variations make discriminative detection of enteric bacteria feasible [56], but complicate antigen characterization. Therefore, LPS serves as an ideal target for early detection and identification of Gram-negative pathogens.

In aqueous solutions, amphiphiles like LPS can present in a micellar conformation [48, 55, 57–59]. This occurs at a concentration specific to the amphiphile [55], and is known as the critical micelle concentration (CMC). At or above the CMC, there is an equilibrium state between monomers, micelles or supramolecular aggregates, depending on environmental conditions [48, 55–57, 60–63]. This amphiphilic biochemistry and structural variability complicates determination of the exact molecular weight of S-form LPS. As such, LPS concentrations are reported in weight per volume, or in endotoxin units (EU), a measure of activity. As degree of endotoxicity can vary according to bacterial origin, a rough estimate of 100 pg = 1 EU is used in many cases to facilitate unit conversion [64, 65].

The large oligosaccharide region on S-form LPS makes the molecule amphipathic [54], which influences the shape of micelles in solution. Lipid A is largely responsible for shaping the LPS micelle [10, 45, 46, 56, 66–68], although other factors can also contribute. Lipid A is conserved within species in the number of fatty acid chains and the degree of saturation [44, 66] within those chains [22, 47, 69]. Shapes for LPS micelles include cubic, lamellar, and hexagonal inverted structures [56, 67, 70, 71]. Whether aggregate or monomeric forms (or both) of LPS is required for innate immune activation is debatable [56, 72, 73]. Since this process occurs in aqueous blood, it is unlikely that the molecule is presented as a monomer, unless associated with serum binding proteins.

Variation in LPS micelles [55] modifies presentation of O-ag-specific epitopes to antibodies, making detection challenging [74, 75]. This is specifically true when the heterogeneous presentation of linear [76] and conformational epitopes [49, 77] present on LPS molecules are considered. The primary structure of LPS varies in the core polysaccharide, within and between species [47, 55]. Core polysaccharides are primarily made up of common sugars such as heptose and 2-deoxy-D-mannoctulosonic acid (a.k.a. KDO), which can be functionalized with phosphate or ethanolamine groups [45, 50, 78]. This feature contributes to varying charge distributions and differential size ratio of the hydrophobic to hydrophilic regions which influences

micelle assembly [10, 59, 79, 80]. Other factors that contribute to micelle shape [10, 79] are pH [61], ion concentration [81–86], and temperature [62]. These biochemical properties drive host-pathogen interactions and should be considered in the design of detection strategies.

4. Detection methods for lipopolysaccharides and similar amphiphiles

There have been many efforts to establish rapid and reliable detection methods for LPS in clinical samples [10, 46] and for testing pharmacological products such as infusion fluids, sterile injectables, medical device implants, and others [87]. These methods can be broadly divided into six overlapping categories: *in vivo* and *in vitro* tests, immunoassays and their derivatives; biological, chemical, and cell-based sensors. These methods span a broad range of sensitivity, but many lack the ability to differentiate between LPS serogroups.

4.1. *Limulus* amoebocyte lysate assay and the rabbit pyrogen test

The first method approved by the US Food and Drug Administration for LPS detection was called the rabbit pyrogen test [88–90], which simply measures the ability of an endotoxin to induce fever in an animal. Any febrile response was attributed to the presence of endotoxin [89–91]. The test, clearly, is activity-based, and nonspecific. In the case of Hepatitis B vaccine manufacturing, the rabbit pyrogen test is still the standard method for determining endotoxin contamination [91], but the test is cost prohibitive and is minimally utilized today, except in some parenteral devices [10].

In 1956, Bang discovered that amoebocytes from *Limulus polyphemus* (a.k.a. horseshoe crab) agglutinate upon addition of endotoxin [46], as a result of a protease cascade [10]. Bang and Levin [46, 92] subsequently used this concept to devise a method for endotoxin detection. Since the lysates of amoebocytes were required, it was called the *limulus* amoebocyte lysate (LAL) assay, and is the gold standard for the detection of lipid A. The LAL assay is prone to variability and can be inhibited through several mechanisms. The United States Pharmacopeia and the Code of Federal Regulations have consequently published guidances for the manufacturing and testing of assays for use on human products [93, 94]. Despite some challenges, the LAL assay is more rapid, cost effective, and reportedly 300 times more sensitive [46] than the rabbit pyrogen test [46].

Variants of the LAL assay use turbidimetric [95], chromogenic [46], or viscosity [10] measurements to determine results [10, 46]. A turbidimetric gel clot has more coagulum, and measures the change in turbidity over time, but does not form a solid clot [46, 95]. The viscosity assay, however, measures the degree of clotting via the change in viscosity. The chromogenic assay can be endpoint or kinetic, and utilizes a *p*-nitroaniline substrate, which is cleaved by an LAL proenzyme, providing a colorimetric readout [46]. The sensitivity of LAL assays is dependent on the sample type, processing method and time, as well as the dilution factor [46]. Additionally, the source of the LAL reagent plays a factor, as it is apparent when comparing the different limits of detection (LoD) reported for endotoxin standards. A survey of the relative sensitivities of the LAL assay is shown in **Table 1**.

| Description | Sample | Detection method | Species | Sensitivity (ng/mL)* | Specific | Source |
|-----------------------------------|-------------------------|---------------------|---|----------------------|----------|--------|
| Rabbit pyrogen | Purified endotoxin | Febrile response | – | – | No | [89] |
| LAL | Plasma | Gelation | Multiple species | 0.5–5.0 | No | [96] |
| LAL | Blood, plasma | Gelation | <i>E. coli</i> | 0.5–5.0 | No | [92] |
| LAL | Serum plasma | Optical density | <i>E. coli</i> | 0.025–0.5 | No | [100] |
| LAL | Urine | Gelation | <i>E. coli</i> | 0.5 | No | [204] |
| LAL | Urine | Optical density | Multiple species | 2.0 | No | [98] |
| LAL | Spinal fluid/ plasma | Optical density | <i>E. coli</i> , <i>Haemophilus influenzae B</i> | 0.1 | No | [101] |
| LAL | Ascites | Gelation | <i>E. coli</i> | 0.5 | No | [104] |
| LAL | Cerebral/synovial | – | <i>E. coli</i> | 1.0 | No | [103] |
| LAL | Seawater | Optical density | <i>E. coli</i> | 2.3 | No | [41] |
| LAL | Purified endotoxin | Gelation | <i>E. coli</i> | 1.0 | No | [95] |
| LAL | Purified endotoxin | Gelation | <i>Salmonella minnesota</i> | 10 ⁻¹¹ | No | [111] |
| LAL | Ground beef | Gelation | <i>Enterobacter aerogenes</i> | – | No | [205] |
| LAL | Ground beef | Gelation | Multiple species | 51.0 ng/g | No | [108] |
| LAL | Milk | Chromogenic | <i>Pseudomonas putida</i> | 0.01 | No | [107] |
| LAL | Purified endotoxin | Gelation | <i>E. coli</i> O114 | 100 | No | [206] |
| LAL-magnetoelastic sensor | Purified LPS | resonant frequency | <i>E. coli</i> O111:B4 | 0.0105 EU/ mL | No | [207] |
| ENDOLisa® (LAL) | Purified endotoxin | Fluorescence | <i>E. coli</i> spp., <i>Salmonella</i> spp. | 0.05–500 EU/ mL | No | [129] |
| ELISA | Milk | Abs at 405 nm | <i>E. coli</i> | 100–200 | | [165] |
| LPS pull down-sandwich ELISA | Pure cultures | Abs at 450 nm | <i>E. coli</i> O157 | – | | [125] |
| LPS pull down-sandwich ELISA | Purified LPS | Abs at 450 nm | <i>Salmonella</i> spp. (31 total) | 1.0 | Yes | [126] |
| Premier EIA <i>E. coli</i> O157 | Stool extract | Spectro-photometric | <i>E. coli</i> O157 | – | Yes | [122] |
| LPS pull down | Purified endotoxin | RIA | <i>E. coli</i> O114 | 300 | No | [206] |
| LPS pull down-ion (NTA-Cu) | Purified LPS | EIS | <i>E. coli</i> O55:B5 | 0.0001–0.1 | No | [161] |
| Diaphorase functionalized surface | Purified LPS | Chemical | <i>E. coli</i> O127:B8 | 50 | Maybe | [87] |

