Review

Optical Immunosensors for Bacteria Detection in Food Matrices

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Abstract: Optical immunosensors are one of the most popular categories of immunosensors with applications in many fields including diagnostics and environmental and food analysis. The latter field is of particular interest not only for scientists but also for regulatory authorities and the public since food is essential for life but can also be the source of many health problems. In this context, the current review aims to provide an overview of the different types of optical immunosensors focusing on their application for the determination of pathogenic bacteria in food samples. The optical immunosensors discussed include sensors based on evanescent wave transduction principles including surface plasmon resonance (SPR), fiber-optic-, interferometric-, grating-coupler-, and ring-resonator-based sensors, as well as reflectometric, photoluminescence, and immunosensors based on surface-enhanced Raman scattering (SERS). Thus, after a short description of each transduction technique, its implementation for the immunochemical determination of bacteria is discussed. Finally, a short commentary about the future trends in optical immunosensors for food safety applications is provided.

Keywords: immunosensor; optical detection; bacteria

1. Introduction

Foodborne diseases affect, according to the World Health Organization (WHO), 1 in 10 people every year worldwide [1], with symptoms ranging from mild diarrhea to severe complications and even death [2]. According to the WHO, it has been estimated that cases of food poisoning have reached up to 600 million, with 420,000 deaths worldwide, amongst which 125,000 were children under the age of 5 [1]. In particular, pathogenic bacteria play a crucial role in food poisoning, with the majority of incidents being caused by 15 pathogenic bacteria including *Listeria monocytogenes*, *Escherichia coli O157:H7*, *Clostridium botulinum*, *Legionella pneumophila*, *Campylobacter jejuni*, *Salmonella* spp., *Staphylococcus aureus*, *Shigella*, *Vibrio vulnificus*, and *Bacillus cereus* [3]. Most of these bacteria are detected in dairy products, fresh vegetables, raw products, and undercooked meat and seafood [4,5].

The efficiency and reliability of the techniques employed to detect these pathogens in food matrices are of paramount importance for the prevention of foodborne diseases. [6,7]. The conventional methods for bacteria detection and identification are based on culturing and colony counting. Those methods are reliable, sensitive, and are considered as the “gold standard” for detecting the presence of bacteria; however, prior to detection, they require several steps such as pre-enrichment, selective enrichment, isolation, and confirmation through biochemical and serological tests [8,9]. Thus, there was an urgent need for rapid bacteria detection techniques, which led to the development of new methods such as molecular and immunological ones [10].

Molecular methods rely on the polymerase chain reaction (PCR) [11,12] or isothermal nucleic acid amplification methods such as nucleic acid sequence-based amplification (NASBA), loop-mediated isothermal amplification (LAMP), strand displacement amplification (SDA), recombinase polymerase amplification (RPA), rolling circle amplification...
(RCA), helicase-dependent amplification, and others [13] to rapidly and effectively identify various bacteria through their genetic fingerprinting. In particular, isothermal nucleic acid amplification methods have the potential for on-site determinations since they do not require thermal cycling with strict temperature control like the standard PCR. Despite this advantage, isothermal nucleic acid amplification methods can be inhibited by several compounds present in foods, like polysaccharides and polyphenolic compounds, leading to false negative results [13]. Moreover, molecular methods cannot discriminate between dead and live bacteria, and this can lead to false positive results.

On the other hand, immunoassays rely on antibodies that recognize specific proteins or lipopolysaccharides of the bacterial external membrane. Chemiluminescent enzyme immunoassay (CL-EIA) [14] and enzyme-linked immunosorbent assay (ELISA) [15–17] are the immunochemical methods most widely employed for rapid bacterial detection and quantification. Immunoassays are characterized by high sensitivity and accuracy, simple sample preparation, and low costs of instrumentation compared to other instrumental methods such as chromatographic ones. Compared to molecular methods, the immunochemical ones are less prone to false negative results, but they can also produce false positive results due to the cross-reactivity of antibodies used with other bacteria species or sub-species of the targeted one. In addition, they are laboratory bound since they involve multiple processing steps and desktop instrumentation [18,19].

The quest for portable analytical devices that can provide reliable results in a short amount of time and that are suitable for on-site applications has been the driving force behind the development of biosensors. Nowadays, the realization of biosensor systems that combine outstanding analytical performance with portability has moved from the sphere of fiction to reality due to significant progress in the field of nanotechnology [20]. Thus, modern biosensor technologies can provide high detection sensitivity and specificity, high-speed analysis, and quantitative results in real time [21]. Biosensors are defined by the IUPAC as analytical devices that provide quantitative or semi-quantitative information by combining a biological recognition element with a physicochemical transducer, which transforms the biorecognition event into a physically detectable signal [22]. The recognition element can be any bioreceptor [23], such as a nucleic acid probe, aptamer, phage, antibody, antigen, whole cell, enzyme, etc., that can bind the target molecule specifically and with high affinity [24–31] (Figure 1a).

![Figure 1](image_url)  
(a) Schematic of a biosensor. (b) The different categories of biosensors.

Immunosensors, i.e., biosensors that rely on antibody–antigen reactions for analyte detection, are the most abundant category of biosensors due to the indispensable ability of antibodies for the highly selective detection of the targeted analytes in complex media and the versatility of available assay formats that could be employed for the determination...
of different kinds of analytes, including bacteria [31]. They can be divided according to the transduction principle into electrochemical [32–35], piezoelectric [36,37], or optical ones [32,38], which can detect biological interactions by recording the variations in the electrical signal, mass, or light, respectively (Figure 1b). Electrochemical immunosensors are cost-efficient devices and have the potential for miniaturization. Although their detection sensitivity is high and the detection limits achieved are suitable for bacteria detection at very low concentrations, especially in cases where labels are involved to enhance the signal, they are prone to interferences from the matrix, which can reduce the reproducibility of the readings and lead to either false positive or false negative results [39]. Immunosensors based on piezoelectric phenomena, even though suitable for label-free detection, are characterized in general by relatively low detection sensitivity [40]. On the other hand, optical immunosensors exhibit high sensitivity and offer simple, fast, and accurate detection of a great variety of analytes, including bacteria [41]. Optical detection methods (Figure 1b) are based on different transduction principles such as light absorbance, total internal reflectance, photoluminescence, fluorescence, light polarization, interferometry, Raman scattering, and surface plasmon resonance [42,43].

The scope of this review is to summarize the achievements of optical immunosensors for foodborne bacteria detection. First, short descriptions of the different detection principles reported so far for bacteria detection will be provided. Then, the application of optical immunosensors for bacteria detection in different food matrices will be extensively presented. Finally, a short commentary on the future trends regarding the prospective applications and challenges of optical immunosensors in food analysis is included.

2. Principles of Optical Immunosensors

2.1. Assay Formats

In principle, most immunosensors offer the potential to monitor antigen–antibody binding in real time. Such direct detection although simpler and faster, since there is no need for additional reaction steps, is usually limited to high-molecular-weight analytes, for which the antigen–antibody reaction results in a measurable change in sensor response [32]. Therefore, in most immunosensors, the assay formats usually applied in microtiter plate solid-phase immunoassays are followed, i.e., the competitive or non-competitive assay format. As schematically depicted in Figure 2, competitive immunoassays are based either on an immobilized antibody (Figure 2A), an immobilized analyte, or an analyte–protein conjugate (Figure 2B). In the first approach, the antibody is immobilized onto the transducer surface, and the concentration of the analyte is defined through its competition with a labeled analyte or analyte–protein conjugate for binding to the antibody. In the second approach, the analyte is immobilized onto the transducer surface (either directly or as an analyte–protein conjugate) and competes with the analyte in the sample for the binding sites of the antibody. Although both approaches are applicable for a given analyte–antibody pair, the second might be advantageous regarding the stability of immobilized biomolecule, i.e., the analyte or analyte–protein conjugate, since antibodies are known to lose a great part of their functionality upon immobilization. The non-competitive or sandwich immunoassay format is suitable for analytes that have at least two antigenic determinants or epitopes in their molecule. This means that at least two antibodies recognizing two different parts of the analyte are available. This is essential for the realization of a non-competitive assay since, as depicted in Figure 2C, an antibody immobilized onto the transducer surface (usually referred to as the capture antibody) binds the analyte through one epitope, and a second antibody (referred to as the detection or reporter antibody) is attached to a different epitope forming a “sandwich” with the analyte. The detection antibody can be labeled or not depending on the transduction principle involved.
The optical transduction principles can be divided into two main categories: those involving labels and the label-free ones.

2.2.1. Detection Using Labels

A significant number of optical immunosensors are based on the use of labels such as enzymes, fluorescent organic dyes, gold nanoparticles, or quantum dots for the quantification of antibody–antigen interactions and the determination of analyte concentrations in a sample [44]. Using enzymes as labels in immunosensors is endorsed by their successful implementation in standard microtiter plate immunoassays. Enzymes offer significant signal enhancement in a short time; however, their use in combination with optical transducers is rather limited to insoluble substrates that can precipitate on the transducer, thus generating a measurable signal change. Biosensing using fluorescently labeled molecules and optical fibers was amongst the first optical transduction principles to be explored [45]. Apart from the typical cylindrical optical fibers, planar waveguides and capillaries that enable light propagation through total internal reflection have also been employed as transducers for the development of optical fluorescence immunosensors [46–48]. Over the years, metal nanoparticles have been employed as labels and incorporated into immunochromatographic strips or used as liquid phase reagents to obtain semi-quantitative information, relying on visual evaluation, or quantitative results through the implementation of an instrument that could quantitate the color, fluorescence, or chemiluminescence intensity. The latter can be performed using smartphones as detection, signal processing, and transmission apparatus, moving the realization of portable devices a step forward [48].

