

## Advances in Rapid Detection Methods for Foodborne Pathogens

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Received: October 7, 2013  
Revised: December 19, 2013  
Accepted: December 22, 2013

First published online  
December 30, 2013

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pISSN 1017-7825, eISSN 1738-8872

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Food safety is increasingly becoming an important public health issue, as foodborne diseases present a widespread and growing public health problem in both developed and developing countries. The rapid and precise monitoring and detection of foodborne pathogens are some of the most effective ways to control and prevent human foodborne infections. Traditional microbiological detection and identification methods for foodborne pathogens are well known to be time consuming and laborious as they are increasingly being perceived as insufficient to meet the demands of rapid food testing. Recently, various kinds of rapid detection, identification, and monitoring methods have been developed for foodborne pathogens, including nucleic-acid-based methods, immunological methods, and biosensor-based methods, etc. This article reviews the principles, characteristics, and applications of recent rapid detection methods for foodborne pathogens.

**Keywords:** Foodborne pathogen, rapid detection, nucleic-acid-based methods, immunological methods, biosensor-based methods

### Introduction

Foodborne pathogens are microorganisms (*i.e.*, bacteria, viruses, and fungi) as well as a number of parasites, which are capable of infecting humans *via* contaminated food or water [24]. In particular, foodborne bacteria such as *Escherichia coli* O157:H7, *Salmonella enterica*, *Staphylococcus aureus*, *Listeria monocytogenes*, *Campylobacter jejuni*, *Bacillus cereus*, and other Shiga-toxin producing *E. coli* strains (non-O157 STEC), and *Vibrio* spp. are leading causes of foodborne diseases. In recent years, diseases caused by foodborne pathogens have become an important public health problem in the world, producing a significant rate of morbidity and mortality [72]. The global incidence of foodborne disease is difficult to estimate, but it has been reported that roughly 1 in 6 Americans in the United States (or 48 million people) gets sick, 128,000 are hospitalized, and 3,000 die of foodborne diseases annually according to CDC 2011 Estimates [9]. A great proportion of these cases

can be attributed to the contamination of food and drinking water. Additionally, diarrhea is a major cause of malnutrition in infants and young children [110]. Although there are 31 pathogens that have been identified as causing foodborne illnesses, *Norovirus*, *Salmonella*, *Campylobacter*, *Staphylococcus aureus*, *Listeria monocytogenes*, *Clostridium perfringens*, *Toxoplasma gondii*, and *Escherichia coli* O157:H7 have been generally found to be responsible for the vast majority of illnesses, hospitalizations, and deaths [9, 100].

The high prevalence of foodborne diseases in many developing countries suggests major underlying food safety problems; therefore, it is important to detect foodborne pathogens in order to reduce foodborne disease occurrence. Traditional methods for the detection of bacterial pathogens from foods depend on culturing the organisms on agar plates; it is a time-consuming process, taking 2–3 days for initial results, and up to more than 1 week for confirming the specific pathogenic microorganisms. It is obvious that culture and colony counting methods are inadequate. In

order to prevent the spread of infectious diseases, ensure the food safety, and thereby to protect public health, there is an ever-increasing demand for more rapid methods of foodborne pathogen detection.

There is a wide variety of microorganisms that are able to produce toxins causing foodborne diseases, mainly *S. aureus*, *Vibrio cholerae*, *Clostridium botulinum*, *C. perfringens*, *Bacillus cereus*, and *E. coli* O157 [27]. Existing literatures report numerous methods developed for the detection of toxin-related genes and their toxin products. The detection methods of toxin-related genes are nucleic-acid-based methods, such as molecular amplification and hybridization probing. The detection methods of toxin products rely primarily on immunological assays, such as ELISA, lateral flow immunoassay and agglutination tests, and bioassays such as mouse neutralization testing and cytotoxicity assays in tissue culture, as well as biosensor-based assay [27, 90].

Recently, many researchers are focusing on the progress of rapid methods for foodborne pathogens. Novel molecular techniques for pathogens are being developed on various aspects of detection, such as sensitivity, rapidity, and selectivity, discrimination of the viable cell, and also suitability for *in situ* analysis. The immunological methods permit the rapid and sensitive analysis of a range of pathogens and toxins, especially with potential for on-site analysis. The emerging biosensor methods can detect foodborne pathogens in a much shorter time with sensitivity and selectivity comparable to the conventional methods and can potentially be used in the future as stand-alone devices for on-site monitoring. According to the main principle, these rapid detection methods can be classified into the following categories: nucleic-acid-based methods, immunological methods, and biosensor-based methods. The purpose of this paper is to review such rapid methods of detection and identification and to discuss some of the more recent and novel methods for the characterization of foodborne pathogens.

## Nucleic-Acid-Based Methods

One of the advantages of nucleic-acid-based food pathogen detection assays is the high level of specificity, as they detect specific nucleic acid sequences in the target organism by hybridizing them to a short synthetic oligonucleotide complementary to the specific nucleic acid sequence. Several different types of nucleic-acid-based assays, including amplification, hybridization, microarrays, and biochips, have been developed for use as rapid

methods to detect foodborne pathogens [92].

### Simple PCR Method

PCR is the most well-known and established nucleic acid amplification technique for detecting pathogenic microorganisms [19]. In this method, double-stranded DNA is denatured into single strands, and specific primers or single-stranded (ss) oligonucleotides anneal to these DNA strands, followed by extension of the primers complementary to the single-stranded DNA, with a thermostable DNA polymerase. These steps are repeated, resulting in doubling of the initial number of target sequences with each cycle. This quantity of the products of amplification can be visualized as a band on an ethidium-bromide-stained electrophoresis gel. Identification based on PCR amplification of target genes by sequencing is considered to be a reliable technique when properly developed and validated for a certain species. With the distinct advantages of rapidity, specificity, sensitivity, and less samples over culture-based methods, many PCR assays for the detection and validation of foodborne bacteria and viruses in food have been developed and applied in food samples [38].