| Description | Sample | Detection method | Species | Sensitivity (ng/mL) [*] | Specific | Source |
|--|---|-------------------------------|---|----------------------------------|----------|--------|
| LPS pull down-SAMs with synthetic peptide | Purified LPS | Electro-chemistry | <i>E. coli</i> ATCC 35218 | 21.8 pg/mL | No | [189] |
| LPS pull down-SAMs with aptamer | Purified LPS | EIS | <i>E. coli</i> O55:B5 | 0.1–1.0 | Maybe | [159] |
| LPS pull down-gold electrode w/ aptamer | Purified LPS | EIS and cyclic voltammetry | <i>E. coli</i> O55:B5 | 0.001–1.0 | No | [160] |
| LPS aptamer sandwich | Purified LPS | Electro-chemistry | – | 10 fg/mL | Maybe | [188] |
| LPS pull down-gold electrodes w/ PmB | Purified LPS | EIS | <i>E. coli</i> O111:B4 | 0.2 | No | [162] |
| Polydiacetylene liposomes | Purified LPS (5 groups) | Change in Abs | <i>E. coli</i> spp, <i>Salmonella</i> spp | 2.22 mg/mL | Yes | [191] |
| Impedance entothelial biosensor | Purified LPS in culture medium [*] | Resistivity of cell monolayer | – | 500 | No | [169] |
| Macrophage microarrays on gold electrodes | Purified LPS in culture medium | FTIR | <i>E. coli</i> O111:B4 | 0.1 µg/mL | No | [200] |
| Primary culture HDME cells | Purified LPS | Fluorescence | <i>E. coli</i> O111:B4 | 1.0 µg/mL | No | [171] |
| Engineered cells secrete alkaline phosphatase | Purified LPS in culture medium [*] | Electro-chemistry | – | 0.1 | No | [170] |
| LPS pull down-PmB | Purified LPS | Evanescent sensing | <i>E. coli</i> O128:B12 | 25 | No | [75] |
| LPS pull down-TLR4/MD2 on gold electrodes | Purified LPS | Electro-chemistry | <i>E. coli</i> O55:B5 | 0.0005 EU/mL | No | [180] |
| LPS pull down-membrane insertion | Purified LPS (3 groups) | Evanescent sensing | <i>E. coli</i> | 420 | Yes | [74] |
| LPS pull down-antibody | Pure cultures in ground beef | Evanescent sensing | <i>E. coli</i> O157 | – | Yes | [166] |
| LPS pull down-proanthocyanidin | FITC-labeled LPS | Fluorescence | <i>E. coli</i> O55:B5 | – | No | [192] |
| Copolythiophene interacts with LPS | Purified LPS | Fluorescence | <i>E. coli</i> O55:B5 | 2.5E ⁻⁵ –2.0 µM | No | [164] |
| Polydiacetylene liposomes | – | Fluorescence | – | 0.1 µM | No | [190] |
| Peptide-based fluorescence | Purified LPS | FRET-increase | <i>E. coli</i> O111:B4 | 0.15–2.0 µM | No | [194] |
| Pyrenyl-derived long-chain quaternary ammonium probe | Purified LPS | Fluorescence | <i>E. coli</i> O55:B5 | 100 nM | No | [193] |

| Description | Sample | Detection method | Species | Sensitivity (ng/mL)* | Specific | Source |
|--|------------------------------|------------------|---|----------------------|----------|--------|
| LPS pull down-peptide on Graphene Oxide | Purified LPS (4 groups) | Fluorescence | Several species | 130 pM | No | [195] |
| LPS pull down-PmB capture | Purified LPS spiked in blood | Acoustic sensing | <i>E. coli</i> O55:B5 | 1.0 | No | [196] |
| LPS pull down-CD14 capture | Biotin-LPS | Luminescence | – | 10.0 | No | [176] |
| LPS pull down Polyaniline + ConA lectin | Purified LPS and LTA | EIS | <i>E. coli</i> , <i>S. aureus</i> | 50.0 | No | [208] |
| Aptamer sandwich on beads | Purified LPS | Fluorescence | <i>E. coli</i> O55:B5 | 0.01 | Maybe | [163] |
| LPS pull down endotoxin neutralizing protein | Purified LPS | Capacitance | <i>E. coli</i> | 10 ⁻¹³ M | No | [181] |
| LPS pull down CramoLL lectin | Purified LPS (4 types) | EIS | <i>E. coli</i> <i>Salmonella</i> <i>Klebsiella</i> <i>Serratia</i> | 25.0 µg/mL | No | [185] |

*Unless otherwise indicated.

Table 1. Overview of sensitivities and specificities for LPS detection methods.

In 1970, Levin discovered that samples tested in whole blood would not render a positive result [92], but if plasma was extracted in chloroform and diluted 1–10%, then endotoxin activity could be detected in the 0.5–5 ng/mL range [92, 96]. Levin correctly assumed that components of whole blood were bound to endotoxin, thereby inhibiting the reaction with the LAL reagent [46, 92, 97], or changing the reaction kinetics [46]. This is evident when the amphiphilic nature of LPS and the aqueous nature of blood are considered. In addition to blood and plasma [46, 92, 96], the LAL assay has been used in urine [46, 98], cerebral spinal fluid, synovial fluid, ascites fluid, vaginal and cervical fluids, broncho-alveolar lavage samples, seawater [46], bovine milk [99], and beef tissue [100, 101]. Virtually all of these have reported ng/mL LoDs, for endotoxin, but none are serogroup-specific. Researchers have used heat [46, 102], chemical treatment with chloroform [103], acids [104, 105], alkali [106, 107], or ether [108] to improve sensitivity with some success when using heat or chemical extraction of the endotoxin [46, 109]. However, the results show poor reproducibility between researchers (**Table 1**). Yin and Galanos [106] reported a sensitivity of 10⁻¹¹ ng/mL for *Salmonella* spp., while Cooper et al. [89] reported 1.0 ng/mL for *E. coli* endotoxin. This disparity leaves a lot of questions and draws attention to the fact that small changes in preparation, heat or chemical treatments, usage of plastics instead of silanized glass, or addition of surfactants can result in altered assay sensitivity. This variation can also be explained by the variable biosynthesis of lipid A, as shown with *Salmonella* [47, 110]. Additionally, LAL can yield false positives upon reacting with other polysaccharides or β-(1,3)-glucans [10, 46] and depends on the source of

bacteria, as LPS/endotoxin can vary in toxicity [10, 56, 79, 68], in regards to immune stimulation [41, 46, 111].

Thus, the LAL assay and rabbit pyrogen test, both based on the native immune responses of the horseshoe crab or rabbit, exhibit significant variability in outcomes. Despite these, the LAL is still very useful for quickly detecting contamination. For example, in 1981, Jay [101] used the LAL test to determine both microbial counts and endotoxin load in 153 samples of store bought ground beef with a mean sensitivity of 7.9 µg/mL (endotoxin/beef sample) in 1 h. In 1985, Nachum and Shanbrom [46] used a chromogenic LAL system to detect between 2 and 175 ng/mL of endotoxin in 324 patient urine samples, with the assay taking between 2 and 4 h. Timely detection is valuable to both patient care and product viability. Despite being an ideal test for the presence of endotoxin, determining identity of pathogens still requires culture or enrichment.

4.2. Immunoassays for LPS detection and antibody selection

Developed in 1971 [112, 113], the enzyme-linked immunosorbent assays (ELISAs) are based on the immune reaction between antigen and antibody, with each assay being tailored for the unique antigen being tested. ELISAs were evaluated for *Salmonella* O-ags very early in development [114]. However, ELISAs for lipoglycans such as LPS suffer from low sensitivity and reproducibility [115–117]. One of the primary reasons for sensitivity issues is the amphipathic biochemistry of these molecules, leading to inconsistent binding on ELISA plates [118], and variable conformations of epitope binding sites [12, 119].

There exist two primary types of LPS-ELISAs, which detect either the LPS antigen, or LPS antibody titers. With the former, the plate surface is typically coated with a primary capture antibody specific to LPS, or with the sample to be tested [118]. After antigen capture, an epitope-specific antibody is used to detect LPS. The detection antibody can be directly labeled with an enzyme [113] or secondary antibody for colorimetric detection [120, 121]. In 1998, Mackenzie et al. [122] reported on the effectiveness of a commercial assay to screen stool samples for *E. coli* O157 antigens, and found that re-testing samples provided inconsistent results. It was speculated that this was due to inefficient washing of the microwells, yet the amphiphilic antigen preparation and its presentation to antibodies could have contributed to assay inconsistencies. It was also not considered that LPS is notorious for nonspecific and inconsistent binding on microplates [10, 116, 118]. In clinical samples, the association of LPS with host carrier molecules may affect its ability to adhere to capture surfaces [123, 124], as proteins will preferentially bind to the plate. Some groups have also reported cross reactivity or false positives with LPS sandwich ELISAs [125, 126]. Choi et al. [126] developed a sensitive capture ELISA with 24 species of *Salmonella*, but cross reactivity was observed. To mitigate cross reactivity, attempts have been made to substitute antibodies with other ligands. Grallert et al. [127, 128] coated microplates with proteins isolated from bacteriophages, which are specific to core polysaccharides in order to capture LPS, followed by detection with Factor C (a component of the LAL assay). This sandwich ENDOLisa[®], a microplate assay for direct detection of endotoxin, reports sensitivity between 0.05 and 500 EU/mL. This technology is sold as the Endotoxin Sample Preparation (ESPTM) kit, and is one of the few kits available for direct

detection of endotoxin in blood or serum [129]. However, the assay is unable to differentiate between serogroups.

The second type of ELISA measures LPS antibody titers to screen for Gram-negative bacterial infections. Here, the surface of the plate is functionalized with the antigen to pull down antibodies (Immunoglobulins A, G, and M (IgG, IgA, IgM)) from serum. Since this method is based on adaptive immunity, there is a lag between initial exposure to the pathogen, and increased antibody titers [130], making early detection difficult. This assay is not specific for active infection, but has been used to monitor population health and track epidemiology of infections. Screening has been used to detect exposure of military personnel to *Shigella* [131], obstetric patients with *Chlamydia* spp. [132], patients with *Salmonella* [133], and other pathogens [114, 134–137]. Suthienkul et al. [136] used an indirect ELISA to passively adsorb LPS onto polystyrene plates, and measure associated IgG/IgM titers in cholera patients. The results indicated discrepancies between the titers of IgG and IgM in young versus older patients, which could either be due to the inconsistency of LPS coating or associated with cross reactivity [138]. Suthienkul also acknowledged that antibody levels in infants screened could be inherited from the mother [136, 139].

Functionalizing ELISA plates with amphiphilic LPS is a technical challenge [12], since the surfaces are optimized for protein binding. In the late 1970s, it was discovered that polymyxin B (an antibiotic, PmB) interacted with LPS monomers in a 1:1 ratio [86, 140], and can be used to functionalize surfaces for Gram-negative detection [119]. However, PmB recognizes the conserved lipid A group of LPS, and does not allow for discriminative detection. Takahashi et al. [118] showed that precoating the plate with high molecular weight poly-L-lysine increases surface adsorption and allows for detection of 1 µg/mL LPS, with no cross reactivity. Others have studied the effects of ions such as calcium and magnesium [141], trichloroacetic acid [142], mixing the antigen in chloroform/ethanol, and drying on the plate surface [135], or complexing LPS with a protein such as bovine serum albumin [143] to improve performance and reproducibility. Functionalization of ELISA plates with proteins known to bind LPS, such as high- or low-density lipoproteins (HDL, LDL), chylomicrons, and LPS-binding protein (LBP) have also been evaluated [123, 124] and offers promise for the reliable detection of LPS antigen in complex samples.