2.2.2. Label-Free Detection

Label-free optical biosensing, on the other hand, is based almost exclusively on monitoring refractive index changes due to immunochemical reactions taking place on top of the optical transducer [37]. There are two main categories of label-free optical transduction principles: the refractometric and the reflectometric ones [49]. In refractometric immunosensors, the light that is transmitted through a waveguide creates an electromagnetic field, known as an evanescent field (Figure 3), which extends in the medium above the biosensor surface. This field is influenced by refractive index changes over the transducer surface resulting from the biomolecular layer thickness increase due to immuno reactions. Thus, when the evanescent wave field is coupled back to the transducer, a change in the intensity, polarization, or phase of the waveguided light is observed, which is proportional to the concentration of analyte in the sample, enabling its quantitative determination [50–52]. Transducers based on surface plasmon resonance (SPR), fiber optics, grating couplers, interferometers, and ring resonators fall into this category of optical sensors (Figure 4).
This field is influenced by refractive index changes over the transducer surface resulting from the biomolecular layer thickness increase due to immunoreactions. Thus, when the evanescent wave field is coupled back to the transducer, a change in the intensity, polarization, or phase of the waveguided light is observed, which is proportional to the concentration of analyte in the sample, enabling its quantitative determination [50–52]. Transducers based on surface plasmon resonance (SPR), fiber optics, grating couplers, interferometers, and ring resonators fall into this category of optical sensors (Figure 4).

Reflectometric sensors are based on light reflection by a stack of materials with different refractive indices leading to the creation of an interference spectrum. The most widely explored reflectometric sensing method is the one introduced in 1991 by Gauglitz et al. [53], which is known as reflectometric interference spectroscopy (RIfS). RIfS transducers are made of a glass substrate on top of which a thin layer of transparent dielectric material has been deposited. Immunochemical reactions that take place on top of the dielectric layer are evidenced as shifts in the reflected interference spectrum. This spectrum is created due to the reflection of the light beam at each interface of different refractive indices with a slightly different angle leading to either constructive or destructive interference. The spectrum shift is directly correlated with the increase in the biomolecular layer thickness due to immunoreactions, and therefore, it can be correlated with the analyte concentration in the sample through a calibration curve. Since the first report on RIfS sensors, a lot of progress
has been made in the direction of reducing the cost and size of the instrumentation and increasing the multiplexing capabilities, e.g., by monitoring, instead of the whole spectrum, specific wavelengths [54] or even a single wavelength [55]. In addition, materials other than glass have been employed as transducers including porous silicon with or without thermally grown oxide [56], porous silicon–carbon composites [57], other porous materials such as TiO₂ [58], or non-transparent substrates such as silicon with a transparent dielectric composed of silicon dioxide [59–61] or silicon nitride [62].

3. Application of Optical Sensors for Bacteria Detection

3.1. Evanescent-Wave-Based Biosensors

Surface plasmon resonance (SPR)-based immunosensors are the optical biosensors most frequently used for the single or multiplex, label-free detection of foodborne pathogenic bacteria due to their high detection sensitivity and ability to monitor binding reactions in real-time. The SPR phenomenon relies on the excitation of metal-free electrons (surface plasmons) when polarized light strikes a metal layer (usually gold) deposited on the surface of an optically transparent material (prism, grating coupler, or dielectric waveguide) at a certain angle. The excited plasmons create an evanescent wave field at the solution/gold interface. This wave is very sensitive to refractive index changes at the gold layer surface occurring due to a biomolecular reaction, and as a result, the angle of incident light has to change during the course of the reaction to preserve the surface plasmon wave, providing the means to monitor these reactions in real time. Thus, it is possible to monitor both the immobilization of specific biomolecules (e.g., antibodies) as well as the binding of analytes in real time (Figure 5) [41,44].

![SPR response during the binding of a bacteria-specific antibody onto the biosensor surface followed by capture of the bacteria by the immobilized antibody.](image)

The first report regarding the detection of bacteria with an SPR sensor dates back to 1998 [63], and it was based on a sandwich immunoassay for the detection of *Escherichia coli* O157:H7 in buffer. The sensor was modified with protein A or protein G, and a mouse monoclonal or a rabbit polyclonal antibody, respectively, was then immobilized. Depending on the antibody used for detection, LODs in the range 5–7 × 10⁵ cfu/mL have been achieved. *Vibrio cholerae* O1 was also identified in buffer with an SPR biosensor functionalized with a protein G layer [64]. The sensor chip was modified with a self-assembled monolayer of a mixture of 11-mercaptoundecanoic acid and hexanethiol on which protein G was covalently bound and used to immobilize a monoclonal antibody specific to *V. cholerae* O1. The LOD of the assay was 10⁵ cfu/mL. The same antibody immobilization approach was employed to immobilize an antibody against *Legionella pneumophila* onto SPR chips, achieving an LOD of 10⁵ cfu/mL in buffer [65]. *Salmonella enterica serovar Enteritidis* and *Escherichia coli* were detected in spiked skim milk by a direct binding assay on SPR chips modified with protein
G to which the anti-bacteria specific antibodies were then bound [66]. The LOD achieved after 1 h assay was 25 cfu/mL for *E. coli* and 23 cfu/mL for *Salmonella*.

The detection of *Salmonella* groups B, D, and E in buffer with SPR has also been reported, employing a sandwich assay format and using antibody pairs from different animals [67]. It was found that the LOD improved 200 times compared to direct detection [67]. The benefits of the sandwich immunoassay format for bacteria detection have also been demonstrated in a study for the detection of *Staphylococcus aureus* with SPR in buffer, where the LOD was improved from $1 \times 10^7$ to $1 \times 10^5$ cfu/mL when a sandwich immunoassay format was followed instead of direct detection [68]. In another report, where the direct binding assay was compared to a sandwich assay for the detection of *E. coli* O157:H7 in buffer, a 1,000-fold improvement in sensitivity was reported, leading to an LOD of $10^3$ cfu/mL [69]. The immobilization of the polyclonal antibody against *E. coli* was performed through covalent bonding to a monolayer of mixed thiol-terminated polyethylene glycol with thiol-terminated polyethylene-carboxylic acid. In another report, the free amine groups of protein A were converted to thiol groups, through a reaction with 2-iminothiolane, to facilitate its immobilization onto SPR chips, which are then modified with an antibody against *Salmonella paratyphi* [70]. An LOD of $10^2$ cfu/mL was achieved in buffer employing the antibody-modified chip in a direct binding assay.

In another study, the SPR chip was modified with brushes of poly(carboxybetaine acrylamide) to reduce the non-specific binding of bacteria to its surface, while antibody-modified gold nanoparticles were used as labels to increase the detection sensitivity [71]. This immunosensor could detect *E. coli* O157:H7 in hamburger and cucumber samples at concentrations as low as $57$ and $17$ cfu/mL and *Salmonella* spp. at $7.4 \times 10^3$ and $11.7 \times 10^3$ cfu/mL, respectively [71]. Gold nanoparticles modified with an antibody against *Campylobacter jejuni* were also employed as labels in an SPR sandwich immunoassay that allowed for the detection of this bacterium in buffer at concentrations as low as $4 \times 10^4$ cfu/mL [72]. A gold-labeled secondary antibody was also employed to increase the detection sensitivity in a sandwich SPR for *L. monocytogenes* by two–four orders of magnitude compared to the direct binding assay, providing an LOD of $10^2$ cfu/mL in buffer [73]. In another study, in order to achieve signal enhancement, a precipitate 3,3',5,5'-tetramethylbenzidine (TMB) substrate was used in combination with an antibody labeled with horse radish peroxidase (HRP) to detect bacteria captured by an antibody attached to an SPR chip through covalent bonding to a self-assembled monolayer of mercaptoundecanoic acid [74]. Following this approach, 250% signal enhancement was achieved with respect to the assay not employing the HRP/TMB system, leading to an LOD of $10^4$ cfu/mL for the detection of *E. coli* in spinach leaves [74].

SPR chips were modified in a plasma reactor in the presence of cyclopropylamine vapors to induce reactive moieties containing nitrogen which were in turn used to immobilize antibodies using glutaraldehyde activation [75]. The chips were employed to detect *Salmonella typhimurium* in buffer by a direct binding assay that provided an LOD of $10^5$ cfu/mL.

Along with the optimization of the SPR assays for bacteria detection, efforts have been devoted to sample treatment methods aiming either to improve detection sensitivity or alleviate non-specific matrix effects. For example, the performance of an SPR sensor for the detection of live, heat-killed, or detergent-lysed *E. coli* O157:H7 cells was investigated, and LODs of $10^6$, $10^5$, and $10^4$ cfu/mL, respectively, were reported [76]. The differences observed were ascribed to changes in cell size and morphology upon treatment with ethanol, whereas treatment with detergent probably led to fragmentation of cells to smaller particles that were recognized more efficiently by the antibody. The effect of the sample preparation method was also evident in another study for the detection of *E. coli* O157:H7 in different food samples [77]. In this study, milk, apple juice, and ground beef patties were spiked with *E. coli* O157:H7 at various concentrations and then analyzed with a portable SPR instrument commercialized by Texas Instruments Inc. under the tradename SPREETA™. The sensor chip was modified with neutravidin to enable the immobilization
of biotinylated antibodies against *E. coli* O157:H7. The spiked milk and apple juice samples were run without pretreatment, whereas the ground beef sample was extracted with buffer and homogenized prior to analysis. The LODs achieved ranged from $10^2$ to $10^3$ cfu/mL depending on the sample analyzed. In another report, where a sandwich SPR assay for *Salmonella* with an LOD of $1.25 \times 10^5$ cells/mL was developed [78], the authors claimed that the presence of milk did not affect the assay performance, alleviating the need for sample preparation or clean-up steps.

It has been suggested that the detection of whole bacteria using SPR generally results in lower sensitivity compared to other techniques, due to the limited interaction of bacteria with the evanescent wave electromagnetic field (the size of the bacteria is multiple times the penetration depth of the evanescent wave field) and the small difference in the refractive index between the bacterial cytoplasm and the surrounding aqueous medium [79]. Thus, instead of running over the sensor the bacteria-containing sample, it is first incubated with the pathogen-specific antibody, and after the separation of the free antibody from the bound antibody, the free antibody is quantified. This assay format is known as subtraction inhibition assay (SIA) and has been applied for the detection of *L. monocytogenes* [80], *E. coli* O157:H7 [81], and *B. anthracis* spores in buffer [82]. The LODs reported were $1 \times 10^5$, $3.0 \times 10^4$, and $10^4$ cfu/mL, respectively, and were one order of magnitude lower than those achieved with the direct binding assay. The SIA format was also applied for the detection of fungal cells that are considerably larger than bacterial ones [83]. Thus, it has been applied for the detection of *Phytophthora infestans* [83] and the detection of *Puccinia striiformis* in buffer with LODs of $2.2 \times 10^6$ sporangia/mL and $3.1 \times 10^5$ urediniospores/mL, respectively [84]. A similar assay format was also applied for the SPR detection of *Cryptosporidium parvum* oocysts in water with an LOD of $1 \times 10^2$ oocysts/mL [85].