PCR is also used for toxins detection by amplifying specific genes that encode bacterial toxins. PCR methods for toxin detection have been developed for a number of bacterial species, including *V. cholera*, *B. cereus*, *E. coli*, and *S. aureus*. In addition to PCR, a number of gene-specific hybridization probes have been designed and used for the detection of toxin genes in foodborne pathogens [77].

### Multiplex PCR

Simultaneous amplification of more than one locus is required for a rapid detection of multiple microorganisms in a single reaction. It is a methodology referred to as multiplex PCR (mPCR), in which several specific primer sets are combined into a single PCR assay [10]. Apparently, the design of the primers is a key factor in the development of a multiplex PCR assay. There may be some interaction between the multiple primer sets, so the primer concentrations may have to be adjusted in order to generate reliable yields of all the PCR products. Meanwhile, the primer sets should be designed with a similar annealing temperature, while providing a method to distinguish between amplicons following thermal cycling. Today, mPCR can also be useful to define the structure of certain microbial communities and to evaluate community dynamics, such as during fermentation or in response to environmental variations. Kong *et al.* [46] described a rapid mPCR method allowing for the simultaneous detection of six commonly encountered

waterborne pathogens in a single tube. The target genes used were the aerolysin (*aero*) gene of *Aeromonas hydrophila*, the invasion plasmid antigen H (*ipaH*) gene of *Shigella flexneri*, the attachment invasion locus (*ail*) gene of *Yersinia enterocolitica*, the invasion plasmid antigen B (*ipaB*) gene of *Salmonella* Typhimurium, the enterotoxin extracellular secretion protein (*epsM*) gene of *Vibrio cholerae*, and a species-specific region of the 16S-23S rDNA (*Vpara*) gene of *Vibrio parahaemolyticus*.

Park *et al.* [75] established a mPCR assay for the simultaneous detection of *Escherichia coli* O157:H7, *Salmonella* spp., *Staphylococcus aureus*, and *Listeria monocytogenes*, in one tube. The mPCR employed the *Escherichia coli* O157:H7 specific primer Stx2A, *Salmonella* spp. specific primer Its, *S. aureus* specific primer Cap8A-B, and *Listeria monocytogenes* specific primer Hly. Amplification with these primers produced products of 553, 312, 405, and 210 bp, respectively. Recently, Mukhopadhyay *et al.* [66] used *fliCh*, and *iap* gene-specific primers to establish a multiplex-PCR assay for the simultaneous detection of *Escherichia coli* O157:H7 and *Listeria monocytogenes*. Meanwhile, they developed a modified method of enrichment and harvesting, leading to a highly sensitive and rapid single-reaction PCR detection of both pathogens.

### Quantitative PCR

Quantitative PCR (qPCR), also called real-time PCR, is an approach capable of continuously monitoring the PCR product formation throughout the reaction; it offers rapid, simultaneous amplification and sequence-specific-based detection of target genes and is increasingly being applied in food microbiology [41]. Using this method allows quantifying one specific microorganism in food and studying its behavior as a consequence of the influence of the environment (*i.e.*, food composition, temperature, pH, oxygen, *etc.*) by studying expression of suitable target genes. Moreover, the real-time monitoring of the process means no need for post-amplification treatment of the samples, such as gel electrophoresis, reducing the time of analysis. Gomez *et al.* [33] developed a qPCR to quantify the total aerobic bacteria and fungi on fresh produce, using as reference the centrifugation water (CW) that comes up during processing instead of the food matrix itself. On average, 35% of the natural bacterial population and 64% of inoculated bacteria were recovered in the CW. Enumeration of cell number by qPCR did not differ significantly from plate assay and therefore, may replace it. This method could be an alternative to plate assays in order to get reliable information about the aerobic bacterial load of

fresh-cut commodities in less than 5 h.

Derzelle *et al.* [22] developed a multiplex qPCR assay capable of detecting all known *stx* gene variants, including the highly divergent subtype *stx2f*, and evaluated its performance in combination with two different internal amplification controls. The new screening method was tested with artificially and naturally contaminated food samples and compared with two *stx*-specific assays used routinely in their laboratory: a PCR-ELISA method and a real-time PCR system, which followed the recommendations from the International Organization for Standardization Technical Specification (ISO/TS) 13136 defining a method for the detection of the main pathogenic Shiga-toxin producing *Escherichia coli* (STEC) in foodstuffs. The results showed that the newly developed qPCR method performed equally as well as the PCR-ELISA test and the *stx*-IAC real-time PCR test when applied to the same 353 naturally contaminated test portions (99.7% concordance).

Fusco *et al.* [28] developed a TaqMan and a SYBR Green real time PCR assay for reliable identification and quantitative detection of *S. aureus* strains harboring the enterotoxin gene cluster, regardless of their variants. Using optimized qPCR conditions, the assay was able to quantitatively detect at least about  $1 \times 10^3$  and  $1 \times 10^4$  CFU of the pathogen per milliliter raw milk (10 and 100 CFU equivalents of  $egc^+$  *S. aureus* per reaction mixture) by the SYBR Green and TaqMan qPCR assay, respectively.