Other limitations for LPS detection include the fact that many LPS antigens have not been isolated [144] and thus are not available for the development of screening assays, limiting accessibility of specific antibodies as well [145–150]. However, there is also a need to refine methods for selection of tailored antibodies. While there are variations [10], ELISA plates are typically functionalized with whole dead bacteria to screen monoclonal antibody cultures [145, 146, 148], giving rise to potentially cross reactive clones [10, 144] that are then screened against a multitude of bacterial strains [146, 149, 150]. It is noted that it is impossible to screen clones against all epitopes of LPS, even amongst the many *E. coli* serotypes. In 2000, Jauho et al. [12] addressed this issue by covalently linking purified LPS O-ags to polystyrene ELISA plates using anthraquinone and UV irradiation. This technique could prove useful in developing serogroup-specific antibodies against LPS, as conserved antigens like lipid A and core polysaccharide are absent. Alternative methods for antibody screening have utilized immunoblotting [144, 149, 151–153] and flow cytometry [154–156]. In addition, ELISAs can suffer from high background due to nonspecific interactions limiting their sensitivity [10, 122, 135, 157]. Particularly,

endogenous endotoxin present in reagents, on glassware, or plastics [158], may contribute to false positive results. Factors identified above have to be carefully considered in the development of ELISAs targeting LPS.

4.3. Biological and chemical-based LPS sensing

Many advanced methods such as electrochemical impedance spectroscopy (EIS) [159–161], anti-microbials [75, 162], aptamers [163], synthetic polymers [164], optical immunoassays [122, 125, 165], waveguide technology [75, 166, 167], lipid bilayers [9, 74, 168], and *in vitro* [169–171] assays have been applied for LPS detection. These technologies involve functionalizing biosensors with proteins or molecules to pull down LPS from a sample.

LBP [10], a relatively small protein (~60 kDa) that transports LPS in blood, shuttles the antigen to the cluster of differentiation 14 (CD14) protein in the extracellular matrix, or to the membrane of immune cells, such as macrophages [10]. After LPS binds CD14, it is passed to the hydrophobic binding pocket of myeloid differentiation factor 2 (MD-2) [7, 10], a necessary cofactor for the activation of TLR4. Also, the serum carrier lipoproteins (HDL and LDL), are carriers for LPS in blood. In addition to these, LPS has been demonstrated to bind aptamers [159, 160], various peptides [87, 109, 162, 172], and metal/cation complexes [84, 86, 161, 173–175]. Such carrier moieties are exploited in the development of novel detection methods for LPS, as outlined below.

For electrochemical (EC) sensing of LPS, a recognition ligand (similar to ELISA) and a transducer are required to measure the variation in signal [161]. For fluorescence-based sensing, a receptor captures LPS, while another molecule emits a fluorescent signal when bound to the antigen. Burkhardt et al. [176] used solubilized LBP to transfer LPS to a CD14 functionalized surface, with a LoD of 10 ng/mL using an electro-chemiluminescent assay. This method enforces the role of LBP as a lipid transfer protein, as demonstrated by Wurfel et al. [177, 178] and shows that CD14 can bind monomeric LPS in the absence of TLR4 [179]. Highly sensitive (LoD = 0.0005 EU) EC sensors have also been developed using a recombinant TLR4 + MD-2 complex for recognition of LPS [180]. Yet, these assays are unable to discriminate between LPS serogroups. Priano et al. [10] developed a competitive EC assay using recombinant endotoxin-neutralizing protein (ENP) on a dextran matrix, with a detection range of 1–100 ng/mL. ENP has also been used in a capacitive biosensor with an extremely low LoD (1.0×10^{-13} M) [181]. The sensitivity differences may be due to variations in surface functionalization. Priano et al. [10] used the dextran matrix, and Limbut et al. [181] used self-assembled monolayers, which provide low background interference [182–184]. Inoue and Takano [10] used a recombinant factor C in an EC hybrid LAL biosensor, with a sensitivity range of 5×10^{-4} –1.0 EU/mL [10]. Kato [87] and Iijima [10] labeled PmB with ferrocene-bound LPS in solution, and captured it on a nanocarbon-film electrode with a detection range of 2–50 ng/mL in 5 minutes [10]. Ding et al. [162] functionalized an electrode with PmB and performed EIS with a detection range of 0.2–0.8 ng/mL which is more sensitive, but has a smaller range. A broader detection range was demonstrated by Rahman et al. [172] who functionalized interdigitated electrodes with PmB and tested 0.1–1000 µg/mL of LPS O111:B4 in food samples, using impedance spectroscopy. Sugar binding proteins, such as lectins and polyaniline coated electrodes, have been used for detecting LPS [10], as with an EIS sensor functionalized with the lectin, cramoLL, with a detection range of 25–200 µg/mL [185].

Several assays have been developed using aptamers as the detection ligand. Su et al. [160, 186, 187] used aptamers attached to gold nanoparticles to detect LPS using EIS, with an impressive detection limit of 0.1 pg/mL [10]. Aptamers have also been used in a magnetic aptasensor to detect LPS in medias containing BSA, sucrose, glucose, or RNA [163], and provide a detection range of 0.01– 1.0×10^6 ng/mL (LPS O55:B5) by flow cytometry within 1 minute. Bai et al. [188] developed an EC sensor where aptamers that bind LPS were hybridized with capture probes, which were hybridized to complementary DNA sequences on gold nanoparticles with a very sensitive range (10 fg/mL up to 50 ng/mL). However, multiple aptamer libraries against O-ag would be essential before this method could be implemented for serogroup discrimination. Modifications to improve sensitivity include use of SAMs to functionalize sensors with peptides [189], PmB [162], antibodies [10], and aptamers [159]. Despite optimal surface capture methods, some of these assays suffer from poor detection limits or range of performance [10, 159].

Investigators have utilized the interaction of LPS with synthetic systems such as copolythiophene copolymers [164] and polydiacetylene liposomes [190, 191]. Johnson et al. [192] demonstrated an endotoxin capture technique by functionalizing a bead matrix with proanthocyanidins and binding with fluorescein isothiocyanate-labeled LPS [192]. Pyrenyl-derived quaternary ammonium probes, developed by Zeng et al. [193] exhibited fluorescence when bound to LPS and detected nanomolar concentrations, while fluorescently labeled CD14 synthetic peptides demonstrated an increase in Förster resonance energy transfer when bound to LPS, but were only able to detect μ M concentrations [194]. Lim et al. [195] used a functionalized graphene oxide to develop a fluorescence quench-recovery method for LPS, targeting the lipid A component. Thompson et al. [196] designed a tandem system to both detect (LoD = 1.0 ng/mL) and filter LPS from blood using piezoelectric quartz discs functionalized with PmB.

Other methods have taken advantage of the amphipathic nature of LPS. Harmon et al. [197] demonstrated that disrupting the hydrophobic association of LPS with liposomes increases the sensitivity of the LAL assay. Stromberg et al. [74, 198] were able to detect 4.20 μ g/mL of amphiphilic LPS O157 in beef lysates on a waveguide biosensor using a technique called membrane insertion, which has previously been applied to other amphiphiles such as LAM and phenolic glycolipids [8, 9, 199]. Membrane insertion uses the natural association of amphiphiles with a lipid bilayer to facilitate detection and fluorescent detection of a labeled antibody is performed within an evanescent field [168, 199]. Many biosensors report exquisite sensitivity, even down to the picogram [164] and femtomolar [9, 168, 199] range, but very few are capable of physiological presentation of amphiphiles to facilitate discriminative detection of O-ag groups [74, 167, 198].

4.4. Cell-based LPS detection systems

Cell systems are ideal for recognizing endotoxin, although interpreting the signal response can be challenging. Bouafsoun et al. [169] functionalized the surface of an impedance biosensor with endothelial cells, and measured the decrease in impedance with LPS binding, with a sensitivity of 500 ng/mL. Veiseh et al. [200] patterned macrophage cells onto gold electrodes to detect LPS concentrations of 0.1–10 μ g/mL. However, cells were concurrently stained with necrosis and apoptosis markers in parallel studies, and no staining effect could be seen in cells using concentrations less than 10 μ g/mL. This is an interesting effect, as in many *in vitro* studies,

cytokine response is induced at much lower concentrations of endotoxin [97, 201, 202]. It can be deduced that Veiseh used serum supplemented media in the experiments, and the lipoproteins and LBP in serum could have a protective effect on cells [46, 202, 203], and attenuated assay sensitivity. The most sensitive cell-based assay was developed by Inoue et al. [170] with a LoD of 0.1–1.0 ng/mL. Here, cells were engineered to secrete alkaline phosphatase in the presence of LPS, and patterned on the surface of an amperometric biosensor to measure voltage change upon LPS binding. Cell-based *in vitro* assays are prone to errors and contamination, so developing a robust and fieldable assay based on this technology is not plausible. However, by studying LPS in cell-based systems, knowledge about interactions with receptors and cell membranes can be gained, which can facilitate better detection methods.

5. Conclusions

Many novel approaches have been used for the detection of amphiphilic LPS, not all of which are functional in physiological matrices or have the required sensitivity or ease of use. One major reason for this is the failure to incorporate the amphiphilic properties of the antigen into assay design. The presentation, conformation, and host-interactions of the antigens should be considered for the development of effective assays. While both LAL and EC assays are the most sensitive for testing endotoxicity, identifying O-ag with a high degree of selectivity remains elusive, and limited to methods that use specific recognition ligands, such as membrane insertion and ELISAs. By far, the greatest limitation has been the lack of sensitive and selective ligands for the serogroup-specific detection of the antigen. Thus, as repositories of these necessary recognition molecules expand to include more serogroups, so too will our ability to selectively detect LPS.