In a different approach for indirect bacteria detection by SPR, a polyclonal antibody against a cell extract enriched for the invasion-associated protein, internalin B, was used to develop an inhibition assay for *Listeria monocytogenes* [86]. After incubation of bacteria containing solutions with the antibody, the mixture was injected over a SRP chip modified with purified-recombinant internalin B, and the signal was inversely proportional to the *L. monocytogenes* concentration achieving an LOD of $2 \times 10^5$ cells/mL.

In addition to single bacteria detection, multiplexed bacteria detection with SPR systems has been also explored. Thus, an antibody microarray was developed on an SPR chip for the simultaneous detection of *S. typhimurium*, *E. coli O157:H7*, *Yersinia enterocolitica*, and *Legionella pneumophila* by modifying the chip with protein G to allow for the immobilization of each one of the anti-bacteria specific antibodies at different areas of the chip through spotting [87]. All bacteria were detected simultaneously in buffer samples each at a concentration of $10^5$ cfu/mL. *E. coli O157:H7*, *L. monocytogenes*, *Campylobacter jejuni*, and *S. choleraesuis* were also simultaneously detected both in buffer and apple juice using a multi-channel SPR system [88]. The whole chip surface was modified with streptavidin, and the four bacteria antibodies were immobilized using an eight-channel fluidic device (two channels per antibody; one for the specific and the other for the non-specific signal monitoring). The LODs achieved were $1.4 \times 10^4$ cfu/mL for *E. coli*, $4.4 \times 10^4$ cfu/mL for *S. choleraesuis*, $1.1 \times 10^5$ cfu/mL for *C. jejuni*, and $3.5 \times 10^5$ cfu/mL for *L. monocytogenes*.

An SPR imaging device was combined with an array of antibodies specific to different serotypes of *L. monocytogenes* with the aim of monitoring the growth of live listeria cells in culture [89]. Emphasis was placed on the characterization of the antibodies rather than the analytical performance of the sensor. Similarly, the detection of *Salmonella* with an SPR imaging array was optimized, and LODs of $2.1 \times 10^6$ and $7.6 \times 10^6$ cfu/mL in buffer and chicken carcass rinse were demonstrated, respectively [90]. An SPR imaging sensor was applied for the simultaneous label-free detection of *Salmonella* spp., Shiga-toxin-producing *E. coli* (STEC), and *L. monocytogenes* in chicken carcass rinse [91]. The antibodies specific to each bacterium were immobilized on the same chip, and an LOD for *Salmonella* of $10^6$ cfu/mL was achieved. An SPR imaging sensor (Figure 6) was also implemented for the simultaneous detection of *Listeria monocytogenes* and *Listeria innocua*, achieving an LOD
of $2 \times 10^2$ cfu/mL for both bacteria after 7 h incubation of the sample in the fluidic cell attached to the SPR chip [92].

![Figure 6. Schematic of a resolution-optimized prism-based SPR imaging apparatus [92]. Copyright 2019. Reproduced with permission from the Royal Society of Chemistry.](image)

Despite the fact that SPR has found numerous applications in diverse fields and several companies have commercialized devices based on this transduction principle, the majority of these instruments are suitable for use in a lab. Thus, considerable efforts have been devoted to reducing the equipment size and complexity in order to build up systems appropriate for analysis at the point of need. The SPREETA™ SPR biosensor mentioned above was a successful outcome of such an effort. It included an AlGaAs light-emitting diode (LED, 840 nm), a polarizer, a temperature sensor, two photodiode arrays, and a reflecting mirror combined with a gold-coated glass slide and a silicone rubber gasket of two channels. The instrument was accompanied by software that provided all the information related to the analysis of the SPR curve, the real-time binding, the layer thickness, and the flow cell temperature. In addition to the determination of *E. coli* O157:H7 in various food samples [77], the SPREETA™ SPR biosensor has also been used for the detection of *Campylobacter jejuni* with an LOD of $10^5$ cfu/mL in both buffer and spiked broiler meat samples [93]. SPREETA™ has also been employed to develop a sensor to detect *E. coli* O157:H7 in laboratory cultures [94]. The sensitivity and specificity of detection were determined. Thus, for an assay of 35 min, an LOD for *E. coli* O157:H7 of $8.7 \times 10^6$ cfu/mL was determined in single-bacteria culture, whereas in mixed cultures with non-target bacteria concentrations up to $10^6$ cfu/mL or less, the LOD was $10^7$ cfu/mL. For higher concentrations of non-target bacteria, the sensor’s sensitivity was negatively affected. In another report, application of the SPREETA™ SPR sensor for *E. coli* detection in water with an LOD of 90 cfu/mL was reported for a direct binding assay that lasted less than 30 min [95]. The much lower LOD achieved in this report with respect to previous ones could be attributed to the fact that the specific antibody was immobilized onto the chip surface via streptavidin and not directly. Finally, SPREETA™ was applied to detect *Salmonella typhimurium* at concentrations equal to or higher than $1 \times 10^6$ cfu/mL in chicken [96]. To increase the detection sensitivity of a SPREETA™ sensor for the detection of *E. coli*, Au-coated magnetic nanoparticles were modified with an antibody against *E. coli* and used not only to concentrate *E. coli* cells from lake, river, puddle, and tap water samples but also as labels in a SPR sandwich immunoassay using SPREETA™ chips, which were modified with an anti-*E. coli* antibody, achieving a detection limit of 3 cfu/mL [97].

Apart from SPREETA™, other attempts to create portable instruments based on the SPR principle of detection have been reported in the literature. Thus, a portable instrument that combined microfluidic and SPR technologies on a single platform was applied for the determination of *E. coli* and *S. aureus* in buffer samples [98]. In this setup, an LED was
used to illuminate a gold-covered rectangular prism, and the reflected light was captured by a CMOS sensor and then transferred for processing to a PC. The chip was modified with 11-mercaptoundecanoic acid to facilitate the covalent binding of protein G in which an antibody against the lipopolysaccharide (LPS) of *E. coli* was captured, enabling *E. coli* detection in buffer at a concentration of $3.2 \times 10^5$ cfu/mL. In another attempt, *Salmonella typhimurium* was detected in buffer at concentrations ranging from $10^7$ to $10^9$ cfu/mL within 1 h using an SPR biosensor in which the incident light from a diode laser (instead of an LED) was directed to the gold film by a rotating mirror, and the light reflected from the metal film was captured by a CMOS image sensor [99].

Another approach to surpass the portability limitations of standard SPR instruments is the implementation of a localized SPR or LSPR transduction approach. In LSPR, the continuous metal surface is replaced by noble-metal nanoparticles (nanospheres, nanorods, or nanodisks) of sub-wavelength size around which the surface plasmons are localized [100]. The light that strikes the nanostructures excites the surface plasmons and when resonance is achieved, certain wavelengths are scattered from the nanostructures. Thus, immunoreactions can be monitored in real time as shifts in the resonance wavelength [101]. The advent of LSPR opened up new horizons for the detection of pathogens, especially in the direction of portable systems. Nonetheless, the first report showed that LSPR was less sensitive than the classical SPR configuration [102] or more vulnerable to interferences from the matrix of the samples analyzed [103]. More recent reports, however, show improved detection sensitivity achieved mainly through optimization of the dimensions and stability of the nanoparticles [104]. Thus, an LSPR sensor was developed for the determination of *E. coli O157:H7* in buffer employing spherical gold nanoparticles non-covalently modified with a specific anti-*E. coli* avian antibody. An LOD of 10 cfu/mL was achieved in less than 2 h, making the sensor suitable for *E. coli O157:H7* determination at the point of need [104]. Instead of using non-continuous gold surfaces, structuring of the gold film through its deposition onto a nanostructured fluoropolymer enabled the development of an SPR sensor based on grating-coupled long-range surface plasmons, which were employed for the detection of *E. coli O157:H7* in spiked buffer samples through a sandwich immunoassay implementing metal nanoparticles modified with another anti-*E. coli O157:H7* antibody as labels to achieve an LOD of 50 cfu/mL [105].

The reports regarding bacteria detection in water and food samples with SPR-based immunosensors are summarized in Table 1.

Table 1. SPR-based immunosensors for bacteria detection.

<table>
<thead>
<tr>
<th>Analyte</th>
<th>Detection Principle</th>
<th>Assay Type</th>
<th>Sample Type</th>
<th>Assay Duration</th>
<th>LOD</th>
<th>Ref. No.</th>
</tr>
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<tbody>
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<td><em>E. coli</em></td>
<td>SPR</td>
<td>direct</td>
<td>milk</td>
<td>5–7 min</td>
<td>23 cfu/mL</td>
<td>[66]</td>
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<td><em>S. enteritidis</em></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>25 cfu/mL</td>
<td></td>
</tr>
<tr>
<td><em>E. coli O157:H7</em></td>
<td>SPR</td>
<td>sandwich with biotinylated detection antibody and streptavidin labeled with gold nanoparticles</td>
<td>hamburger/cucumber</td>
<td>~1 h</td>
<td>57/17 cfu/mL</td>
<td>[71]</td>
</tr>
<tr>
<td><em>Salmonella sp.</em></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>7.4 × 10^3/11.7 × 10^3 cfu/mL</td>
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<tr>
<td><em>E. coli</em></td>
<td>SPR</td>
<td>sandwich with detection antibody labeled with peroxidase + TMB substrate</td>
<td>fresh spinach</td>
<td>~2 h</td>
<td>$10^4$ cfu/mL</td>
<td>[74]</td>
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<tr>
<td><em>E. coli O157:H7</em></td>
<td>SPR</td>
<td>direct</td>
<td>milk</td>
<td>30 min</td>
<td>$10^2$–$10^3$ cfu/mL</td>
<td>[77]</td>
</tr>
<tr>
<td><em>S. typhimurium</em></td>
<td>SPR</td>
<td>sandwich</td>
<td>milk</td>
<td>1 h</td>
<td>$1.25 \times 10^3$ cfu/mL</td>
<td>[78]</td>
</tr>
<tr>
<td><em>C. parvum</em></td>
<td>SPR</td>
<td>subtractive inhibition</td>
<td>water</td>
<td>30 min</td>
<td>$10^2$ oocysts/mL</td>
<td>[85]</td>
</tr>
<tr>
<td><em>L. monocytogenes</em></td>
<td>SPR</td>
<td>inhibition</td>
<td>chocolate milk</td>
<td>30 min</td>
<td>$2 \times 10^5$ cfu/mL</td>
<td>[86]</td>
</tr>
</tbody>
</table>
Table 1. Cont.