Recently developed qPCR assays eliminated the post-PCR step by means of real-time monitoring of the PCR product generation, and the multiplex PCR approach has been implemented in the qPCR using a set of TaqMan probes labeled with different fluorescent dyes. Kim *et al.* [45] developed and proposed a multiplex qPCR assay for the simultaneous detection of *V. cholerae*, *V. parahaemolyticus*, and *V. vulnificus*, using *zot*, *vmrA*, and *vuua* as target genes, respectively. The overall procedure took approximately 12 h, including the enrichment culture period; it yielded a method that was faster, simpler, and less costly than conventional culture-based methods. Using enrichment culture with alkaline peptone water and optimized multiplex qPCR assay, they achieved a practical maximum sensitivity ( $10^0$  CFU/g food homogenate) for each target species in all food matrices tested. Therefore, the method was shown to achieve a maximum sensitivity that meets the FDA guidelines ( $10^4$  CFU/g) for acceptable levels of *V. cholerae*, *V. parahaemolyticus*, and *V. vulnificus* in seafood.

### Isothermal Amplification

Although PCR has been widely used in foodborne

pathogens, it requires thermocycling to separate the double strands of DNA; this has limited its application in the low-resource settings. During the past two decades, many novel methods have been developed to amplify nucleic acids under isothermal conditions. These methods include loop-mediated isothermal amplification (LAMP), nucleic acid sequence-based amplification (NASBA), rolling circle amplification (RCA), and strand displacement amplification (SDA). Isothermal amplification has simpler hardware requirements than PCR, as it does not require a thermal cycling system, and may even work with a simple water bath setup. Isothermal amplification techniques have better tolerance than PCR to some inhibitory materials that affect the molecular amplification efficiency [30].

**Loop-mediated isothermal amplification.** Most recently, a novel nucleic acid amplification method known as LAMP has been demonstrated as a rapid, low-cost, easy operating, highly sensitive, and specific detection method applied in several fields [70]. This method relies on an autocycling strand displacement DNA synthesis performed by the *Bst* DNA polymerase large fragment, which is different from PCR in that 4–6 primers are used to target 6–8 specific regions of the target gene. The amplification is performed under isothermal conditions between 59°C and 65°C, and the amplicons are mixtures of many different sizes of stem loop DNAs with several inverted repeats of the target sequence and cauliflower-like structures with multiple loops. The reaction can be accelerated with additional one or two loop primers. LAMP reactions usually result in about 10<sup>3</sup>-fold or higher levels of amplification product with stem-loop DNAs in 60 min than conventional PCR. LAMP products can be observed with the naked eye by employing SYBR Green I dye instead of conventional gel electrophoresis analysis; the color of the solution changes to green in the presence of LAMP amplicons, whereas it remains orange for mixtures with no amplification.

The first foodborne pathogen application of the LAMP method was for the detection of *stxA2* in *Escherichia coli* O157: H7 cells [62]. The mild permeabilization conditions and low isothermal temperatures used in the *in situ* LAMP method caused less cell damage than *in situ* PCR. The results showed that higher-contrast images were obtained with this method than with *in situ* PCR. Chen *et al.* [13] developed and evaluated a LAMP assay for identification and direct detection of acidophilic thermophilic bacteria (ATB) contaminants in pure juices. The LAMP method could detect 2.25 × 10<sup>1</sup> CFU/ml of ATB in juice samples within 2 h.

Recently, derivative LAMP assays, such as reverse-transcription LAMP assay [12], multiplex LAMP assay [40], *in situ* LAMP assay [38, 116], and real-time reverse-transcription LAMP assay [55], have been developed and employed for the detection of various foodborne pathogens, such as *Bacillus anthracis* [79], *Vibrio parahaemolyticus* [69, 113, 120], *Staphylococcus aureus* [114], *Salmonella* [13, 116, 119], *Pseudomonas aeruginosa* [121], *Escherichia coli* O157 [71, 118], and *Listeria monocytogenes* [105].

**Nucleic acid sequence-based amplification.** NASBA is an isothermal amplification reaction for the detection of RNA or DNA, which was developed after PCR had begun gaining widespread attention [18]. The reaction typically consists of three enzymes, including T7 RNA polymerase, RNase H, and avian myeloblastosis virus (AMV) reverse transcriptase (RT), all of which act together to amplify sequences from an original single-stranded RNA template. The reaction also includes buffering agents and two specific primers and takes place at approximately 41°C [35].

NASBA is specific for target RNA or DNA sequences and has been gaining popularity owing to its wide range of applications for pathogen detection in clinical, environmental, and food samples [4]. Simpkins *et al.* [93] showed that NASBA can selectively amplify mRNA sequences from *Salmonella enterica* in a background of genomic DNA and demonstrated that NASBA could be a great means of assessing cell viability. Min and Baeumner [65] developed a NASBA assay for the detection of viable *Escherichia coli*. Baeumner *et al.* [5] confirmed the assay's specificity for viable *Escherichia coli* by demonstrating that heat-killed cells did not produce a signal above the background of the instrumentation. Churrua *et al.* [17] developed a NASBA assay based on molecular beacons used for real-time detection of *Campylobacter jejuni* and *Campylobacter coli* in samples of chicken meat.

Real-time NASBA was proven to be the basis of sensitive and specific assays for detection, quantification, and analysis of RNA (and, in one case, DNA) targets [99]. Molecular beacons were used to generate fluorescence signals with NASBA assays for the detection of *Vibrio cholerae* [29]. More examples of the application of nucleic-acid-based techniques in food and other samples are presented in Table 1.