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Author details

Loreen R. Stromberg^{1,2,3}, Heather M. Mendez^{1,2,3} and Harshini Mukundan^{1,2,3*}

*Address all correspondence to: harshini@lanl.gov

1 Center for Biomedical Engineering, University of New Mexico, Albuquerque, USA

2 Physical Chemistry and Applied Spectroscopy, Los Alamos National Laboratory, Los Alamos, USA

3 New Mexico Consortium, Los Alamos, USA

References

- [1] Love TE, Jones B. Introduction to pathogenic bacteria. Principles of Bacterial Detection: Biosensors, Recognition Receptors and Microsystems. New York, NY: Springer New York; 2008. pp. 3-13
- [2] Kumagai Y, Takeuchi O, Akira S. Pathogen recognition by innate receptors. The Journal of Infectious Diseases. 2008;**14**:86-92
- [3] Broz P, Monack DM. Newly described pattern recognition receptors team up against intracellular pathogens. Nature Reviews Immunology. 2013;**13**:551-565
- [4] Miyake K. Innate recognition of lipopolysaccharide by Toll-like receptor 4-MD-2. Trends in Microbiology. 2004;**12**:186-192
- [5] Deisingh AK, Thompson M. Biosensors for the detection of bacteria. Canadian Journal of Microbiology. 2004;**50**:69-77
- [6] Kawai T, Akira S. TLR signaling. Seminars in Immunology. 2007;**19**:24-32
- [7] Kumar H, Kawai T, Akira S. Pathogen recognition by the innate immune system. International Reviews of Immunology. 2011;**30**:16-34
- [8] Sakamuri RM, Price DN, Lee M, Cho SN, Barry CE, Via LE, et al. Association of lipoarabinomannan with high density lipoprotein in blood: Implications for diagnostics. Tuberculosis. 2013;**93**:301-307
- [9] Sakamuri RM, Capek P, Dickerson TJ, Barry CE, Mukundan H, Swanson BI. Detection of stealthy small amphiphilic biomarkers. Journal of Microbiological Methods. 2014;**103**: 112-117
- [10] Su W, Ding X. Methods of endotoxin detection. Journal of Laboratory Automation. 2015;**20**: 354-364
- [11] Alexander C, Rietschel ET. Bacterial lipopolysaccharides and innate immunity. Journal of Endotoxin Research. 2001;**7**:167-202
- [12] Jauho ES, Boas U, Wiuff C, Wredstrøm K, Pedersen B, Andresen LO, et al. New technology for regiospecific covalent coupling of polysaccharide antigens in ELISA for serological detection. Journal of Immunological Methods. 2000;**242**:133-143
- [13] Munford RS. Sensing Gram-Negative bacterial lipopolysaccharides: A human disease determinant? Infection and Immunity. 2008;**76**:454-465
- [14] Schreiber A, Humbert M, Benz A, Dietrich U. 3D-Epitope-Explorer (3DEX): Localization of conformational epitopes within three-dimensional structures of proteins. Journal of Computational Chemistry. 2005;**26**:879-887
- [15] Beveridge TJ. Structures of Gram-negative cell walls and their derived membrane vesicles. Journal of Bacteriology. 1999;**181**:4725-4733

- [16] Joly-Guillou M-L. Clinical impact and pathogenicity of *Acinetobacter*. *Clinical Microbiology and Infection, European Journal of Clinical Microbiology & Infectious Diseases*. 2005;**11**:868-873
- [17] Wiersinga WJ, van der Poll T, White NJ, Day NP, Peacock SJ. Melioidosis: Insights into the pathogenicity of *Burkholderia pseudomallei*. *Nature Reviews Microbiology*. 2006;**4**:272-282
- [18] Mattoo S, Foreman-Wykert AK, Cotter PA. Mechanisms of *Bordetella* pathogenesis. *Frontiers in Bioscience*. 2001;**6**:E168–E186
- [19] Brooks BW, Devenish J, Lutze-Wallace CL, Milnes D, Robertson, RH, Berlie-Surujballi G. Evaluation of a monoclonal antibody-based enzyme-linked immunosorbent assay for detection of *Campylobacter fetus* in bovine preputial washing and vaginal mucus samples. *Veterinary Microbiology*. 2004;**103**:77-84
- [20] Lazcka O, Del Campo FJ, Muñoz FX. Pathogen detection: A perspective of traditional methods and biosensors. *Biosensors & Bioelectronics*. 2007;**22**:1205-1217
- [21] Ketley JM. Pathogenesis of enteric infection by *Campylobacter*. *Microbiology*. 1997;**143**:5-21
- [22] Lüderitz O, Freudenberg MA, Galanos C, Lehmann V, Rietschel ET, Shaw DH. Lipopolysaccharides of Gram-Negative Bacteria. *Current Topics in Membranes and Transport*. Elsevier; 1982. pp. 79-151
- [23] Belland RJ, Ouellette SP, Gieffers J, Byrne GI. *Chlamydia pneumoniae* and atherosclerosis. *Cell Microbiology*. 2004;**6**:117-127
- [24] Leimbach A, Hacker J, Dobrindt U. *E. coli* as an All-Rounder: The Thin Line Between Commensalism and Pathogenicity. Berlin, Heidelberg: Springer; microbiology and immunology. 2013;**358**:3-32
- [25] Moran AP, Lindner B, Walsh EJ. Structural characterization of the lipid A component of *Helicobacter pylori* rough- and smooth-form lipopolysaccharides. *Journal of Bacteriology*. 1997;**179**:6453-6463
- [26] Yamaoka Y, Graham DY. *Helicobacter pylori* virulence and cancer pathogenesis. *Future Oncology*. 2014;**10**:1487-1500
- [27] Livorsi DJ, Macneil JR, Cohn AC, Baretta J, Zansky S, Petit S, et al. Invasive *Haemophilus influenzae* in the United States, 1999-2008: Epidemiology and outcomes. *Journal of Infection*. Elsevier Ltd. 2012;**65**:496-504
- [28] Meatherall BL, Gregson D, Ross T, Pitout JDD, Laupland KB. Incidence, risk factors, and outcomes of *Klebsiella pneumoniae* bacteremia. *American Journal of Medicine*. 2009;**122**:866-873
- [29] Newton HJ, Ang DKY, van Driel IR, Hartland EL. Molecular pathogenesis of infections caused by *Legionella pneumophila*. *Clinical Microbiology Reviews*. 2010;**23**:274-298
- [30] Laura Perez Vidakovics M, Riesbeck K. Virulence mechanisms of *Moraxella* in the pathogenesis of infection. *Current Opinion in Infectious Diseases*. 2009;**22**:279-285

- [31] Stephens DS. Biology and pathogenesis of the evolutionarily successful, obligate human bacterium *Neisseria meningitidis*. *Vaccine*. 2009;**27**:B71–B77
- [32] Mahajan-Miklos S, Tan MW, Rahme LG, Ausubel FM. Molecular mechanisms of bacterial virulence elucidated using a *Pseudomonas aeruginosa*-*Caenorhabditis elegans* pathogenesis model. *Cell*. 1999;**96**:47-56
- [33] Mobley HL, Belas R. Swarming and pathogenicity of *Proteus mirabilis* in the urinary tract. *Trends Microbiology*. 1995;**3**:280-284
- [34] Mather AE, Reid S, Maskell DJ, Parkhill J, Fookes MC, Harris S, et al. Distinguishable epidemics within different hosts of the multidrug resistant zoonotic pathogen *Salmonella Typhimurium* DT104. *Science*. 2013;**341**(6153):1514
- [35] Caboni M, Pédrón T, Rossi O, Goulding D, Pickard D, Citiulo F, et al. An O antigen capsule modulates bacterial pathogenesis in *Shigella sonnei*. Koehler TM, editor. *PLoS Pathogens*. 2015;**11**:e1004749
- [36] Fábrega A, Vila J. *Yersinia enterocolitica*: Pathogenesis, virulence and antimicrobial resistance. *Medicina Clinica. SEGO*. 2012;**30**:24-32
- [37] Bennett IL, Cluff LE. Bacterial pyrogens. *Pharmacological Reviews*. 1957;**9**:427-475
- [38] Westphal O, Westphal U. The history of pyrogen research. *Microbiology*. 1977;**1977**: 221-238
- [39] Beutler B, Rietschel ET. Innate immune sensing and its roots: The story of endotoxin. *Nature Reviews Immunology*. 2003;**3**:169-176
- [40] Olins AL, Warner RC. Physicochemical studies on a lipopolysaccharide from the cell wall of *Azotobacter vinelandii*. *Journal of Biological Chemistry*. 1967;**242**(21):4994-5001
- [41] Schromm AB, Bradenburg K, Loppnow H, Zähringer U, Rietschel ET, Carroll SF, et al. The charge of endotoxin molecules influences their conformation and IL-6-inducing capacity. *Journal of Immunology*. 1998;**161**:5464-5471
- [42] Bishop RE. Fundamentals of endotoxin structure and function. In: Herwald RW, editor. *Contributions to Microbiology*. Basel: Karger; 2005. pp. 1-27
- [43] Meredith TC, Aggarwal P, Mamat U, Lindner B, Woodard RW. Redefining the requisite lipopolysaccharide structure in *Escherichia coli*. *ACS Chemical Biology*. 2006;**1**:33-42
- [44] Raetz CRH, Guan Z, Ingram BO, Six DA, Song F, Wang X, et al. Discovery of new biosynthetic pathways: The lipid A story. *Journal of Lipid Research*. 2009;**50**(Suppl):S103-S108
- [45] Raetz CRH, Whitfield C. Lipopolysaccharide endotoxins. *Annual Review of Biochemistry*. 2002;**71**:635-700
- [46] Hurley JC. Endotoxemia: Methods of detection and clinical correlates. *Clinical Microbiology Reviews*. 1995;**8**:268-292