<table>
<thead>
<tr>
<th>Analyte</th>
<th>Detection Principle</th>
<th>Assay Type</th>
<th>Sample Type</th>
<th>Assay Duration</th>
<th>LOD</th>
<th>Ref. No.</th>
</tr>
</thead>
<tbody>
<tr>
<td>L. monocytogenes</td>
<td>SPR imaging</td>
<td>direct</td>
<td>apple juice</td>
<td>30 min</td>
<td>3.5 × 10^3 cfu/mL</td>
<td></td>
</tr>
<tr>
<td>E. coli O157:H7</td>
<td>SPR imaging</td>
<td>direct</td>
<td>carcass rinse</td>
<td>~20 min</td>
<td>1.4 × 10^4 cfu/mL</td>
<td>[88]</td>
</tr>
<tr>
<td>C. jejuni</td>
<td>SPR imaging</td>
<td>direct</td>
<td>chicken carcass rinse</td>
<td>~20 min</td>
<td>7.6 × 10^6 cfu/mL</td>
<td>[90]</td>
</tr>
<tr>
<td>S. typhimurium</td>
<td>SPR imaging</td>
<td>direct</td>
<td>water</td>
<td>~17 min</td>
<td>90 cfu/mL</td>
<td>[95]</td>
</tr>
<tr>
<td>Salmonella spp. Shiga-toxin producing</td>
<td>SPR imaging</td>
<td>direct</td>
<td>lake, river, puddle, and tap water</td>
<td>20 min</td>
<td>3 cfu/mL</td>
<td>[97]</td>
</tr>
<tr>
<td>Escherichia coli L. monocytogenes</td>
<td>SPR imaging</td>
<td>direct</td>
<td>chicken carcass rinse</td>
<td>3 min</td>
<td>10^6 cfu/mL</td>
<td>[96]</td>
</tr>
</tbody>
</table>

3.1.2. Fiber Optic Immunosensors

Fiber optic immunosensors rely on the immobilization of immunoreagents onto a part of the optical fiber from which the cladding layer has been removed to allow interaction of the waveguided photons, through the evanescent wave field, with the analyte in the solution surrounding the fiber (Figure 7). In order to increase the evanescent field effect, fiber tapering is applied either in the form of a tapered tip or of a continuous tapered fiber [45,106]. Tapered tips are created by gradually reducing the diameter at the end of an optical fiber down to nanometers. In order to obtain the highest possible sensitivity due to reactions, the recognition biomolecules are immobilized on the tip region with the smallest diameter where the evanescent field is stronger. Continuous tapered fibers usually have a biconical taper, comprising a region of decreasing diameter, a region of constant diameter called the waist, and a region of increasing diameter [107]. Sensing takes place on the waist region where the evanescent field exhibits its higher intensity, whereas the emitted light is collected from the region of increasing diameter [108]. Fiber optic biosensors have been widely employed in the field of foodborne pathogen detection due to their convenience, small size, lack of electromagnetic interference, cost-effectiveness, high sensitivity, and accuracy [109].

Figure 7. Main configurations of fiber optic sensors: (a) de-cladded optical fiber, (b) tapered optical fiber, (c) tapered tip, and (d) U-shaped optical fiber probe.

The first label-free approach for the detection of pathogens was realized using a U-bent optical fiber sensor [110]. Bending a de-cladded fiber into a U-shaped structure enhances the penetration depth of evanescent waves and, hence, the sensitivity of the probe. This system could detect E. coli in concentrations lower than 10^3 cfu/mL in buffer with an assay
duration of 1 h. A similar approach employing a plastic fiber optic sensor with a U-shaped sensing probe functionalized with an antibody against *E. coli* detection in buffer, resulting in an LOD of $10^5$ cfu/mL for an assay duration of 10 min per sample [111]. Upon exposure of the sensor to bacteria solutions, the output signal decreased with time due to the attachment of the bacteria onto the probe surface, which resulted in an increase in the refractive index value close to the probe.

Several fiber optic immunosensors employing labels have also been reported for bacteria detection. Thus, tapered fiber tips have been used for the detection of *Salmonella* in culture medium [112] and *E. coli* O157:H7 in ground beef [113] with LODs of $10^4$ and $10^5$ cfu/mL, respectively. The first employed silica fibers with tapered tips were modified with mercaptosilane to facilitate the covalent bonding of an anti-*Salmonella* antibody, while a second antibody labeled with a fluorescent dye was used as a detection antibody. In the second report [113], polystyrene fibers were first coated with biotinylated bovine serum albumin and then reacted with streptavidin and a biotinylated anti-*E. coli* antibody. A fluorescently labeled antibody was also used for detection in this case. Polystyrene fibers were integrated into a portable instrument commercialized under the name RAPTOR™ to develop an instrument that could be used for on-site determinations. This instrument was used for the detection of *S. typhimurium* in rinse water from sprouted alfalfa seeds through modification of the fibers first with streptavidin and then with a biotinylated antibody [114]. A second fluorescently labeled antibody was used for detection, achieving an LOD of $10^3$ cfu/mL or 50 cfu/g of sprouted alfalfa seeds after 67 h of bacteria enrichment. The RAPTOR™ biosensor has also been used to detect *Enterococcus faecalis* in ambient water, with an LOD of $5.0 \times 10^5$ cells/mL [115], and *L. monocytogenes* in several food matrices, with LODs ranging from $10^3$ to $4.3 \times 10^5$ cfu/mL [116–118]. In all cases, sandwich immunoassays were implemented with the exception of [117] where a fluorescently labeled aptamer (aptamer A8) specific for internalin A, an invasin protein of *L. monocytogenes*, was used for detection. The sensor was applied for the detection of *L. monocytogenes* in hot dog and bologna sausages at concentrations down to 10 cfu/g after 24 h of enrichment [116] as well as $10^2$ cfu/g in ready-to-eat meat products (sliced beef, chicken, and turkey) after 18 h of enrichment [117]. A version of RAPTOR™ that supported multiplexed determinations was applied for the detection of *L. monocytogenes*, *E. coli* O157:H7, and *S. enterica* in several meat products [119]. The LODs achieved were 50 cfu/mL for *S. enterica* and $10^5$ cfu/mL for *L. monocytogenes*, and the sensor could detect the three bacteria (*Salmonella, E. coli*, and *Listeria*) in ready-to-eat beef, chicken, and turkey meats inoculated with each pathogen at 100 cfu/25 g after enrichment for 18 h.

Fluorescence resonance energy transfer, i.e., the non-radiative energy transfer from a fluorescent donor molecule to an acceptor one when these two are in close proximity, has been also implemented for the detection of *S. typhimurium* with an optical fiber tip sensor in ground beef samples [120]. The anti-*Salmonella* antibody was labeled with the donor fluorophore (AlexaFluor 546) and protein G was labeled with the acceptor fluorophore (Alexa Fluor 594). Upon binding of *S. typhimurium* to the antibody, the induced conformation changes reduced the distance between the donor and acceptor molecules, resulting in an increase in emitted fluorescence and achieving an LOD of $10^5$ cfu/g for the samples.

In recent years, fiber optic immunosensors based on surface modifications with nanomaterials have shown significant improvements compared to conventional fiber optic sensors regarding detection speed and sensitivity. For example, a fiber optic biosensor modified with zinc oxide (ZnO) nanorods for the detection of *E. coli* in water with an LOD of $10^3$ cfu/mL was developed [121].

Fiber optic sensors in which the exposed fiber core is coated with a gold layer to take advantage of the SPR phenomenon have also been used for bacteria detection. Such a sensor has been employed for the detection of *Legionella pneumophila* by a direct assay after modification of the fiber gold-covered area with 11-mercaptoundecanoic to allow the covalent bonding of an anti-*L. pneumophila* antibody [122]. An LOD of 10 cfu/mL was achieved for a direct assay that lasted 1 h. In another report, a fiber optic SPR sensor was
modified with MoS$_2$ nanosheets on which the specific antibodies were attached, and an LOD of 94 cfu/mL for E. coli was achieved compared to 391 cfu/mL received from fibers without MoS$_2$ nanosheets [123]. The reports regarding bacteria detection in water and food samples with fiber-optic-based immunosensors are summarized in Table 2.

Table 2. Fiber optic sensors for bacteria detection.

<table>
<thead>
<tr>
<th>Analyte</th>
<th>Detection Principle</th>
<th>Assay Type</th>
<th>Sample Type</th>
<th>Assay Duration</th>
<th>LOD</th>
<th>Ref. No.</th>
</tr>
</thead>
<tbody>
<tr>
<td>E. coli O157:H7</td>
<td>fiber optic fluorescence</td>
<td>sandwich</td>
<td>ground beef</td>
<td>2.5 h</td>
<td>$10^3$ cfu/mL</td>
<td>[113]</td>
</tr>
<tr>
<td>S. typhimurium</td>
<td>plastic fiber optic fluorescence</td>
<td>sandwich</td>
<td>sprouted alfalfa seeds rinse water</td>
<td>~30 min</td>
<td>$10^3$ cfu/mL, 50 cfu/g (67 h)</td>
<td>[114]</td>
</tr>
<tr>
<td>E. faecalis</td>
<td>plastic fiber optic fluorescence</td>
<td>indirect</td>
<td>ambient water</td>
<td>2.5 h</td>
<td>$10^5$ cfu/mL</td>
<td>[115]</td>
</tr>
<tr>
<td>L. monocytogenes</td>
<td>plastic fiber optic fluorescence</td>
<td>sandwich</td>
<td>hot dog bologna</td>
<td>2.5 h</td>
<td>$4.3 \times 10^3$ cfu/mL, 10 cfu/g (24 h)</td>
<td>[116]</td>
</tr>
<tr>
<td>L. monocytogenes</td>
<td>plastic fiber optic fluorescence</td>
<td>sandwich</td>
<td>meat products</td>
<td>4.0 h</td>
<td>$10^5$ cfu/mL, 10$^2$ cfu/g (18 h)</td>
<td>[117]</td>
</tr>
<tr>
<td>L. monocytogenes</td>
<td>plastic fiber optic fluorescence</td>
<td>sandwich</td>
<td>frankfurter</td>
<td>12 min</td>
<td>$5 \times 10^5$ cfu/mL</td>
<td>[118]</td>
</tr>
<tr>
<td>E. coli O157:H7</td>
<td>plastic fiber optic fluorescence</td>
<td>sandwich</td>
<td>beef chicken turkey</td>
<td>4.0 h</td>
<td>$10^3$ cfu/mL, 4 cfu/g (18 h)</td>
<td>[119]</td>
</tr>
<tr>
<td>S. typhimurium</td>
<td>FRET-based optical fiber</td>
<td>direct</td>
<td>buffer ground pork</td>
<td>5 min</td>
<td>$10^3$ cells/mL, $10^5$ cfu/g</td>
<td>[120]</td>
</tr>
<tr>
<td>E. coli</td>
<td>fiber optic with a gold layer and ZnO nanorods</td>
<td>direct</td>
<td>water</td>
<td>3 s</td>
<td>$10^3$ cfu/mL</td>
<td>[121]</td>
</tr>
<tr>
<td>E. coli O157:H7</td>
<td>fiber optic SPR</td>
<td>direct</td>
<td>drinking water</td>
<td>~15 min</td>
<td>94 cfu/mL</td>
<td>[123]</td>
</tr>
</tbody>
</table>

* Enrichment time required to achieve the relevant LOD.