## Immunological Methods

Immunological detection based on antigen-antibody bindings is widely used for determining foodborne

**Table 1.** Nucleic-acid-based techniques employed for pathogen detection.

Techniques	Detected pathogens	Limit of detection	Assay time	References
Multiplex PCR	<i>Escherichia coli</i> O157:H7, <i>Salmonella</i> , and <i>Shigella</i>	$8 \times 10^1$ CFU/g (or CFU/ml) in apple cider, cantaloupe, lettuce, tomato, and watermelon; $8 \times 10^1$ CFU/g in alfalfa sprouts	30 h <sup>a</sup>	[52]
	<i>Salmonella</i> spp., <i>Listeria monocytogenes</i> , and <i>Escherichia coli</i> O157:H7	$10^3$ CFU/ml for each pathogen by pure culture, 1 cell per 25 g of inoculated pork sample	30 h	[44]
	<i>Escherichia coli</i> O157:H7, <i>Salmonella</i> , and <i>Shigella</i>	$10^5$ CFU/g for <i>Escherichia coli</i> O157:H7, $10^3$ CFU/g for <i>Salmonella</i> , and $10^4$ CFU/g for <i>Shigella</i>	3 h	[106]
	<i>Salmonella</i> spp., <i>Listeria monocytogenes</i> , and <i>Escherichia coli</i> O157:H7	5 CFU/25 g of inoculated sample after 20 h of enrichment		[43]
	O26, O103, O111, O145, sorbitol fermenting (SF) O157 and non-sorbitol fermenting (NSF) O157	Minced beef and sprouted seeds enrichment broths were inoculated with $5 \times 10^4$ CFU/ml STEC O157, and raw-milk cheese enrichment broths with $5 \times 10^5$ CFU/ml STEC O157	24 h	[102]
Quantitative PCR	<i>Listeria monocytogenes</i>	Detect as few as 100 CFU/g and quantify as few as 1,000 CFU/g	3 h	[83]
	<i>Salmonella</i>	$10^3$ to $10^4$ CFU/ml of inoculums in broth without enrichment, <10 CFU/ml of inoculum in broth after 18 h enrichment		[68]
	<i>Shigella</i>	0.12 to 0.74 CFU per reaction	24 h	[53]
	<i>Listeria monocytogenes</i> and <i>Staphylococcus aureus</i>	7 CFU/g in coleslaw for <i>L. monocytogenes</i> and 2 CFU/g in raw minced meat for <i>S. aureus</i>		[61]
LAMP	<i>Shigella</i> and enteroinvasive <i>Escherichia coli</i>	8 CFU per reaction	2 h	[95]
	<i>Streptococcus pneumoniae</i>	10 or more copies of purified <i>S. pneumoniae</i> DNA	1 h	[88]
	<i>Salmonellae</i>	3.4 to 34 viable <i>Salmonella</i> cells in pure culture and $6.1 \times 10^5$ to $6.1 \times 10^4$ CFU/g in spiked produce samples	3 h	[14]
NASBA	<i>Vibrio parahaemolyticus</i>	$5.3 \times 10^2$ CFU/ml	1 h	[112]
	<i>Chlamydia pneumoniae</i>	10 molecules of <i>in vitro</i> wild-type <i>C. pneumoniae</i> RNA and		[56]
	<i>Chlamydomytila pneumoniae</i>	0.1 inclusion-forming unit (IFU) of <i>C. pneumoniae</i>		
	<i>Aspergillus fumigatus</i>	$10^4$ copies/ml of RNA and $10^0$ cells		[117]
	<i>Mycobacterium tuberculosis</i>	$1 \times 10^2$ CFU/ml	< 5 h	[31]

<sup>a</sup>Enrichment.

pathogens. These assays rely mainly on the specific binding of an antibody to an antigen. A variety of antibodies have been employed in different assay types for the detection of foodborne pathogens and microbial toxins. The suitability of the antigen-antibody complex depends mainly on the antibodies' specificity. In order to ensure the reliable detection of foodborne pathogens using antibody-based methods, the influence of stress on antibody reactions should be thoroughly examined and understood first, as the physiological activities in cells are often altered in response

to a stress [36]. Most polyclonal antibodies, derived from either rabbit or goat serum, contain a collection of antibodies with different cellular origins and, therefore, somewhat different specificities. Monoclonal antibodies are often more useful than polyclonal antibodies for specific detection of a molecule, since they provide an indefinite supply of a single antibody. With the development of monoclonal antibodies, immunological detection of microbial contamination has become more specific, sensitive, reproducible, and reliable, as many commercial immunological assays are



available for the detection of a wide variety of microbes and their products [50].

### Enzyme-Linked Immunosorbent Assay

One of the most widely used immunological assays for foodborne pathogens detection is enzyme-linked immunosorbent assay (ELISA), which is a very accurate and sensitive method for detecting antigens or haptens [101]. Traditional ELISA typically involves chromogenic reporters and substrates that produce some kind of observable color change to indicate the presence of antigen or analyte. The most powerful ELISA format is called the “sandwich” assay, because the antigen from the enrichment cultures to be measured is bound between two primary antibodies: the capture antibody and the detection antibody. The sandwich format is used because it is sensitive and robust. The walls of wells in microtiter plates are the most commonly used solid support; however, ELISAs have also been designed using dipsticks, paddles, membranes, pipet tips, and other solid matrices [26]. Bolton *et al.* [6] described the BIOLINE *Salmonella* ELISA test for *Salmonella* spp., which was a rapid, easy, and convenient assay for the detection of *Salmonella* in foods and feeds. The limit of detection of the ELISA test kit was as low as 1 CFU/25 g sample with at least 4 of the 20 matrixes tested, and was found to be applicable to all sample types tested. The BIOLINE *Salmonella* ELISA test kit was granted AOAC-RI performance tested status.

Many foodborne toxins detection rely mainly on the presence of immunological reactions that are used to detect toxins. ELISA, the most commonly used in toxins detection, has been generated for staphylococcal enterotoxins A, B, C, and E and found to have detection levels of less than 0.5 µg/100 g in ground beef. ELISA has also been employed for the detection of botulinum toxins and enterotoxins produced by *E. coli* [27].

### Lateral Flow Immunoassay

Although ELISA has been widely used in many laboratories, this method still requires various equipments and trained personnel. Therefore, rapid and cheap, yet still reliable methods that can be conducted and interpreted at the site of the contamination are needed. More and more on-site immunological techniques based on lateral flow immunoassays such as dipstick, immunochromatography, and immunofiltration are gaining attention in the area of pathogen, mycotoxin, and disease detection in the food industry and medicine [67].