- [47] Erridge C, Bennett-Guerrero E, Poxton IR. Structure and function of lipopolysaccharides. *Microbes and Infection*. 2002;**4**:837-851
- [48] Aurell CA, Wistrom AO. Critical aggregation concentrations of Gram-negative bacterial lipopolysaccharides (LPS). *Biochemical and Biophysical Research Communications*. 1998;**253**:119-123
- [49] Plested JS, Makepeace K, Jennings MP, Gidney MA, Lacelle S, Brisson J, et al. Conservation and accessibility of an inner core lipopolysaccharide epitope of *Neisseria meningitidis*. *Infection and Immunity*. 1999;**67**:5417-5426
- [50] Silipo A, Molinaro A. The diversity of the core oligosaccharide in lipopolysaccharides. *Subcellular Biochemistry*. Dordrecht: Springer Netherlands. 2010;**53**:69-99
- [51] Nevola JJ, Stocker BA, Laux DC, Cohen PS. Colonization of the mouse intestine by an avirulent *Salmonella typhimurium* strain and its lipopolysaccharide-defective mutants. *Infection and Immunity*. 1985;**50**:152-159
- [52] Murray GL, Attridge SR, Morona R. Regulation of *Salmonella typhimurium* lipopolysaccharide O antigen chain length is required for virulence; identification of FepE as a second Wzz. *Molecular Microbiology*. 2003;**47**:1395-1406
- [53] De Bagüés M, Elzer PH, Jones SM. Vaccination with *Brucella abortus* rough mutant RB51 protects BALB/c mice against virulent strains of *Brucella abortus*, *Brucella melitensis*, and *Brucella ovis*. *Infection and Immunity*. 1994;**62**(11):4990-4996
- [54] Stenutz R, Weintraub A, Widmalm G. The structures of *Escherichia coli* O-polysaccharide antigens. *FEMS Microbiology Reviews*. 2006;**30**:382-403
- [55] Goldman RC, Leive L. Heterogeneity of antigenic-side-chain length in lipopolysaccharide from *Escherichia coli* 0111 and *Salmonella typhimurium* LT2. *European Journal of Biochemistry*. 1980;**107**:145-153
- [56] Bradenburg K, Schromm AB, Gutschmann T. Endotoxins: Relationship between structure, function, and activity. *Structure*. Dordrecht: Springer Netherlands. 2004;**53**:53-67
- [57] Bergstrand A, Svanberg C, Langton M, Nydén M. Aggregation behavior and size of lipopolysaccharide from *Escherichia coli* O55:B5. *Colloids and Surfaces B: Biointerfaces*. 2006;**53**:9-14
- [58] Santos NC, Silva AC, Castanho MARB, Martins Silva J, Saldanha C. Evaluation of lipopolysaccharide aggregation by light scattering spectroscopy. *ChemBioChem*. 2003;**4**:96-100
- [59] Israelachvili J. *Intermolecular and Surface Forces*. 2nd ed. London: Academic Press; 1991
- [60] Ruckenstein E, Nagarajan R. Critical micelle concentration. Transition point for micellar size distribution. *Journal of Physical Chemistry (American Chemical Society)*. 1975;**79**:2622-2626

- [61] Din ZZ, Mukerjee P, Kastowsky M, Takayama K. Effect of pH on solubility and ionic state of lipopolysaccharide obtained from the deep rough mutant of *Escherichia coli*. *Biochemistry*. 1993;**32**:4579-4586
- [62] Sasaki H, White SH. Aggregation behavior of an Ultra-Pure lipopolysaccharide that stimulates TLR-4 receptors. *Biophysical Journal*. 2008;**95**:986-993
- [63] Moroi Y. CMC range due to polydispersity of micelles. *Journal of Colloid and Interface Science*. 1991;**141**:581-583
- [64] Krüger D. Assessing the quality of medicinal products containing ingredients obtained by gene technology. *Drugs Made in Germany*. 1989;**32**(2):64-67
- [65] Hirayama C, Sakata M. Chromatographic removal of endotoxin from protein solutions by polymer particles. *Journal of Chromatography B Analytical Technologies Biomedical and Life Sciences*. 2002;**781**:419-432
- [66] Netea MG, van Deuren M, Kullberg BJ, Cavaillon J-M, Van der Meer JWM. Does the shape of lipid A determine the interaction of LPS with Toll-like receptors? *Trends in Immunology*. 2002;**23**:135-139
- [67] Seydel U, Schromm AB, Blunck R, Bradenburg K. Chemical structure, molecular conformation, and bioactivity of endotoxins. *Chemical Immunology*. 2000;**74**:5-24
- [68] Nagarajan R. Molecular packing parameter and surfactant Self-Assembly: The neglected role of the surfactant tail †. *Langmuir*. 2002;**18**:31-38
- [69] Rietschel ET, Brade H, Brade L, Kaca W, Kawahara K, Lindner B, et al. Newer aspects of the chemical structure and biological activity of bacterial endotoxins. *Progress in Clinical and Biological Research*. 1985;**189**:31-51
- [70] Jucker BA, Harms H, Zehnder A. Polymer interactions between five gram-negative bacteria and glass investigated using LPS micelles and vesicles as model systems. *Colloids and Surfaces B: Biointerfaces*. 1998;**11**:33-45
- [71] Petsch D, Anspach FB. Endotoxin removal from protein solutions. *Journal of Biotechnology*. 2000;**76**:97-119
- [72] Mueller M, Lindner B, Kusumoto S, Fukase K, Schromm AB, Seydel U. Aggregates are the biologically active units of endotoxin. *Journal of Biological Chemistry*. 2004;**279**:26307-26313
- [73] Vasselon T, Hailman E, Thieringer R, Detmers PA. Internalization of monomeric lipopolysaccharide occurs after transfer out of cell surface CD14. *Journal of Experimental Medicine*. 1999;**190**:509-521
- [74] Stromberg LR, Hengartner NW, Swingle KL, Moxley RA, Graves SW, Montañó GA, et al. Membrane insertion for the detection of lipopolysaccharides: Exploring the dynamics of Amphiphile-in-Lipid Assays. Gasset M, editor. *PLoS ONE*. 2016;**11**:e0156295
- [75] James EA, Schmeltzer K, Ligler FS. Detection of endotoxin using an evanescent wave fiber-optic biosensor. *Applied Biochemistry and Biotechnology*. 1996;**60**:189-202

- [76] Kannenberg EL, Perotto S, Bianciotto V, Rathbun EA, Brewin NJ. Lipopolysaccharide epitope expression of *Rhizobium* bacteroids as revealed by *in situ* immunolabelling of pea root nodule sections. *Journal of Bacteriology*. 1994;**176**:2021-2032
- [77] Haselhorst T, Espinosa J-F, Jiménez-Barbero J, Sokolowski T, Kosma P, Brade H, et al. NMR experiments reveal distinct Antibody-Bound conformations of a synthetic disaccharide representing a general structural element of bacterial lipopolysaccharide epitopes. *Biochemistry*. 1999;**38**:6449-6459
- [78] Hershberger C, Binkley SB. Chemistry and Metabolism of 3-Deoxy-d-mannoctulosonic acid I. Stereochemical determination. *Journal of Biological Chemistry*. 1968;**243**(7): 1578-1584
- [79] Brandenburg K, Andrä J, Müller M, Koch MHJ, Garidel P. Physicochemical properties of bacterial glycopolymers in relation to bioactivity. *Carbohydrate Research*. 2003;**338**:2477-2489
- [80] Aurell CA, Hawley ME, Wistrom AO. Direct visualization of Gram-negative bacterial lipopolysaccharide self-assembly. *Molecular Cell Biology Research Communications*. 1999;**2**:42-46
- [81] Adams PG, Lamoureux L, Swingle KL, Mukundan H, Montañó GA. Lipopolysaccharide-induced dynamic lipid membrane reorganization: Tubules, perforations, and stacks. *Biophysical Journal*. 2014;**106**:2395-2407
- [82] van Alphen L, Verkleij A, Burnell E, Lugtenberg B. ³¹P nuclear magnetic resonance and freeze-fracture electron microscopy studies on *Escherichia coli*. II. Lipopolysaccharide and lipopolysaccharide-phospholipid complexes. *Biochimica et Biophysica Acta (BBA) - Biomembranes*. 1980;**597**:502-517
- [83] Coughlin RT, Haug A, McGroarty EJ. Physical properties of defined lipopolysaccharide salts. *Biochemistry*. 1983;**22**:2007-2013
- [84] Coughlin RT, Tonsager S, McGroarty EJ. Quantitation of metal cations bound to membranes and extracted lipopolysaccharide of *Escherichia coli*. *Biochemistry*. 1983;**22**: 2002-2007
- [85] Naumann D, Schultz C, Sabisch A, Kastowsky M, Labischinski H. New insights into the phase behaviour of a complex anionic amphiphile: Architecture and dynamics of bacterial deep rough lipopolysaccharide membranes as seen by FTIR, X-ray, and molecular modelling techniques. *Journal of Molecular Structure*. 1989;**214**:213-246
- [86] Schindler M, Osborn MJ. Interaction of divalent cations and polymyxin B with lipopolysaccharide. *Biochemistry*. 1979;**18**:4425-4430
- [87] Kato D, Iijima S, Kurita R, Sato Y, Jia J, Yabuki S, et al. Electrochemically amplified detection for lipopolysaccharide using ferrocenylboronic acid. *Biosensors & Bioelectronics*. 2007;**22**:1527-1531
- [88] McClosky WT, Price CW, Van Winkle W, Welch H, Calvery HO. Results of first U. S. P. Collaborative study of pyrogens. *Journal of the American Pharmaceutical Association (Scientific edition)*. 1943;**32**:69-73