3.1.3. Interferometric Immunosensors

Interferometric immunosensors are another category of devices that have been implemented for the detection of different bacteria. These sensors can detect refractive index changes down to $10^{-8}$ RIU and have demonstrated excellent analytical performance regarding the determination of analytes in complex matrices [124,125]. The most popular configurations of interferometric sensors are Mach–Zehnder (MZI) [125–128], Young (YI) [129], Hartman [130–132], and bimodal interferometers [133,134] (Figure 8).

In Mach–Zehnder interferometers (Figure 8a), a waveguide splits into two arms: one that can detect the variations in the refractive index over its surface through a window in the cladding layer (sensing arm) and the other which is fully covered by the cladding layer and operates as a reference (reference arm) [124,125]. The two arms combine again after some point to a single waveguide, and the output light intensity is monitored. Biomolecular reactions taking place in the sensing arm window change the refractive index and cause a phase difference between the light beams guided in the two arms. Thus, the output light is a cosine function of the input light. This means that the sensitivity to effective refractive index changes would be the maximum at the quadrature points and the minimum in the vicinity of the extrema. Regarding the geometrical characteristics of the transducer, most MZIs are symmetric, i.e., the sensing and the reference arms have equal lengths while asymmetric MZIs, i.e., MZIs with different lengths for the two arms, have been also explored. The majority of MZI-based detection systems implement monochromatic light
sources, i.e., lasers, which complicate the instrument miniaturization and development of portable systems; therefore broad-band light sources have been explored instead of lasers. In this direction, external broad-band light sources have been coupled to MZIs integrated on the substrate [126,127], or silicon light-emitting diodes (LED) integrated onto the same silicon chip with planar silicon nitride waveguides have been implemented [125,127]. It should be noted that the detection sensitivities in terms of the refractive index achieved with these configurations were comparable to those of MZIs implementing lasers as light sources.

A Young interferometer (YI) also consists of a waveguide divided into two arms by means of a Y-junction (Figure 8b). The critical difference between MZIs and YIs is that in the second case, the two waveguides do not combine again but the output light interferes in air and the “interferogram” created is depicted on a CCD array. Thus, in YIs, the changes in the effective refractive index over the sensing arm due to binding reactions that cause the phase difference between the two interfering beams are recorded as a shift in the interference fringes [124]. There are considerably fewer reports of integrated YIs as compared to MZIs; nonetheless, it has been demonstrated that YI-based sensors can achieve an LOD \(9 \times 10^{-9}\) in terms of the bulk refractive index and therefore, they are supposed to be more sensitive than the other interferometric sensors as well as SPR.

Hartman interferometers are based on planar waveguides in which the two modes of light, TE and TM, propagate and interact with the adlayer on the same path (Figure 8c) [124]. Changes in the refractive index cause phase shifts of the two polarizations that are not equal because the sensitivity of the two modes to refractive index changes differs.

Bimodal interferometers are the fourth and most recently developed category of interferometric sensors (Figure 8d) [124]. In a bimodal interferometer, the transducer is a single waveguide with two different zones: the first supporting a single mode and the second supporting two modes (fundamental and first-order modes). Those two modes interfere and propagate until they reach the waveguide’s output. When refractive index changes occur at the waveguide surface, due to binding reactions, the interference pattern at

---

**Figure 8.** Schematic representation of (a) Mach–Zehnder, (b) Young, (c) Hartman, and (d) bimodal interferometer [124]. The arrows indicate the incoming and outgoing light beam. Copyright 2014. Reproduced with permission from the Elsevier B.V.
the waveguide output also changes since the velocity with which the two modes propagate depends on the refractive index of the waveguide adlayer.

The first report for bacteria detection based on interferometric sensors was the immunochemical detection of *S. typhimurium* with a Hartman interferometer in buffer [130]. The sensor could detect $5 \times 10^8$ cfu/mL of Salmonella in a direct assay that lasted 40 min. An integrated two-channel Hartman interferometer was also applied for the detection of *S. typhimurium* in spiked chicken rinse fluid [132]. Detection by direct binding of Salmonella cells to antibody-modified waveguides was compared to a sandwich assay. Both configurations provided an LOD of $10^4$ cfu/mL for an assay duration of 10 min, which corresponded to 20 cfu/mL of chicken rinse fluid after 12 h of enrichment.

Mach–Zehnder interferometers (MZIs) have also been employed for bacteria detection. Thus, MZIs integrated onto silicon chips were modified with an antibody against *L. monocytogenes* following a chemical activation protocol specific to silicon nitride so as to limit antibody attachment to the sensing window areas [126]. The protocol consisted of chip treatment with HF for the creation of amine groups onto the silicon nitride followed by reaction with glutaraldehyde to enable the covalent bonding of antibody molecules through their free amine group. An LOD of $10^5$ cfu/mL was achieved for a direct binding assay of 15 min. In another report, the MZIs’ waveguide was formed by patterning a photoresist layer deposited on a glass coverslip [127]. The immobilization of an antibody against *E. coli* was performed after modification of the sensing arm with an aminosilane and subsequent activation with glutaraldehyde. The assay duration was 10 min and the LOD was $10^5$ cfu/mL. In another report, a chip integrating ten MZIs along with the respective silicon light-emitting diodes was employed for the simultaneous detection of *S. typhimurium* and *E. coli* through a competitive immunoassay format [128]. The chip was activated with aminosilane, and then the liposaccharides of *S. typhimurium* and *E. coli* were spotted onto the sensing arm windows of different MZIs of the chip and immobilized through physical adsorption. MZIs spotted with the blocking protein (bovine serum albumin) were used as reference sensors. For the assay, mixtures of calibrators or samples with the bacteria-specific antibodies were run over the chip followed by reaction with biotinylated secondary antibodies and streptavidin for signal enhancement. Following this format, LODs of 40 cfu/mL for *S. typhimurium* and 110 cfu/mL for *E. coli* in both water and milk samples were achieved for a 10-min assay [128]. It was also calculated that 7.5 h enrichment was necessary to detect 1 cfu/25 mL of *Salmonella* spp. and *E. coli* in order to comply with the EU legislation (CE 1441/2007), which requires the absence of these bacteria (zero tolerance) in 25 g of milk or drinking water.

Regarding Young interferometers there are no reports for the detection of bacteria, although they have been employed for the immunochemical detection of viruses, and more specifically of herpes simplex virus type 1 (HSV-1), achieving an LOD of 850 particles/mL [129]. On the other hand, the label-free detection of *B. cereus* and *E. coli* with a bimodal interferometric immunosensor has also been reported [132]. The immobilization of antibodies was carried out either by physical adsorption on aminosilane-modified chips or by covalent binding to chips modified with a carboxysilane after conversion of surface carboxyl groups to active ester groups. The device could detect 70 cfu/mL of *B. cereus* in 12.5 min and 40 cfu/mL of *E. coli* in 25 min. The same bimodal interferometric sensor was also applied for the detection of multidrug-resistance bacteria genes without amplification through a DNA hybridization assay [133]. Table 3 summarizes the application and analytical performance of integrated interferometric sensors for the detection of bacteria in water and food samples.

3.1.4. Grating-Coupler-Based Immunosensors

Grating coupler sensors are based on planar waveguides with a grating incorporated into the waveguide to enable coupling and transmission of the incident light in a manner dependent on the refractive index of the medium over the waveguide surface [42]. Thus, binding reactions that occur onto the waveguide surface can be monitored by determining
the incident light in-coupling angle. In addition, since the binding reactions affect the transmitted light (through interaction with the evanescent wave field), the light out-coupling angle is also altered and can also be used to monitor the binding reactions. The second configuration is advantageous compared to the first one since there is no need for precision alignment of the light, thus leading to simpler experimental setups. Over the years, grating coupler sensors have been upgraded through the introduction of two-dimensional grating structures [135] or new configurations such as wavelength-interrogated optical sensors (WIOSs), which implement two gratings for light in- and out-coupling to the waveguide [136], or angle interrogated optical sensors, which use a MEMS micro-mirror to scan the angle of the incident light of the gratings [137]. All these novel configurations have allowed for the development of systems for multiplexed determinations of analytes. Gratings have also been combined with other evanescent wave-based biosensors such as interferometric [138], SPR [139], SPR imaging [140], optical fiber [141], and silicon microring resonators [142] in order to create more flexible and sensitive biosensors.

Table 3. Bacteria detection with integrated interferometric immunosensors.