Lateral flow assays are a form of immunoassay in which the test sample flows along the solid substrate *via* capillary action. After the sample is applied to the test, it encounters a colored reagent (antibody or antigen labeled by colloidal latex or gold particles), which mixes with the sample and transits the substrate, encountering lines or zones that have been pretreated with an antibody or antigen. Depending on the analytes present in the sample, the colored reagent can become bound at the test line or zone [32]. Most lateral flow assays are basically designed to incorporate a visual response about 2–10 min after the application of the sample. Using these techniques allows simplifying the detection and minimizing the manipulations in order to provide accurate results with little or no instrumentation [25].

Delmulle *et al.* [21] developed an immunoassay-based lateral flow dipstick for the rapid detection of aflatoxin B1 in pig feed. The visual detection limit for aflatoxin B1 was 5 µg/kg. Jung *et al.* [42] developed a colloidal immunochromatographic strip for the detection of *Escherichia coli* O157:H7 in enriched samples, reporting that the minimum limit was  $1.8 \times 10^5$  CFU/ml without enrichment and 1.8 CFU/ml after enrichment. Avidin and streptavidin are widely used in (strept)avidin–biotin system technology, which is based on their tight biotin-binding capability. These biotin–(strept)avidin-based methods enable both a signal amplification and a reduction in background activity, resulting in suitable analytical techniques to be used in many fields [84].

The labels used in lateral flow immunoassay are mainly colloidal gold and monodisperse latex, labeled with colored, fluorescent, or magnetic tags. Dyed latexes and paramagnetic particles are available from a variety of sources, including Bangs Laboratories, Dynal, Merck/Estapor, and Magsphere. Magnetic, latex, metal, and semiconductor particles on the nanometer scale have unique optical, electronic, and structural properties that can be used in a variety of detection applications [78].

With the development of semiquantitative and qualitative assays [2] as well as autoreading technologies, such as the Biosite Triage and Response Biomedical RAMP systems, Magnetic Assay Reader (MAR), Cozart’s DDS, or Rapiscan products such as American BioMedica Corporation’s Rapid Reader for their Rapid Screen, lateral flow immunoassay will be applied in ways that have the potential to create entirely new paradigms in high-sensitivity point-of-need testing on-site.

### Immnomagnetic Separation Assay

Immnomagnetic separation (IMS), a procedure that utilizes

immunomagnetic beads (IMBs) as capturing reagents, has been developed for microbial isolation and identification. IMS is analogous to selective cultural enrichment, whereby the growth of other pathogen is suppressed while the target pathogen is allowed to grow. The separation process consists of two fundamental steps; first, the target cells are mixed with immunomagnetic particles for incubation of less than 1 h and separated by an appropriate magnetic separator; then, the magnetic complex is washed several times to remove the contaminants [60]. The use of IMS in assays is increasing because magnetic handling is fast, efficient, and only slightly affects the target analytes. Furthermore, various bioreactive molecules can be conjugated to the IMB surface for the immunoprecipitation, isolation, and identification of biomolecules (such as cells, pathogens, and proteins), or to improve the resolution of magnetic resonance imaging (MRI) [97]. Unlike the several days necessary to perform the conventional microbiological method and additional workup to elucidate the microbial status of any suspected colonies (more than 500 CFU in plate), DeCory *et al.* [20] developed and optimized a protocol for the rapid detection of *Escherichia coli* O157:H7 in aqueous samples by a combined immunomagnetic bead-immunoliposome (IMB/IL) fluorescence assay within a single 8 h work shift. The assay was able to identify samples containing *Escherichia coli* O157:H7 with 100% accuracy. The results highlighted the possible benefits of using immunomagnetic beads in combination with sulforhodamine B-encapsulating immunoliposomes for the rapid detection of *Escherichia coli* O157:H7 in aqueous samples.

The growing importance of mass spectrometry for the identification and characterization of protein toxins produced by foodborne pathogens is a result of the improved sensitivity and specificity of mass-spectrometry-based techniques, especially when these techniques are combined with affinity methods. Schlosser *et al.* [87] reported a novel method based on the use of immunoaffinity capture and matrix-assisted laser desorption ionization-time-of-flight mass spectrometry for selective purification and detection of staphylococcal enterotoxin B (SEB). In this method, an affinity molecular probe was prepared by immobilizing the anti-SEB antibody on the surface of paratoluene-sulfonyl-functionalized monodisperse magnetic particles and was used to selectively isolate SEB. Immobilization and affinity capture procedures were optimized to maximize the density of anti-SEB immunoglobulin G and the amount of captured SEB, respectively, on the surface of the magnetic beads. SEB

could be detected directly “on beads” by placing the molecular probe on the matrix-assisted laser desorption ionization target plate or, alternatively, “off beads” after its acidic elution.

Other technologies relying on the Ab-Ag binding mechanism have also been developed and applied in detection of foodborne pathogens and toxins. Lee and Deininger [49] used IMS and ATP bioluminescence for the selective capture of target bacteria and their quantification, respectively. The method consisted of trapping bacteria on a filter, resuspending them in a small amount of buffer, and washing the suspension with an antibody-coated magnetic bead mixture specific to the bacterial species of interest. A detection limit of about 20 CFU/100 ml was achieved, which was well below the action limits of 300 CFU/ml (daily event), or a 30 day moving average of 126 CFU/100 ml set by US EPA. The entire procedure took less than 1 h to perform without an enrichment step. The study demonstrated that the system combining IMS with ATP bioluminescence was effective and expedient for detecting *Escherichia coli* in beach water.