- [89] Cooper JF, Levin J, Wagner HN. Quantitative comparison of *in vitro* and *in vivo* methods for the detection of endotoxin. *Journal of Laboratory and Clinical Medicine*. 1971;**78**:138-148
- [90] Roberts KJ. Endotoxins: Pyrogens, LAL testing and depyrogenation. Williams KL, editor. CRC Press, Taylor & Fancis Group LLC; 2007. pp. 261-284
- [91] Park C-Y, Jung S-H, Bak J-P, Lee S-S, Rhee D-K. Comparison of the rabbit pyrogen test and Limulus amoebocyte lysate (LAL) assay for endotoxin in hepatitis B vaccines and the effect of aluminum hydroxide. *Biologicals*. 2005;**33**:145-151
- [92] Levin J, Tomasulo PA, Oser RS. Detection of endotoxin in human blood and demonstration of an inhibitor. *Journal of Laboratory and Clinical Medicine*. 1970;**75**:903-911
- [93] Pharmacopeial Convention US. 85> Bacterial Endotoxins Test. 2011. The United States Pharmacopeial Convention (P) \ \usp-netapp2\share\SHARE\USPNF\PRINTQ\pager\xmlIn\AO_20111014080832_M98830.xml
- [94] U.S. Department of Health and Human Services, U.S. Food and Drug Administration. Guidance for Industry. 21 CFR; Dec 8, 1987 pp. 1-54.
- [95] Novitsky TJ, Roslansky PF. Quantification of endotoxin inhibition in serum and plasma using a turbidimetric LAL assay. *Progress in Clinical and Biological Research*. 1985;**189**:181-196
- [96] Levin J, Poore TE, Zauber NP, Zauber NP, Oser RS. Detection of endotoxin in the blood of patients with sepsis due to Gram-negative bacteria. *The New England Journal of Medicine*. 1970;**283**:1313-1316
- [97] Cavaillon JM, Fitting C, Haeffner-Cavaillon N, Kirsch SJ, Warren HS. Cytokine response by monocytes and macrophages to free and lipoprotein-bound lipopolysaccharide. *Infection and Immunity*. 1990;**58**:2375-2382
- [98] Nachum R, Shanbrom E. Rapid detection of Gram-negative bacteriuria by limulus amoebocyte lysate assay. *Journal of Clinical Microbiology*. 1981;**13**:158-162
- [99] Svensson A, Hahn-Hägerdal B. Comparison of a gelation and a chromogenic Limulus (LAL) assay for the detection of Gram-negative bacteria, and the application of the latter assay to milk. *Journal of Dairy Research*. 1987;**54**:267-273
- [100] Jay JM, Margitic S, Shereda AL, Covington HV. Determining endotoxin content of ground beef by the Limulus amoebocyte lysate test as a rapid indicator of microbial quality. *Applied and Environmental Microbiology*. 1979;**38**:885-890
- [101] Jay JM. Rapid estimation of microbial numbers in fresh ground beef by use of the Limulus test. *Journal of Food Protection*. 1981;**44**:275-278
- [102] Goto H, Nakamura S. Dry up method as a revised Limulus test with a new technique for gelatin inhibitor removing. *The Japanese Journal of Experimental Medicine*. 1979;**49**:19-25

- [103] Hartman I, Ziv G, Saran A. Application of the *Limulus* amoebocyte lysate test to the detection of Gram-negative bacterial endotoxins in normal and mastitic milk. *Research in Veterinary Science*. 1976;**20**:342-343
- [104] Reinhold RB, Fine J. A technique for quantitative measurement of endotoxin in human plasma. *Proceedings of the Society for Experimental Biology and Medicine*. 1971. 334-340
- [105] Maxie MG, Valli VE. Studies with radioactive endotoxin. III. Localization of ³H-labelled endotoxin in the formed elements of the blood and detection of endotoxin in calf blood with the *Limulus* amoebocyte lysate. *Canadian Journal of Comparative Medicine-Revue Canadienne De Medecine Comparee*. 1974;**38**:383-390
- [106] Yin ET, Galanos C, Kinsky S, Bradshaw RA, Wessler S, Lüderitz O, et al. Picogram-sensitive assay for endotoxin: Gelation of *Limulus polyphemus* blood cell lysate induced by purified lipopolysaccharides and lipid A from Gram-negative bacteria. *Biochimica et Biophysica Acta*. 1972;**261**:284-289
- [107] Takagi K, Moriya A, Tamura H, Nakahara C. Quantitative measurement of endotoxin in human blood using synthetic chromogenic substrate for horseshoe crab clotting enzyme: A comparison of methods of blood. *Thrombosis Research*. 1981;**23**:51-57
- [108] Niwa M, Hiramatsu T, Waguri O. Proceedings: Quantitative aspects of the gelation reaction of horseshoe crab amoebocyte and bacterial endotoxin. *Symp. Toxins. jpn j of medical sci biology*. 1974;**28**:98-100
- [109] Tsuji K, Martin PA, Gaunnac GL. Recovery of endotoxin from human plasma by acid oxidative treatments as monitored by an automated microtiter plate-chromogenic substrate *limulus* amoebocyte lysate (LAL) assay method. *Progress in Clinical and Biological Research*. 1986;**231**: 443-457
- [110] Karibian D, Deprun C, Caroff M. Comparison of lipids A of several *Salmonella* and *Escherichia* strains by 252Cf plasma desorption mass spectrometry. *Journal of Bacteriology*. 1993;**175**: 2988-2993
- [111] Schletter J, Heine H, Ulmer AJ, Rietschel ET. Molecular mechanisms of endotoxin activity. *Archives of Microbiology*. 1995;**164**:383-389
- [112] Engvall E, Perlmann P. Enzyme-linked immunosorbent assay, ELISA III: Quantitation of specific antibodies by enzyme-labeled anti-immunoglobulin in antigen-coated tubes. *Journal of Immunology*. 1972;**109**:129-135
- [113] Van Weemen BK, Schuurs AHWM. Immunoassay using antigen-enzyme conjugates. *FEBS Letters*. 1971;**15**:232-236
- [114] Carlsson HE, Lindberg AA, Hammarström S. Titration of antibodies to *Salmonella* O antigens by enzyme-linked immunosorbent assay. *Infection and Immunity*. 1972;**6**:703-708
- [115] Nielsen B, Baggesen D, Bager F, Haugegaard J, Lind P. The serological response to *Salmonella* serovars typhimurium and infantis in experimentally infected pigs. The time course followed with an indirect anti-LPS ELISA and bacteriological examinations. *Veterinary Microbiology*. 1995;**47**: 205-218

- [116] Nalepka JL, Greenfield EM. Detection of bacterial endotoxin in human tissues. *Biotechniques*. 2004;**37**:413-417
- [117] Reither K, Saathoff E, Jung J, Minja LT, Kroidl I, Saad E, et al. Low sensitivity of a urine LAM-ELISA in the diagnosis of pulmonary tuberculosis. *BMC Infectious Diseases*. 2009;**9**:141
- [118] Takahashi K, Fukada M, Kawai M, Yokochi T. Detection of lipopolysaccharide (LPS) and identification of its serotype by an enzyme-linked immunosorbent assay (ELISA) using poly-l-lysine. *Journal of Immunological Methods*. 1992;**153**:67-71
- [119] Scott BB, Barclay GR. Endotoxin-polymyxin complexes in an improved enzyme-linked immunosorbent assay for IgG antibodies in blood donor sera to Gram-Negative endotoxin core glycolipids. *Vox Sanguinis*. 1987;**52**:272-280
- [120] Sternberger LA. The unlabeled antibody (PAP) method. *Journal of Histochemistry & Cytochemistry*. 1979;**27**:1657-1657
- [121] Hsu SM, Raine L, Fanger H. The use of antiavidin antibody and avidin-biotin-peroxidase complex in immunoperoxidase technics. *American Journal of Clinical Pathology*. 1981;**75**:816-821
- [122] Mackenzie A, Lebel P, Orrbine E, Rowe PC, Hyde L, Chan F, et al. Sensitivities and Specificities of premier *E. coli* O157 and premier EHEC enzyme immunoassays for diagnosis of infection with verotoxin (Shiga-Like Toxin)-producing *Escherichia coli*. *Journal of Clinical Microbiology*. 1998;**36**:1608-1611
- [123] Vreugdenhil AC, Snoek AM, van 't Veer C, Greve JW, Buurman WA. LPS-binding protein circulates in association with apoB-containing lipoproteins and enhances endotoxin-LDL/VLDL interaction. *Journal of Clinical Investigation*. 2001;**107**:225-234
- [124] Vreugdenhil A, Rousseau CH. Lipopolysaccharide (LPS)-binding protein mediates LPS detoxification by chylomicrons. *Journal of Immunology*. 2003;**170**:1399-1405
- [125] Kerr P, Chart H, Finlay D, Pollock DA, MacKie DP, Ball HJ. Development of a monoclonal sandwich ELISA for the detection of animal and human *Escherichia coli* O157 strains. *Journal of Applied Microbiology*. 2001;**90**:543-549
- [126] Choi D, Tsang RS, Ng MH. Sandwich capture ELISA by a murine monoclonal antibody against a genus-specific LPS epitope for the detection of different common serotypes of salmonellas. *Journal of Applied Bacteriology*. 1992;**72**:134-138
- [127] Schütz M, Meyer R, Grallert H, Miller S. Method for Detecting and for Removing Endotoxin [Internet]. 2010. US Patent Office; US20090017445 A1
- [128] Schütz M, Meyer R, Grallert H, Miller S. Method for identifying and for extracting endotoxin. 2009. US Patent Office; 7,585,620
- [129] Grallert H, Leopoldseder S, Schuett M, Kurze P, Buchberger B. EndoLISA®: A novel and reliable method for endotoxin detection. *Nature Reviews Immunology*. Nature Publishing Group. 2011;**8**:iii-v