<table>
<thead>
<tr>
<th>Analyte</th>
<th>Detection Principle</th>
<th>Assay Type</th>
<th>Sample Type</th>
<th>Assay Duration</th>
<th>LOD</th>
<th>Ref. No.</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>S. typhimurium</em></td>
<td>Hartman interferometer</td>
<td>sandwich and direct</td>
<td>buffer</td>
<td>10 min</td>
<td>$10^4 \text{ cfu/mL}$</td>
<td>$20 \text{ cfu/mL (12 h)}$</td>
</tr>
<tr>
<td><em>S. typhimurium E. coli</em></td>
<td>MZI</td>
<td>competitive with biotinylated secondary antibodies and streptavidin</td>
<td>water milk</td>
<td>10 min</td>
<td>$40 \text{ cfu/mL}$</td>
<td>$110 \text{ cfu/mL}$</td>
</tr>
<tr>
<td><em>E. coli</em></td>
<td>Bimodal interferometer</td>
<td>direct</td>
<td>buffer</td>
<td>25 min</td>
<td>$40 \text{ cfu/mL}$</td>
<td>[133]</td>
</tr>
<tr>
<td><em>B. cereus</em></td>
<td></td>
<td></td>
<td></td>
<td>12.5 min</td>
<td>$70 \text{ cfu/mL}$</td>
<td></td>
</tr>
</tbody>
</table>

* Enrichment time required to achieve the relevant LOD.

A reverse symmetry waveguide sensor with an integrated grating coupler was applied for the detection of *E. coli K12* in buffer by monitoring the adhesion of bacteria cells onto the sensor surface, which was modified with poly-L-lysine, creating a positively charged layer that enabled bacteria binding through electrostatic interactions [143]. An LOD of 60 cells/mm$^2$ was achieved following this approach [143]. Optical fiber long-period grating (LPFG) sensors have also been used for the detection of *Staphylococcus aureus* in buffer through modification of the fiber surface with ionic self-assembled multilayers that facilitated the covalent bonding of antibodies specific to penicillin-binding protein 2a of methicillin-resistant staphylococci. The sensor could discriminate between methicillin-resistant and methicillin-sensitive bacteria, with an LOD of $10^5 \text{ cfu/mL}$ for methicillin-resistant bacteria [144]. Similarly, a long-period fiber grating sensor modified with nano-pitted polyelectrolyte coatings and an antibody was implemented for detection of *S. aureus* in buffer, with an LOD of 224 cfu/mL for a 30-min assay [145]. Another LPF immunosensing platform formed by two identical cascaded chirped long period gratings was applied for the detection of *E. coli* in buffer (Figure 9) [146]. The sensor worked like a Mach–Zehnder interferometer due to the space between the two gratings and could detect *E. coli* at concentrations as low as 7 cfu/mL. Finally, a grating-coupler-based biosensing platform known as Optical Waveguide Lightmode Spectroscopy System (OWLS), commercialized by Microvacuum Ltd. (Microvacuum Ltd.; URL: http://www.owls-sensors.com/, accessed on 18 June 2023), was applied for the label-free immunochemical detection of *S. typhimurium* [147] and *L. pneumophila* [148] in water following a direct binding assay format with LODs of $1.3 \times 10^3$ and $1.3 \times 10^4 \text{ cfu/mL}$, respectively.
3.1.5. Ring-Resonator-Based Immunosensors

Ring resonators rely on the coupling of light propagating along a linear waveguide, through the evanescent wave field, to a circular one on which it propagates in the form of whispering-gallery modes. Any change in the refractive index in the proximity of the ring surface affects the spectral position of the whispering-gallery modes and changes the wavelength of the incident light for which resonance is achieved. As the light propagating in the ring can interact with the molecules on its surface multiple times, ring resonators are expected to provide the same performance (denoted by the Q factor) as that obtained from linear waveguides with many times longer lengths. Thus, by implementing ring resonators as transducers, smaller-sized devices as compared to linear waveguides and denser transducer arrays can be realized. Ring resonators can adopt not only the 2D format of a microdisk [149] or microring [150] but also the 3D format of a microtoroid [151]. Toroids claim higher Q factors than the planar resonators and therefore, higher detection sensitivity is expected. Sensors based on microring resonators have also been explored with regard to bacteria detection. The detection of *E. coli* in buffer with a microring resonator sensor has been reported and the relatively high LOD of $10^5$ cfu/mL was ascribed to suboptimal functionalization of resonators with bacteria-binding antibodies (Figure 10) [152].

![Figure 9](image_url)  
**Figure 9.** Schematic of antibody immobilization and *Escherichia coli* bacteria detection by a cascaded chirped long-period gratings immunosensor [146]. Copyright 2019. Reproduced with permission from AIP Publishing.

![Figure 10](image_url)  
**Figure 10.** Scanning electron micrographs of a small section of the test and control micro-rings on a resonator chip showing specific bacterial binding at $2200 \times$ magnification [152]. Copyright 2007. Reproduced with permission from Elsevier B.V.

Finally, a whispering gallery mode optical microdisk resonator was modified with the phage protein LysK, an endolysin from the staphylococcal phage K that binds strongly to staphylococci and used to detect *S. aureus* in buffer with an LOD of $5 \times 10^6$ cfu/mL [153].

3.2. Reflectrometric Immunosensors

Reflectrometric sensors rely on monitoring shifts in the interference spectrum due to binding reactions taking place on a stack of materials with different refractive indices. The illumination sources usually employed are white light sources.

A commercially available RIfS-based biosensing device was used to detect *L. pneumophila* in buffer by monitoring either the direct capture of bacteria cells via electrostatic
interaction onto the chip surface or through a sandwich assay [154]. The device’s performance was compared to that of SPR, and an LOD of $1 \times 10^5$ cfu/mL was determined in both cases. A white-light-reflectance-spectroscopy-based immunosensor exhibited better performance regarding the rapid and sensitive detection of *S. typhimurium* in drinking water (Figure 11) [155]. The sensor chip consisted of a Si die with a thin SiO$_2$ layer on top, and *S. typhimurium* detection was performed through a competitive immunoassay format between the bacteria in the sample and the *Salmonella* liposaccharide immobilized onto the chip. An LOD of 320 cfu/mL in drinking water was achieved for an assay duration of 15 min.

**Figure 11.** Illustration of the white light reflectance spectroscopy (WLRS) optical setup and sensing principle [155]. Copyright 2021. Reproduced with permission from MDPI.

Porous silicon has been also employed as substrate for detection of bacteria through reflectance spectroscopy. Thus, the label-free detection of *E. coli K12* in buffer was achieved using a sensor based on a nanostructured oxidized porous silicon thin film [156,157]. The sensor surface was functionalized with specific antibodies against *E. coli* through aminosilanization and activation of surface amine groups with bis(N-succinimidyl)carbonate for the coupling of antibodies via their free amine groups [156,157]. The LOD determined was about $10^4$ cells/mL and the assay was completed in 30 min. Optimization of the same sensor, in which a different surface modification was followed (Figure 12), resulted in an LOD of $10^3$ cells/mL in water for an assay of 45 min [158]. In this case, after aminosilanization, the surface was functionalized with glutaraldehyde to introduce aldehyde groups through which streptavidin was bound onto the surface to facilitate immobilization of biotinylated anti-bacteria-specific antibodies.

Another biosensor developed for *E. coli* detection on porous silicon substrates involved surface modification with a hydrogel made of polyacrylamide to which biotinylated specific monoclonal antibodies were immobilized onto the streptavidin covalently bound to the surface after appropriate chemical functionalization [159]. A detection limit in the range of $10^5 - 10^6$ cell/mL was determined for a direct bacteria binding assay of 30 min. Furthermore, for the direct detection of *E. coli*, a biosensor based on the blockage of nanopores created by etching of a Si chip was presented, achieving a detection limit in buffer of $10^3$ cfu/mL [160]. More specifically, when *E. coli* cells were trapped into the chip nanopores, a decrease in effective optical thickness was recorded. Thus, by monitoring the change of effective optical thickness value, it was possible to quantitatively determine the cells captured in the nanopores via indirect Fourier Transformed Reflectometric Interference Spectroscopy.
Figure 12. (a) A top-view high-resolution scanning electron microscope image of a porous SiO₂ film with pores in the range of 60–100 nm. (b) Schematic representation of the steps followed to bio-functionalize the porous SiO₂ surface with IgG, including: (I) 3-aminopropyl-triethoxysilane modification, (II) reaction of amine-terminated porous SiO₂ with one of the aldehyde groups of the cross-linker glutaraldehyde, (III) grafting of streptavidin onto the aldehyde-terminated surface, and (IV) conjugation of biotinylated-IgG (E. coli) via biotin-streptavidin binding [158]. Copyright 2016. Reproduced with permission from Springer Nature.

Finally, an interferometric reflectance imaging system (IRIS) was employed for the label-free detection of E. coli in tap water [161]. The bacteria specific antibody was spotted onto a SiO₂/Si chip modified with a polymer and after incubation for 2 h with the bacteria solutions the bound cells were counted using a low-magnification optical setup accompanied by an appropriate software. Based on experimental data, an extrapolated LOD of 2.2 cfu/mL was calculated which is the lowest, so far, reported for direct bacteria detection. Table 4 summarizes the analytical performance of reflectometric sensors for detection of bacteria in water and food samples.

<table>
<thead>
<tr>
<th>Analyte</th>
<th>Detection Principle</th>
<th>Assay Type</th>
<th>Sample Type</th>
<th>Assay Duration</th>
<th>LOD</th>
<th>Ref. No</th>
</tr>
</thead>
<tbody>
<tr>
<td>S. typhimurium</td>
<td>WLRS</td>
<td>competitive</td>
<td>drinking water</td>
<td>15 min</td>
<td>320 cfu/mL</td>
<td>[155]</td>
</tr>
<tr>
<td>E. coli</td>
<td>reflectance spectroscopy on porous silicon</td>
<td>direct</td>
<td>water</td>
<td>45 min</td>
<td>10⁴ cells/mL</td>
<td>[158]</td>
</tr>
<tr>
<td>E. coli</td>
<td>reflectance spectroscopy imaging</td>
<td>direct</td>
<td>tap water</td>
<td>2 h</td>
<td>2.2 cfu/mL</td>
<td>[161]</td>
</tr>
</tbody>
</table>

3.3. Photoluminescence-Based Immunosensors

Photoluminescence (fluorescence and phosphorescence) is the phenomenon of light emission from a molecule that has been excited by absorption of photons in the visible or UV region [162]. Thus, the decrease in photoluminescence intensity of TiO₂ nanoparticles deposited on glass slides and modified with antibodies against S. typhimurium upon binding of bacteria was exploited in an immunosensor that could detect S. typhimurium in the range from 10⁴ to 10⁵ cells/mL [163]. In another report, a soda lime glass was employed as a waveguide and microarray substrate for the detection of S. typhimurium in several food samples as well as in chicken excretal samples through a sandwich immunoassay with fluorescently labeled detection antibodies [164]. The LOD achieved was 8 × 10⁴ cfu/mL for an assay duration of 15 min and could be improved 10-times by increasing the assay duration to 1 h [164]. This array biosensor was also used to detect Shigella dysenteriae and Campylobacter jejuni in buffer and a variety of food and beverage samples (chicken carcass rinse, ground turkey, buffered milk, and lettuce leaf rinse) at concentrations of 4.9 × 10⁴ and 9.7 × 10⁵ cfu/mL, respectively, by applying sandwich immunoassays that
lasted 25 min [165]. The same sensor was applied to detect the bacterium *Campylobacter jejuni* following a 25-min sandwich immunoassay in a number of different food matrices (ground turkey, sausage, ham, carnation non-fat dried milk, and vanilla fat-free yogurt) with an LOD of 500 cells/mL [166]. Finally, the sensor was applied to detect *Escherichia coli* in less than 30 min in various spiked food matrices (ground beef, turkey sausage, carcass wash, and apple juice) with LODs in the range 1–5 × 10⁴ cells/mL [167].