## Biosensor-Based Methods

Biosensors have recently been defined as analytical devices incorporating a biological material (*e.g.*, tissue, microorganisms, organelles, cell receptors, enzymes, antibodies, nucleic acids, natural products, *etc.*), a biologically derived material (*e.g.*, recombinant antibodies, engineered proteins, aptamers, *etc.*), or a biomimic (*e.g.*, synthetic catalysts, combinatorial ligands, and imprinted polymers) intimately associated with or integrated within a physicochemical transducer or transducing microsystem, which may be optical, electrochemical, thermometric, piezoelectric, magnetic, or micromechanical [48]. Biosensors are devices for pathogen detection and generally consist of at least three elements, including a biological capture molecule (*e.g.*, probes or antibodies), a method of converting capture molecule-target interactions into a signal, and a data output system [48, 100]. The greatest advantageous aspects of biosensors are those that enable fast or real-time detection, portability, and multi-pathogen detection for both field and laboratory analyses. The advantages of fast or real-time detection can provide almost immediate interactive information on the food materials, which enable users to take corrective measures before consumption or further contamination occurs [82]. It has been reported that biosensors have been developed and applied to the microbial analysis of foodborne pathogens, including

**Table 2.** Different modes of biosensor-based foodborne pathogen detection.

Mode of detection	Analyte	Limit of detection	Assay time	References
Optical biosensor	<i>Escherichia coli</i> O157:H7	$5 \times 10^5$ cells/ml	45 min	[80]
	<i>Escherichia coli</i> O157:H7, <i>Yersinia enterocolitica</i> , <i>Salmonella</i> Typhimurium, and <i>Listeria monocytogenes</i>	$10^4$ CFU/ml for all bacterial species		[59]
	<i>Escherichia coli</i> O157:H7	$10^6$ cells/ml		[37]
	<i>Salmonella</i> Typhimurium	$10^5$ CFU/ml	12 h	[89]
Surface plasmon resonance biosensor	<i>Escherichia coli</i> O157:H7	$3 \times 10^5$ CFU/ml	5 to 7 min	[92]
	<i>Escherichia coli</i> O157:H7	$10^2$ CFU/ml	2 min	[108]
	<i>Escherichia coli</i> O157:H7	$8.7 \times 10^6$ CFU/ml	35 min	[64]
	<i>Salmonella</i> Typhimurium	$1 \times 10^6$ CFU/ml		[47]
	<i>Salmonella enteritidis</i> and <i>Escherichia coli</i>	25 CFU/ml for <i>Escherichia coli</i> and 23 CFU/ml for <i>Salmonella</i>	<1 h	[109]
Piezoelectric biosensors	<i>Escherichia coli</i> O157:H7			[111]
	<i>Bacillus anthracis</i>	300 spores/ml		[5]
	<i>Escherichia coli</i> O157:H7, staphylococcal enterotoxin B			[73]
Immunosensors	<i>Escherichia coli</i> O157:H7	$4.12 \times 10^2$ CFU/ml	<45 min	[51]
	<i>Salmonella</i> Typhi	$10^5$ cells/ml	90 min	[94]
	<i>Salmonella</i>	2.43 log CFU/ml	4 h	[63]
Electrochemical biosensors	<i>Escherichia coli</i> O157:H7	$10^2$ CFU/ml		[54]
	<i>Salmonella</i> Typhi		1 h 15 min	[81]
	<i>Bacillus cereus</i>	35–88 CFU/ml	6 min	[74]

*Escherichia coli* O157:H7, *Staphylococcus aureus*, *Salmonella*, and *Listeria monocytogenes*, as well as various microbial toxins such as staphylococcal enterotoxins and mycotoxins [3]. Different modes of biosensor-based foodborne pathogen detection are given in Table 2.

### Optical Biosensors

Optical biosensors are a powerful alternative to conventional analytical techniques, due to their particularly high specification and sensitivity, as well as their small size and cost-effectiveness [58]. Biosensor detection typically relies on an enzyme system, which catalytically converts analytes into products that can be oxidized or reduced at a working electrode and maintained at a specific potential. One of the best advantages of this optical transducer is the low cost and the use of biodegradable electrodes. An optical biosensor is a compact analytical device containing a biological sensing element integrated or connected to an optical transducer system [23]. Optical biosensor technology

can be classified into several subclasses based on absorption, reflection, refraction, Raman, infrared, chemiluminescence, dispersion, fluorescence, and phosphorescence. In the past decade, various kinds of optical biosensors for the rapid detection of pathogens, toxins, and contaminants in the food industry have been developed [100]. The main advantage of this technique is the real-time binding reaction detection, allowing kinetic evaluation of affinity interactions and, in addition, the low cost of the instrumentation required. Optical biosensors require a suitable spectrometer to record the spectral chemical properties of the analyte. A common method that employs the techniques of optical detection using reflectance spectroscopy for detection of foodborne pathogens is surface plasmon resonance (SPR).

SPR is a collective oscillation of free charges (conduction electrons) present at the interface of two media (metal–dielectric) with permittivities of opposite sign [1]. Receptors or antibodies initially immobilized on the surface of a thin film of precious metal, deposited on the reflecting surface



of an optically transparent waveguide, are used to capture the various target pathogens. The sensing surface is located above or below a high-index resonant layer and a low-index coupling layer. When a visible or near-infrared radiation (IR) is passed through the waveguide in the correct manner, the interaction of light with the electron cloud in the metal generates a strong resonance. Binding of the pathogen to the metal surface causes a shift in resonance to longer wavelengths, and the corresponding amount of shift reflects the concentration of bound pathogens [3]. The main drawbacks of current SPR technique lay in its complexity (specialized staff is required), high cost of equipment, and the large size of most currently available instruments. For this reason, the miniature SPR instrument and disposable cartridge and biochip were developed for real-time genetic detection in a cost-effective manner. This system included a disposable SPRLAMP cartridge made of PMMA with a PC prism and a simple SPR imaging system with temperature control for LAMP amplification [16].