- [130] Alberts B. *Molecular Biology of the Cell*. 5th ed. Garland Science; 2008. pp. 1539-1557
- [131] Hyams KC, Malone JD, Bourgeois AL, Hawkins R, Hale TL, Murphy JR. Serum antibody to lipopolysaccharide antigens of *Shigella* species among U.S. military personnel deployed to Saudi Arabia and Kuwait during Operations Desert Shield and Desert Storm. *Clinical and Diagnostic Laboratory Immunology*. 1995;2:700-703
- [132] Blatz R, Aseffa A, Gedefaw M, Rühle HJ, Forberg J, Christian AR. Prevalence of Chlamydia specific antibodies among obstetric and gynecological outpatients in Gondar, North-west Ethiopia. *Ethiopia Medical Journal*. 2001;39:293-303
- [133] House D, Wain J, Ho VA, Diep TS, Chinh NT, Bay PV, et al. Serology of typhoid fever in an area of endemicity and its relevance to diagnosis. *Journal of Clinical Microbiology*. 2001;39:1002-1007
- [134] Chaicumpa W, Ngren-ngarmert W, Kalambaheti T, Ruangkunaporn Y, Chongsanguan M, Tapchaisri P, et al. Monoclonal antibody-based dot-blot ELISA for the detection of *Salmonella* in foods. *Asian Pacific Journal of Allergy and Immunology*. 1995;13:159-166
- [135] Freudenberg MA, Fomsgaard A, Mitov I, Galanos C. ELISA for antibodies to lipid A, lipopolysaccharides and other hydrophobic antigens. *Infection*. 1989;17:322-328
- [136] Suthienkul O, Poomchart A, Kositanont U, Siripanichgon K, Vathanophas K. ELISA for seroepidemiological study of exposure to *Vibrio cholerae* of population in Krabi Province, Thailand. *Asian Pacific Journal of Allergy and Immunology*. 1992;10:55-60
- [137] Chaicumpa W, Ruangkunaporn Y, Burr D, Chongsanguan M, Echeverria P. Diagnosis of typhoid fever by detection of *S typhi* antigen in urine. *Journal of Clinical Microbiology*. 1992;30:2513-2515
- [138] Covinsky M, Laterza O, Pfeifer JD, Farkas-Szallasi T, Scott MG. An IgM lambda antibody to *Escherichia coli* produces false-positive results in multiple immunometric assays. *Clinical Chemistry*. 2000;46:1157-1161
- [139] Ben-Hur H, Gurevich P, Elhayany A, Avinoach I, Schneider D, Zusman I. Transport of maternal immunoglobulins through the human placental barrier in normal pregnancy and during inflammation. *International Journal of Molecular Medicine*. 2005;16:401-407
- [140] Morrison DC, Jacobs DM. Binding of polymyxin B to the lipid A portion of bacterial lipopolysaccharides. *Immunochemistry*. 1976;13:813-818
- [141] Mattsby-Baltzer I, Jakobsson A, Sörbo J. Endotoxin is angiogenic. *International Journal of Experimental Pathology*. 1994;75:191-196
- [142] Hardy E, Ohlin M, Llano M. Enhanced ELISA sensitivity using TCA for efficient coating of biologically active lipopolysaccharides or lipid A to the solid phase. *Journal of Immunological Methods*. 1994;176:111-116

- [143] Weintraub A, Widmalm G, Jansson PE, Jansson M. *Vibrio cholerae* O139 Bengal possesses a capsular polysaccharide which may confer increased virulence. *Microbial Pathogenesis*. 1994;**16**:235-241
- [144] Stromberg LR, Stromberg ZR, Banisadr A, Graves SW, Moxley RA, Mukundan H. Purification and characterization of lipopolysaccharides from six strains of non-O157 Shiga toxin-producing *Escherichia coli*. *Journal of Microbiological Methods*. 2015;**116**:1-7
- [145] Brooks BW, Lutze-wallace CL, Blais B, Gauthier M, Deschênes M. Monoclonal antibodies to lipopolysaccharide O antigens of enterohemorrhagic *Escherichia coli* strains in serogroups O26, O45, O103, O111, O121, and O145. *Journal of Food Protection*. 2015;**78**:1252-1258
- [146] Westerman RB, He Y, Keen JE, Littledike ET, Kwang J. Production and characterization of monoclonal antibodies specific for the lipopolysaccharide of *Escherichia coli* O157. *Journal of Clinical Microbiology*. 1997;**35**:679-684
- [147] Coughlin RT, Bogard WC. Immunoprotective murine monoclonal antibodies specific for the outer-core polysaccharide and for the O-antigen of *Escherichia coli* O111: B4 lipopolysaccharide (LPS). *Journal of Immunology*. 1987;**139**(2):557-561
- [148] Rivera-Betancourt M, Keen JE. Murine monoclonal antibodies against *Escherichia coli* O4 lipopolysaccharide and H5 flagellin. *Journal of Clinical Microbiology*. 2001;**39**:3409-3413
- [149] Mutharia LM, Crockford G, Bogard WC, Hancock RE. Monoclonal antibodies specific for *Escherichia coli* J5 lipopolysaccharide: Cross-reaction with other Gram-negative bacterial species. *Infection and Immunity*. 1984;**45**:631-636
- [150] Bogard WCW, Dunn DLD, Abernethy KK, Kilgariff CC, Kung PCP. Isolation and characterization of murine monoclonal antibodies specific for gram-negative bacterial lipopolysaccharide: Association of cross-genus reactivity with lipid A specificity. *Infection and Immunity*. 1987;**55**:899-908
- [151] de Jongh-Leuvenink J, Vreede RW, Marcelis JH, de Vos M, Verhoef J. Detection of antibodies against lipopolysaccharides of *Escherichia coli* and *Salmonella* R and S strains by immunoblotting. *Infection and Immunity*. 1985;**50**:716-720
- [152] Gibb AP, Barclay GR, Poxton IR, di Padova F. Frequencies of lipopolysaccharide core types among clinical isolates of *Escherichia coli* defined with monoclonal antibodies. *Journal of Infectious Diseases*. 1992;**166**:1051-1057
- [153] Porat YB, Zan-Bar I, Ravid A. Quantitative dot-blot assay for low titer anti-lipopolysaccharide antibodies in human plasma. *Journal of Immunological Methods*. 1995;**180**:213-218
- [154] Evans ME, Pollack M, Hardegen NJ, Koles NL, Guelde G, Chia JK. Fluorescence-activated cell sorter analysis of binding by lipopolysaccharide-specific monoclonal antibodies to Gram-negative bacteria. *Journal of Infectious Diseases*. 1990;**162**:148-155

- [155] Evans ME, Pollack M, Koles NL, Hardegen NJ, Panopoulos D. Lipopolysaccharide heterogeneity in *Escherichia coli* J5 variants: Analysis by flow cytometry. *Journal of Infectious Diseases*. 1992;**166**:803-811
- [156] Nelson D. Flow cytometry in bacteriology. *Reviews in Medical Microbiology*. 1993;**4**: 215-221
- [157] Péterfi Z, Kocsis B. Comparison of blocking agents for an elisa for Lps. *Journal of Immunoassay*. 2000;**21**:341-354
- [158] Gorbet MB, Sefton MV. Endotoxin: The uninvited guest. *Biomaterials*. 2005;**26**:6811-6817
- [159] Kim S-E, Su W, Cho M, Lee Y, Choe W-S. Harnessing aptamers for electrochemical detection of endotoxin. *Analytical Biochemistry*. Elsevier Inc. 2012;**424**:12-20
- [160] Su W, Lin M, Lee H, Cho M, Choe W-S, Lee Y. Determination of endotoxin through an aptamer-based impedance biosensor. *Biosensors & Bioelectronics*. Elsevier B.V. 2012;**32**:32-36
- [161] Cho M, Chun L, Lin M, Choe W, Nam J, Lee Y. Sensitive electrochemical sensor for detection of lipopolysaccharide on metal complex immobilized gold electrode. *Sensors and Actuators B: Chemical*. Elsevier B.V. 2012;**174**:490-494
- [162] Ding SJ, Chang BW, Wu CC, Chen CJ. A new method for detection of endotoxin on polymyxin B-immobilized gold electrodes. *Electrochemistry Communications*. 2007;**9**: 1206-1211
- [163] Zuo M-Y, Chen L-J, Jiang H, Tan L, Luo Z-F, Wang Y-M. Detecting endotoxin with a flow cytometry-based magnetic aptasensor. *Analytical Biochemistry*. 2014;**466**:38-43
- [164] Lan M, Wu J, Liu W, Zhang W, Ge J, Zhang H, et al. Copolythiophene-derived colorimetric and fluorometric sensor for visually supersensitive determination of lipopolysaccharide. *Journal of the American Chemical Society*. 2012;**134**:6685-6694
- [165] Mohammed AH, McCallus DE, Norcross NL. Development and evaluation of an enzyme-linked immunosorbent assay for endotoxin in milk. *Veterinary Microbiology*. 1988;**18**:27-39
- [166] DeMarco DR, Lim DV. Detection of *Escherichia coli* O157: H7 in 10-and 25-gram ground beef samples with an evanescent-wave biosensor with silica and polystyrene waveguides. *Journal of Food Protection*. 2002;**65**:596-602
- [167] Lamoureux L, Adams P, Banisadr A. An optical biosensor for detection of pathogen biomarkers from Shiga toxin-producing *Escherichia coli* in ground beef samples. *Proceedings of SPIE*. 2015; 931004
- [168] Mukundan H, Price DN, Goertz M, Parthasarathi R, Montañó GA, Kumar S, et al. Understanding the interaction of Lipoarabinomannan with membrane mimetic architectures. *Tuberculosis*. Elsevier Ltd; 2012;**92**:38-47