A homogeneous FRET immunosensor using antibodies conjugated to graphene oxide quantum dots and graphene oxide sheets was designed for the detection of *C. jejuni* cells in food samples [168]. The antibody conjugated to graphene oxide quantum dots interacted with the graphene oxide sheets through π-π stacking, leading to fluorescence quenching. When *C. jejuni* was selectively captured by the antibody, this interaction was disrupted, and the fluorescence emission increased proportionally to the concentration of bacteria in the sample. The assay was completed in 1.5 h and the LOD for the detection of *C. jejuni* to poultry liver was 100 cfu/mL. In Table 5, the analytical performance of photoluminescence-based sensors for the detection of bacteria in water and food samples are presented.

Table 5. Photoluminescence-based immunosensors for bacteria detection.

<table>
<thead>
<tr>
<th>Analyte</th>
<th>Detection Principle</th>
<th>Assay Type</th>
<th>Sample Type</th>
<th>Assay Duration</th>
<th>LOD</th>
<th>Ref. No.</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>S. typhimurium</em></td>
<td>fluorescence</td>
<td>sandwich</td>
<td>sausage, cantaloupe, whole liquid egg, alfalfa sprouts, chicken carcass rinse, chicken excretal samples</td>
<td>15 min</td>
<td>8 × 10⁴ cfu/mL, 8 × 10³ cfu/mL or cfu/g</td>
<td>[164]</td>
</tr>
<tr>
<td><em>Shigella dysenteriae</em></td>
<td>fluorescence</td>
<td>sandwich</td>
<td>buffer, chicken carcass rinse, ground turkey, buffered milk, lettuce leaf rinse</td>
<td>25 min</td>
<td>4.9 × 10⁴ cfu/mL, 9.7 × 10² cfu/mL</td>
<td>[165]</td>
</tr>
<tr>
<td><em>C. jejuni</em></td>
<td>fluorescence</td>
<td>sandwich</td>
<td>ground turkey, sausage, ham, carnation non-fat dried milk, vanilla fat-free yogurt</td>
<td>25 min</td>
<td>500 cells/mL</td>
<td>[166]</td>
</tr>
<tr>
<td><em>E. coli</em></td>
<td>fluorescence</td>
<td>sandwich</td>
<td>ground beef, turkey, sausage, carcass wash, apple juice</td>
<td>30 min</td>
<td>1–5 × 10⁴ cells/mL</td>
<td>[167]</td>
</tr>
<tr>
<td><em>C. jejuni</em></td>
<td>homogeneous FRET</td>
<td>direct</td>
<td>poultry liver</td>
<td>1.5 h</td>
<td>100 cfu/mL</td>
<td>[168]</td>
</tr>
</tbody>
</table>

3.4. Surface Enhanced Raman Scattering (SERS)-Based Immunosensors

SERS-based biosensors combine Raman inelastic scattering of incident light with signal enhancement provided by nanostructured noble metal substrates [169]. More specifically, the Raman signal of molecules adsorbed onto the SERS surfaces is enhanced by a factor of 10⁴–10⁸ due to the strong electromagnetic field generated on the surface of these substrates. This enhanced scattering phenomenon results in characteristic peaks due to resonance with the vibrational modes of the molecules, which are unique for each molecule. Thus, in SERS-based bioanalysis, the target molecules can either be detected directly after their attachment to the nanostructured surfaces (Figure 13a), through their binding to surface-anchored recognition elements, such as antibodies (Figure 13b), or through sandwich immunoassays employing SERS-active labels for signal enhancement (Figure 13c) [170–173]. SERS active
labels or tags are prepared by conjugating the analyte-specific antibodies to nanoparticles along with Raman reporter molecules, which are low-molecular-weight moieties with strong and distinguishable Raman signals. All of these approaches have been implemented for bacteria detection through SERS.

**Figure 13.** Schematic illustration of SERS-based approaches for detection (a) without any specific recognition molecule, (b) through binding to a surface-immobilized antibody, or (c) through sandwich immunoassays employing a surface-immobilized capture antibody and a detection antibody labeled with a SERS tag.

Knauer et al. developed direct label-free SERS-based immunochemical methods for the detection of *L. pneumophila* and *S. typhimurium* in a single run [174], and of *E. coli* separately [175]. Regarding the simultaneous determination of *S. typhimurium* and *L. pneumophila*, glass substrates were modified with an epoxy-silane and then reacted with a diamine-polyethylene glycol to introduce amine groups onto the surfaces, which were then implemented for the covalent bonding of antibodies specific against the two bacteria by spotting of the respective solutions at different areas of the substrate. After bacteria binding, the surfaces were incubated with an Ag colloid preparation that aggregated onto the immobilized bacteria, creating “hot spots” which provided a strong Raman signal. The assay lasted 65 min, and the LODs achieved were $10^6$ and $10^8$ cells/mL for *L. pneumophila* and *S. typhimurium*, respectively, in the water samples [174]. For *E. coli* detection, the antibody-modified substrates were combined with a flow-through system, enabling the detection of *E. coli* strains in water samples at concentrations down to $4.3 \times 10^3$ cfu/mL [175].

An LOD of 10 cfu/mL in spiked ground beef homogenates was achieved for *E. coli* O157:H7 with a SERS-based non-competitive immunoassay that combined antibody-modified magnetic particles and gold nanoparticles modified with an anti-bacterium antibody and a SERS tag molecule [176]. At first, the bacteria solution was incubated with the antibody-labeled magnetic nanoparticles, and after magnetic separation, the bound bacteria were incubated with the SERS-tagged antibody-modified gold nanoparticles. The immunocomplexes formed were separated from free antibodies using a membrane filter on which the Raman signal was determined [176].

A similar immunoassay format was applied to the simultaneous detection of *E. coli* O157:H7 and *S. aureus*, with LODs of 10 and 25 cfu/mL, respectively [177]. In this case, magnetic beads and SERS active gold nanoparticles functionalized with specific antibody pairs against each bacterium were used, and the simultaneous determination was based on implementation of a different SERS tag for each bacterium. After the formation of immunocomplexes in the liquid phase, a magnetic field was applied and the Raman signals from the two different tags were quantified [177]. The sensor was applied for the detection of the two bacteria in spiked samples of bottled water and milk.

In another report, the capture antibodies were immobilized on magnetite-gold nanoparticles to enable separation and concentration of the *E. coli* O157 cells from the liquid, followed by reaction with gold nanoparticles modified with antibodies and Raman tags [178]. This method provided rapid separation and detection (less than one hour) of *E. coli* in apple juice, achieving an LOD of $10^2$ cfu/mL. A similar approach was applied for the isolation and detection of multiple pathogenic bacteria through the implementation of lectin functionalized silver coated magnetic nanoparticles prior to reaction with SERS-tagged silver.
nanoparticles functionalized with antibodies specific to each one of the targeted bacteria. Thus, *E. coli*, *S. typhimurium*, and *methicillin-resistant S. aureus* isolation and detection in buffer were achieved at concentrations as low as 10 cfu/mL [179].

Gold-coated magnetic nanoparticles (gold-coated MnFe$_2$O$_4$ nanoparticles) were conjugated with antibodies against *S. aureus* and used as SERS tags, as well as for bacteria capture and separation from spiked buffer samples, resulting in an assay with an LOD of 10 cfu/mL [180]. Spherical and rod-shaped gold nanoparticles modified with Raman tags and an antibody against *E. coli* were compared as labels in a sandwich immunoassay with capture antibody immobilized onto a gold-coated glass slide [181]. The LODs determined in buffer using the gold nanorods and the spherical gold nanoparticles as labels were 4 and 5 cfu/mL, respectively.

Rod-shaped gold-covered magnetic nanoparticles modified with an antibody against *E. coli* have also been investigated as labels in a liquid phase assay for the detection of *E. coli* in buffer at concentrations as low as 35 cfu/mL [182]. The difference in the LOD of this report compared to the previous one that used the same label [181] was attributed to the lower capture efficiency of magnetic gold nanorod particles as compared to the gold-coated glass slide surface. Thus, when the same group implemented gold-coated magnetic spherical nanoparticles modified with an anti-*E. coli* antibody in combination with rod-shaped gold nanoparticles modified with Raman tags and an anti-*E. coli* antibody, an LOD for *E. coli* detection in water samples of 8 cfu/mL was achieved [183].

In another detection approach, SERS was combined with a microfluidic dielectrophoretic device to detect *Salmonella enterica serotype Choleraesuis* and *Neisseria lactamica* in buffer [184]. The SERS labels employed were silica-coated dye-induced aggregates of a small number of gold nanoparticles, denoted as nanoaggregate-embedded beads, and they were modified with antibodies specific to each bacterium to allow their online detection with an LOD of 70 cfu/mL.