Wang *et al.* [107] developed a SPR immunosensor for the detection of *Escherichia coli* O157:H7 by means of a new subtractive inhibition assay. Their results showed that the signal was inversely correlated with the concentration of *E. coli* O157:H7 cells in a range from  $3.0 \times 10^4$  to  $3.0 \times 10^8$  CFU/ml, where the limit of detection was  $3.0 \times 10^4$  CFU/ml. The limit of detection subtractive inhibition assay method was reduced by one order of magnitude, compared with direct SPR by immobilizing antibodies on the chip and ELISA for *E. coli* O157:H7 (limit of detection: both  $3.0 \times 10^5$  CFU/ml).

Several commercial instruments using SPR techniques are available from companies such as BIAcore and Biosensing Instruments Inc. Commercial SPR instruments have a detection limit of  $10^5$  CFU/ml for *Listeria monocytogenes*. The commercially available low-cost SPREETA SPR biosensor was reported to detect an *E. coli* O157:H7 enterotoxin, *stx1*, with a detection limit of 300 pmol compared with a bulk acoustic wave sensor [96].

### Piezoelectric Biosensors

Piezoelectric biosensors, which are capable of sensitive detection of minute amounts of analytes according to a linear relationship between the deposited mass and its frequency response, are an effective alternative to established label-free optical sensors, such as surface plasmon resonance spectroscopy and interferometry [11]. Piezoelectric biosensors have been widely used, and their performance for studies of affine interactions was extensively referred.

Olfactory sensing of specific volatile organic compounds

released by the bacterial pathogens is one of the more outstanding ways to determine contamination in food products. Sankaran *et al.* [86] used a computational simulation to determine the biomimetic peptide-based sensing material to be deposited on the quartz crystal microbalance (QCM) sensor for detecting specific gases (alcohols) at low concentrations in food samples. The results showed that the developed QCM sensors were sensitive to 1-hexanol as well as 1-pentanol as predicted by the simulation algorithm. The estimated lower detection limits of the QCM sensors for detecting 1-hexanol and 1-pentanol were 2–3 ppm and 3–5 ppm, respectively. This report demonstrated the applicability of a simulation-based peptide sequence that mimics the olfactory receptor for sensing specific gases.

Salmain *et al.* [85] successfully designed a direct, label-free immunosensor for the rapid detection and quantification of staphylococcal enterotoxin A (SEA) in buffered solutions, using the quartz crystal microbalance with dissipation (QCM-D) as a transduction method. With the optimized sensing layer, a standard curve for the direct assay of SEA was established from QCM-D responses within a working range of 50–1,000 or 2,000 ng/ml, with a detection limit of 20 ng/ml. The total time for analysis was 15 min. The study indicated that such systems had a considerable amount of potential for the rapid and reliable detection of targets at trace amounts of pathogens in various environments.

### Immunosensors

Immunosensors, which are based on specific antibody-antigen interactions, detect antigen binding to antibodies by immobilizing the reaction to the surface of a transducer, which converts surface change parameters into a detectable electric signal [32]. It is difficult to measure immunological reactions in real time owing to the diffusion limitations of antigens to immobilized antibodies, particularly for low levels of contaminants. However, most immunosensors produce results within 20–90 min, which is close to real time compared with conventional techniques and classical ELISAs. Moreover, the results of immunosensors are read *via* digital signals and are not as dependant on personal factors such as bias, fatigue, level of training, or visual disorders. However, this property is also shared by microtiter plate spectrophotometric immunoassays [98]. Chen [15] reported a new conductometric immune-biosensor for the detection of staphylococcal enterotoxin B (SEB) based on immobilization of horseradish peroxidase (HRP)-labeled SEB antibody (HRP-anti-SEB) onto a nanogold/chitosan-multiwalled carbon nanotube (Au/CTS-MWNT)-functionalized biorecognition interface. The results showed

that under optimal conditions, the proposed immune-biosensor exhibited a good conductometric response relative to SEB concentration in a linear range from 0.5 to 83.5 ng/ml, with a correlation coefficient of 0.998.

### Electrochemical Biosensors

Electrochemical-based detection methods are further transduction-based systems that have been used for identifying and quantifying foodborne pathogens. Electrochemical biosensors can be classified into amperometric, potentiometric, impedimetric, and conductometric responses, based on observed parameters such as current, potential, impedance, and conductance, respectively [100]. Electrochemical biosensors developed for the simultaneous multiplexed analysis of foodborne pathogens primarily use electrochemical impedance spectroscopy as the transduction technique, thus providing label-free, on-line, high-throughput devices for bacterial detection [76]. Impedance spectroscopy is a powerful method for the study of conducting materials and interfaces. Through this technique, a cyclic function of small amplitude and variable frequency is applied to a transducer, and the resulting current is used to calculate the impedance at each of the probed frequencies [48]. Impedance biosensors for the detection of foodborne pathogens are based on the measurement of changes in the electrical properties of bacterial cells when they are attached to, or associated with, the electrodes [115]. Furthermore, the advantages in microfabrication techniques have enabled the use of microfabricated microarray electrodes for impedance detection, and the miniaturization of impedance microbiology into a chip assay.

Louie *et al.* [57] developed an impedance-based, fieldable biosensor system to detect *Escherichia coli* O157:H7 and *Salmonella* spp. The portable biosensor system used a variety of disposable analyte-specific sensor modules, each of which could be used to quantitatively determine specific analytes. The response for each sensor was rapid, and stable readings could be obtained in less than 1 min. Despite the development of a portable reagentless impedance biosensor that allows rapid detection of specific foodborne pathogens, however, no real foodborne or clinical sample application was considered.