- [169] Bouafsoun A, Othmane A, Jaffrezic-Renault N. Impedance endothelial cell biosensor for lipopolysaccharide detection. *Materials Science and Engineering: C*. 2008;**28**:653-661
- [170] Inoue KY, Yasukawa T, Shiku H, Matsue T. Cell-based electrochemical assay for endotoxin using a secreted alkaline phosphatase reporter system. *Electrochemistry*. 2008;**76**:525-528
- [171] Unger RE, Peters K, Sartoris A, Freese C, Kirkpatrick CJ. Human endothelial cell-based assay for endotoxin as sensitive as the conventional *Limulus* Amebocyte Lysate assay. *Biomaterials*. 2014;**35**:3180-3187
- [172] Rahman MSA, Mukhopadhyay SC, Yu P-L, Goicoechea J, Matias IR, Gooneratne CP, et al. Detection of bacterial endotoxin in food: New planar interdigital sensors based approach. *Journal of Food Engineering*. Elsevier Ltd. 2013;**114**:346-360
- [173] Ganesh V, Bodewits K, Bartholdson SJ, Natale D, Campopiano DJ, Mareque-Rivas JC. Effective binding and sensing of lipopolysaccharide: Combining complementary pattern recognition receptors. *Angewandte Chemie International Edition*. 2009;**48**:356-360
- [174] Beveridge TJ, Koval SF. Binding of metals to cell envelopes of *Escherichia coli* K-12. *Applied and Environmental Microbiology*. 1981;**42**(2):325-335
- [175] Langley S, Beveridge TJ. Effect of O-side-chain-lipopolysaccharide chemistry on metal binding. *Applied and Environmental Microbiology*. 1999;**65**:489-498
- [176] Burkhardt M, LopezAcosta A, Reiter K, Lopez V, Lees A. Purification of soluble CD14 fusion proteins and use in an electrochemiluminescent assay for lipopolysaccharide binding. *Protein Expression and Purification*. 2007;**51**:96-101
- [177] Wurfel MM, Wright SD. Lipopolysaccharide-binding protein and soluble CD14 transfer lipopolysaccharide to phospholipid bilayers: Preferential interaction with particular classes of lipid. *Journal of Immunology*. 1997;**158**:3925-3934
- [178] Fitzgerald KA, Rowe DC, Golenbock DT. Endotoxin recognition and signal transduction by the TLR4/MD2-complex. *Microbes and Infection*. 2004;**6**:1361-1367
- [179] Yu B, Hailman E, Wright SD. Lipopolysaccharide binding protein and soluble CD14 catalyze exchange of phospholipids. *Journal of Clinical Investigation*. 1997;**99**:315-324
- [180] Yeo TY, Choi JS, Lee BK, Kim BS, Yoon HI, Lee HY, et al. Electrochemical endotoxin sensors based on TLR4/MD-2 complexes immobilized on gold electrodes. *Biosensors & Bioelectronics*. Elsevier B.V. 2011;**28**:139-145
- [181] Limbut W, Hedström M, Thavarungkul P, Kanatharana P, Mattiasson B. Capacitive biosensor for detection of endotoxin. *Analytical and Bioanalytical Chemistry*. 2007;**389**: 517-525
- [182] Anderson AS, Dattelbaum AM, Montaña GA, Price DN, Schmidt JG, Martinez JS, et al. Functional PEG-modified thin films for biological detection. *Langmuir*. 2008;**24**: 2240-2247

- [183] Kwon SJ, Kim E, Yang H, Kwak J. An electrochemical immunosensor using ferrocenyl-tethered dendrimer. *Analyst*. 2006;**131**:402-406
- [184] Senaratne W, Andruzzi L, Ober CK. Self-Assembled monolayers and polymer brushes in biotechnology: Current applications and future perspectives. *Biomacromolecules*. 2005;**6**:2427-2448
- [185] Oliveira MDL, Andrade CAS, Correia MTS, Coelho LCBB, Singh PR, Zeng X. Impedimetric biosensor based on self-assembled hybrid cystein-gold nanoparticles and CramoLL lectin for bacterial lipopolysaccharide recognition. *Journal of Colloid and Interface Science*. 2011;**362**:194-201
- [186] Su W, Kim SE, Cho M, Nam JD, Choe WS, Lee Y. Selective detection of endotoxin using an impedance aptasensor with electrochemically deposited gold nanoparticles. *Innate Immunity*. 2013;**19**:388-397
- [187] Su W, Cho M, Nam J-D, Choe W-S, Lee Y. Aptamer-Assisted gold Nanoparticles/PEDOT platform for ultrasensitive detection of LPS. *Electroanalysis*. 2013;**25**:380-386
- [188] Bai L, Chai Y, Pu X, Yuan R. A signal-on electrochemical aptasensor for ultrasensitive detection of endotoxin using three-way DNA junction-aided enzymatic recycling and graphene nanohybrid for amplification. *Nanoscale*. 2014;**6**:2902-2908
- [189] Zuzuarregui A, Souto D, Pérez-Lorenzo E, Arizti F, Sánchez-Gómez S, Martínez de Tejada G, et al. Novel integrated and portable endotoxin detection system based on an electrochemical biosensor. *Analyst*. 2015;**140**:654-660
- [190] Wu J, Zawistowski A, Ehrmann M, Yi T, Schmuck C. Peptide functionalized polydiacetylene liposomes act as a fluorescent Turn-On sensor for bacterial lipopolysaccharide. *Journal of the American Chemical Society*. 2011;**133**:9720-9723
- [191] Rangin M, Basu A. Lipopolysaccharide identification with functionalized polydiacetylene liposome sensors. *Journal of the American Chemical Society*. 2004;**126**:5038-5039
- [192] Johnson BJ, Delehanty JB, Lin B, Ligler FS. Immobilized proanthocyanidins for the capture of bacterial lipopolysaccharides. *Analytical Chemistry*. 2008;**80**:2113-2117
- [193] Zeng L, Wu J, Dai Q, Liu W, Wang P, Lee C-S. Sensing of bacterial endotoxin in aqueous solution by supramolecular assembly of pyrene derivative. *Organic Letters*. 2010;**12**:4014-4017
- [194] Voss S, Fischer R, Jung G, Wiesmüller K-H, Brock R. A fluorescence-based synthetic LPS sensor. *Journal of the American Chemical Society*. 2007;**129**:554-561
- [195] Lim SK, Chen P, Lee FL, Moochhala S, Liedberg B. Peptide-assembled graphene oxide as a fluorescent turn-on sensor for lipopolysaccharide (endotoxin) detection. *Analytical Chemistry*. 2015;**87**:9408-9412
- [196] Thompson M, Blaszykowski C, Sheikh S, Romaschin A. A true theranostic approach to medicine: Towards tandem sensor detection and removal of endotoxin in blood. *Biosensors & Bioelectronics*. 2015;**67**:3-10

- [197] Harmon P, Cabral-Lilly D, Reed RA, Maurio FP, Franklin JC, Janoff A. The release and detection of endotoxin from liposomes. *Analytical Biochemistry*. 1997;**250**:139-146
- [198] Stromberg LR. Differential interactions of lipopolysaccharides with lipid bilayers: Applications for pathogen detection. [Doctoral Dissertation]. 2016. 1-296
- [199] Sakamuri RM, Wolfenden MS, Anderson AS, Swanson BI, Schmidt JS, Mukundan H. Novel optical strategies for biodetection. *Proceedings of SPIE*. 2013. 881209
- [200] Veiseh M, Veiseh O, Martin MC, Bertozzi C, Zhang M. Single-cell-based sensors and synchrotron FTIR spectroscopy: A hybrid system towards bacterial detection. *Biosensors & Bioelectronics*. 2007;**23**:253-260
- [201] Shanmugam A, Rajoria S, George AL, Mittelman A, Suriano R, Tiwari RK. Synthetic Toll like receptor-4 (TLR-4) agonist peptides as a novel class of adjuvants. *PLoS ONE*. 2012;**7**:e30839
- [202] Lamping N, Dettmer R, Schröder NW, Pfeil D, Hallatschek W, Burger R, et al. LPS-binding protein protects mice from septic shock caused by LPS or Gram-negative bacteria. *Journal of Clinical Investigation*. 1998;**101**:2065-2071
- [203] Chien JY, Jerng JS, Yu CJ, Yang PC. Low serum level of high-density lipoprotein cholesterol is a poor prognostic factor for severe sepsis. *Critical Care Medicine*. 2005;**33**:1688-1693
- [204] Cooperstock MS, Tucker RP, Baublis JV. Possible pathogenic role of endotoxin in Reye's syndrome. *The Lancet*. 1975;**1**:1272-1274
- [205] Jay JM. Limulus lysate endotoxin assay as a test of microbial quality of ground Beef. *Journal of Applied Bacteriology*. 1977;**43**:99-109
- [206] Kimura H. Measurement of endotoxin. II. Comparison of reactivities measured by radioimmunoassay and with the Limulus test. *Acta Medica Okayama*. 1976;**30**:257-270
- [207] Ong KG, Leland JM, Zeng K, Barrett G, Zourob M, Grimes CA. A rapid highly-sensitive endotoxin detection system. *Biosensors & Bioelectronics*. 2006;**21**:2270-2274
- [208] da Silva JSL, Oliveira MDL, de Melo CP, Andrade CAS. Impedimetric sensor of bacterial toxins based on mixed (Concanavalin A)/polyaniline films. *Colloids and Surfaces B: Biointerfaces*. 2014;**117**:549-554