SERS detection was also combined with lateral flow strip biosensors. Thus, *L. monocytogenes* and *S. typhimurium* were simultaneously detected with a lateral flow sandwich immunoassay employing gold nanoparticles modified with a Raman tag and specific antibodies against each one of the targeted bacteria [185]. The LODs achieved were 75 cfu/mL for both bacteria, and the strip assay was applied for bacteria detection in milk samples. Another lateral flow strip biosensor employing SERS labels combined with recombinase polymerase amplification (RPA) was applied for the simultaneous determination of *S. enteritidis* and *L. monocytogenes* [186]. The method made use of forward primers labeled with digoxin and fluorescein for *S. enteritidis* and *L. monocytogenes*, respectively, whereas the reverse primers were labeled with biotin. Thus, when the RPA product was applied to the sample pad, it was run along with gold nanoparticles modified with streptavidin and Raman tags toward the two test lines where antibodies against digoxin and fluorescein have been spotted, resulting in the creation of the respective colored lines (Figure 14). The LODs achieved in buffer were 27 and 19 cfu/mL for *S. enteritidis* and *L. monocytogenes*, respectively [186]. The method was applied for the detection of the two bacteria in milk, chicken breast, and beef samples with slightly increased LODs, which were 31, 35, and 35 cfu/mL for *S. Enteritidis* and 36, 29, and 22 cfu/mL for *L. monocytogenes*, respectively. The same approach was employed to detect *Escherichia coli* O157:H7, with an LOD of $5 \times 10^4$ cfu/mL in milk, chicken breast, and beef [187].

In Table 6, data regarding bacteria detection with SERS-based biosensors in food and water samples are presented.
Figure 14. (a) Schematic illustration of: (A) preparation of gold nanoparticles modified with streptavidin and Raman tags (MBA), and (B) the multiplex lateral flow SERS assay. (b) Results from lateral flow strips for the simultaneous detection of S. enteritidis (test line 1) and L. monocytogenes (test line 2). The test lines could be observed by the naked eye at a concentration of $1.9 \times 10^2$ cfu/mL of L. monocytogenes and $2.7 \times 10^3$ cfu/mL of S. enteritidis. The SERS signal of the test lines could be detected at a concentration of $1.9 \times 10^1$ cfu/mL of L. monocytogenes and $2.7 \times 10^1$ cfu/mL of S. enteritidis. (B) Specificity of the lateral flow strip assay against three L. monocytogenes and three S. enteritidis bacteria [186]. Copyright 2017. Reproduced with permission from the American Chemical Society.

Table 6. SERS-based immunosensors for bacteria detection.

<table>
<thead>
<tr>
<th>Analyte</th>
<th>Detection Principle</th>
<th>Assay Type</th>
<th>Sample Type</th>
<th>Assay Duration</th>
<th>LOD</th>
<th>Ref. No</th>
</tr>
</thead>
<tbody>
<tr>
<td>L. pneumophila</td>
<td>SERS</td>
<td>direct</td>
<td>water</td>
<td>65 min</td>
<td>$10^6$ cfu/mL</td>
<td>[174]</td>
</tr>
<tr>
<td>S. typhimurium</td>
<td>SERS</td>
<td>direct</td>
<td>water</td>
<td>&gt;60 min</td>
<td>$4.3 \times 10^3$ cfu/mL</td>
<td>[175]</td>
</tr>
<tr>
<td>E. coli O157:H7</td>
<td>SERS</td>
<td>sandwich</td>
<td>ground beef</td>
<td>~2 h</td>
<td>10 cfu/mL</td>
<td>[176]</td>
</tr>
<tr>
<td>E. coli S. aureus</td>
<td>SERS</td>
<td>sandwich</td>
<td>bottled water</td>
<td>~2 h</td>
<td>10 cfu/mL</td>
<td>25 cfu/mL</td>
</tr>
<tr>
<td>E. coli O157</td>
<td>SERS</td>
<td>sandwich</td>
<td>apple juice</td>
<td>&lt;1 h</td>
<td>$10^6$ cfu/mL</td>
<td>[178]</td>
</tr>
<tr>
<td>E. coli</td>
<td>SERS</td>
<td>sandwich</td>
<td>water</td>
<td>70 min</td>
<td>8 cfu/mL</td>
<td>[183]</td>
</tr>
<tr>
<td>L. monocytogenes</td>
<td>LFIA SERS</td>
<td>sandwich</td>
<td>milk</td>
<td>10 min</td>
<td>75 cfu/mL</td>
<td>[185]</td>
</tr>
<tr>
<td>S. typhimurium</td>
<td>SERS</td>
<td>sandwich</td>
<td>buffer chicken</td>
<td>30 min</td>
<td>27/19 cfu/mL</td>
<td>35/29 cfu/mL</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>breast beef</td>
<td></td>
<td>35/22 cfu/mL</td>
<td>31/36 cfu/mL</td>
</tr>
<tr>
<td>E. coli O157: H7</td>
<td>LFIA SERS</td>
<td>sandwich</td>
<td>milk</td>
<td>15 min</td>
<td>$5 \times 10^4$ cfu/mL</td>
<td>[187]</td>
</tr>
</tbody>
</table>

4. Conclusions and Outlook

This review has outlined the principles and applications of antibody-based optical sensors in the detection of food pathogenic bacteria. Although there is an abundance of relative publications, it seems that very few of the available sensing principles have been
exploited for the detection of bacteria in real food samples. An additional reason for that can be the fact that the LODs achieved in most cases cannot cover the requirements set by the existing regulations, according to which highly dangerous and contagious bacteria like *Salmonella* and *L. monocytogenes* should not be detected in 25 g of food samples (e.g., Commission Regulation (EC) No 2073/2005).

Thus, although fiber optic sensors employing fluorescent labels have been the first to be implemented for bacteria detection in food samples, the great majority of references, especially the most recent ones, rely on SPR sensors. The reason for that is probably due to the availability of different instruments based on the SPR detection principle, some of which are commercially available. Nevertheless, the LODs achieved in most of the cases ranged from $10^3$ to $10^5$ cfu/mL, which are considered high for direct application to food analysis, and they should be combined with a sample enrichment procedure that adds a few hours to the total time required from sampling to answer.

There are a few reports, however, that report LODs of a few or a few tens of cfu/mL [66,95,97,103–105], from which only one is performed in a standard benchtop SPR instrument [66], and two others with a commercially available portable SPR instrument (SPREETA™) [95,97], which is not currently in the market. The first two reports were based on direct binding assays for the label-free detection of targeted bacteria [66,95], whereas in the third, gold magnetic particles were employed as labels to drop the LOD to 3 cfu/mL [97]. The rest of the reports implement the localized SPR (LSPR) principle either in the form of gold nanoparticles [103,104] or in the form of nanostructured gold film [105]. In addition to higher detection sensitivity, LSPR is also considered more easily adaptable to portable low-cost devices, and it remains to be seen what would be carried out in this direction in the near future.

Regarding the majority of fiber-optic-based immunosensors, the LODs achieved for bacteria detection also ranged from $10^3$ to $10^5$ cfu/mL. LODs of less than 100 cfu/mL were achieved only when fibers were combined with SPR via covering their sensing area with a gold film or gold nanoparticles or films of other materials (e.g., MoS$_2$ nanosheets) [122,123]. Since fiber optic experimental setup can also be reduced in size and cost, the combination with SPR might also be proved a viable solution for portable devices in the future.

Integrated interferometers, particularly in the form of MZIs [128] and bimodal interferometers [133], have shown adequate sensitivity for bacteria detection (LODs in the range of a few tens of cfu/mL) combined with short assay times and great potential for multiplexed determinations. They also seem to be the most promising candidate in the direction of portable devices suitable for point-of-need determinations. On the other hand, microring resonators or microtoroids, despite their claimed high detection sensitivity, are the optical transducers less frequently employed for bacteria detection, whereas a few existing reports present LODs as non-competitive to other types of optical biosensors [152,153]. The same seems to be true for most of the immunosensors based on grating couplers for which there is only one report, with an LOD of a few cfu/mL in which an optical fiber grating device was implemented [144].

Immunosensors based on reflectance spectroscopy have also demonstrated potential for the detection of bacteria at concentrations as low as 2.2 cfu/mL [161], whereas the assay was performed in a 24-well plate with the interferometric reflectance imaging system (IRIS) chip placed at the bottom of each well and lasted over 2 h. A more compact system based on reflectance spectroscopy achieved an LOD of 320 cfu/mL with a 15-min assay, allowing for the detection of bacteria in food samples after a pre-enrichment step of approximately 8 h [155]. The most impressive performance regarding the percentage of reports that mention LODs of less than 100 cfu/mL present immunosensors based on SERS [176–182,184–186]. However, with the exception of two reports where Raman spectroscopy is combined with a lateral flow immunoassay [182,184], for which the assay duration was less than 30 min, most sensors required more than 1 h to complete the assay.

Regarding the assay type implemented for bacteria detection with optical immunosensors, although direct binding onto antibody-modified transducers has been widely used, it
has rarely led to high detection sensitivity combined with a short assay time. Thus, competitive or non-competitive immunoassay formats have been employed to increase detection sensitivity and/or decrease assay duration. This might complicate the development of portable devices a bit since it requires the integration of optical sensors with microfluidic modules, pumps, and valves, which will provide for the automated execution of the assay steps. In addition, this integration should be performed in such a way that it will not increase either the device size or the cost.

In the direction of developing portable devices based on optical transducers, the tools offered by the continuously evolving smartphone gadgets, either as light sources or for the detection of optical signals or the ability to run the instrument software in such a device, process the data, and transfer them wirelessly to central facilities, are considered a significant asset.

Although the current technological limitations are more than certain to be surpassed in the near future, there are some aspects of optical immunosensors that need to be further addressed prior to their application for bacteria detection at the point of need. For example, the study of relevant literature reveals that sensitive bacteria detection with immunochemical techniques is often challenging due to lack of appropriate antibodies. Thus, in many instances, optical immunosensors have been developed using in-house produced antibodies that are not widely available. Even if antibodies with appropriate binding characteristics for the specific and sensitive detection of a particular bacterium are commercially available, their suitability for the detection of bacteria in processed food has to be investigated since the structure of bacteria epitopes could change dramatically when the food has been processed under certain temperature or pH conditions. In addition, in many instances, antibodies cannot discriminate viable from non-viable cells, providing false positive results.

Despite the above-mentioned limitations of optical immunosensors for bacteria detection, they remain one of our best hopes for sensitive portable devices that would be available at affordable prices with low operation costs but most importantly in a shorter time compared to the established techniques for bacteria detection in food. The long time required from sampling to testing by most of the established methods (especially the microbiological ones) is and will remain the driving force behind the development of biosensors for application in food safety. Even if the necessity for sample enrichment remains, suppression of the time required from days to a few hours will help to test a higher amount of raw food materials and ready-to-eat food by both the industry and the food-safety control departments.

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