There seems to be a lot of interest in the development of integrated biosensors for the detection of multiple biologically relevant species. A miniaturized biosensor device composed of a probe, sampler, detector, amplifier, and logic circuitry for monitoring infectious pathogens is an attractive alternative to existing instrumentation. Normal biosensors and biochips employ only one type of bioreceptor

as probes (*i.e.*, either nucleic acid, enzyme, or antibody probes). The multifunctional biochip (MFB) is an integrated multi-array biochip, designed by combining integrated circuit elements, an electro-optics excitation/detection system, and bioreceptor probes into a self-contained and integrated microdevice [103]. The MFB is a superior system that can detect multiple specific analytes simultaneously and offer information on both gene mutation (with DNA probes) and protein expression (with antibody probes) simultaneously. Vo-Dinh *et al.* [103] described a MFB, which used two different types of bioreceptors, including nucleic acid and antibody probes, on a single platform. The multifunctional capability of the MFB device for biomedical diagnostics was illustrated by the measurements of DNA probes specific to gene fragments of *Bacillus anthracis* and antibody probes targeted to *Escherichia coli*. The results showed that the calibration curves for monitoring pathogenic species illustrated the capability of the device for medical diagnostics and for quantitative detection of pathogenic agents.

Cao *et al.* [8] described a rapid and sensitive DNA target detection using enzyme amplified electrochemical detection based on a microchip. They employed a biotin-modified DNA, which reacted with avidin-conjugated horseradish peroxidase (avidin-HRP), in order to obtain the HRP-labeled DNA probe, and hybridized it with its complementary target. After hybridization, the mixture containing dsDNA-HRP, excess ssDNA-HRP, and remaining avidin-HRP was separated. With this protocol, the limits of quantification for the hybridization assay of 21- and 39-mer DNA fragments were  $8 \times 10^{-12}$  M and  $1.2 \times 10^{-11}$  M, respectively. The method was applied satisfactorily in the analysis of *Escherichia coli* genomic DNA.

Wang *et al.* [104] described a new diagnostic assay for the rapid detection of methicillin-resistant *Staphylococcus aureus* (MRSA) by combining nucleic acid extraction and isothermal amplification of target nucleic acids in a magnetic bead-based microfluidic system. LAMP amplification of the target genes was performed *via* the incorporation of a built-in micro temperature control module, followed by spectrophotometric analysis of the optical density of the LAMP amplicons. The results showed that the limit of detection for MRSA in clinical samples was approximately 10 fg/ml by performing this diagnostic assay in the magnetic bead-based microfluidic system within 60 min.

### Future Perspectives

Traditional foodborne pathogen detection methods,

although sensitive enough, are often too time-consuming for practical use, taking days to a week to perform. Therefore, new methods that overcome this performance limitation are required. Recently, several methods have been explored and developed for the rapid detection of foodborne pathogens. However, most of them still require improvement in sensitivity, selectivity, or accuracy to be of any practical use.

Nucleic-acid-based methods have high sensitivity and require a shorter time than conventional culture-based techniques for detection of foodborne pathogens and toxins, but most of them require trained personnel and expensive instruments, which limit their use in a practical environment. The emerging isothermal amplification methods such as LAMP and NASBA may have a good prospect for detection of pathogens and toxins in resource-limit settings. The development of nucleic-acid-based methods and immunological methods helped improve the time required to yield results. The specificity and the sensitivity of immunological methods depend on the binding strength of the specific antibody to its antigen, and they work well for food matrixes without interfering factors such as other non-target cells, DNA, and proteins. Biosensors-based methods are easy to perform without training, and yield results in real-time detection of foodborne pathogens and toxins with high sensitivity and selectivity comparable to the culture-based methods. However, they still need to be improved in food matrixes detection.

All assays available for food diagnostics require some degree of sample preparation, which is a very important factor for rapid and conventional detection methods, and also a bottleneck for the advanced rapid methods. More studies regarding the separation techniques of microorganisms from the food matrix are required, as well as for sample concentration prior to detection by immunological, nucleic-acid-based, or biosensor assays. Preconcentration is the preferred choice, as it can enhance sensitivity several folds by increasing the number of target organisms per unit volume at a relatively low cost. Several available modes of preconcentration are used, including filtration, size-fractionation, centrifugation, and immunomagnetic separation, or combinations of these methods.

The possibilities of combining various rapid methods, including nucleic-acid-based methods, immunological-based methods, and biosensor-based methods should be further exploited. With the correct application of a number of these technologies simultaneously, broader ranging and more accurate technologies could be developed. Antibodies can be modified to capture specific cells, which may then be

detected by a nucleic-acid-based method. Various nucleic acid amplified products can be quantified using immunoassays. The trend in immunoassays and nucleic-acid-based methods should result in the quantitative detection of microorganisms and the simultaneous determination of more than one pathogen or toxin. For immunological-based methods, further study of the application of biosensor chips may result in multiplex analyte assays. Biosensors must prove that they are capable of reaching at least the same detection levels as traditional methods (between 10 and 100 CFU/ml) in order to strengthen their appeal in food microbiology applications, not to mention the cost-effectiveness and time efficiency. Despite the numerous research efforts made during the past decades and in recent years for foodborne pathogen detection, current technologies still entail room for improvement. Since foodborne pathogens are mostly present in very low numbers (<100 CFU/g) and in the presence of millions of other bacteria, they are not easily detected. Therefore, a detection method that is reliable, accurate, rapid, simple, sensitive, selective, and cost-effective would be ideal. Such methods of pathogen detection would offer a great commercial advantage in the food industry and related fields. Moreover, the trend of crossing various methods will generate novel devices or methodologies to strengthen the advantages of rapid detection methods.

In summary, there are a host of promising applications in the field of rapid and automated detection methods for foodborne pathogens. Given the broad applicability and the great potential of such methods, there is still a great chance for further developments in the near future.

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