Comprehensive REVIEWS in Food Science and Food Safety

Culture-Independent Rapid Detection Methods for Bacterial Pathogens and Toxins in Food Matrices

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Abstract: Rapid detection of bacterial pathogens and toxins in foods is necessary to provide real-time results to mitigate foodborne illness outbreaks. Cultural enrichment methods, although the most widely used, are time-consuming and therefore inadequate for rapid pathogen detection from food samples. The development of novel "rapid" detection methods has decreased detection time dramatically. This review presents an overview of detection methods for various foodborne pathogens, including *Listeria monocytogenes*, *Salmonella enterica*, and shiga toxin-producing *Escherichia coli*, and bacterial toxins in food matrices, with emphasis on those methods which do not require cultural enrichment. Discussed methods include nucleic acid-, immunological-, and biosensor-based techniques. A summary of each type of detection method is given, including referenced methods from the literature. Since these discussed methods do not require cultural enrichment, there is a higher probability of interference from the food matrices. Therefore, the review also discusses the potential interference of food components on detection methods and addresses preprocessing strategies to overcome matrix associated inhibition and to concentrate low quantities of pathogens and toxins in food. Development of rapid and sensitive detection technologies advances and ensures public health safety and security.

Keywords: biosensors, detection, foodborne bacterial pathogens, food matrices, food safety

Introduction

Foodborne pathogens and toxins

Foodborne illness has become a major threat to public health, as roughly 1 in 6 Americans (or 48 million people) gets sick each year, estimated by the Centers for Disease Control and Prevention (CDC 2011). Foodborne diseases are generally caused by the consumption of food or water contaminated with pathogens which include bacteria, viruses, fungi, toxins, and parasites (Dwivedi and Jaykus 2011). There are 31 known pathogens, which cause foodborne illness, and bacterial pathogens account for the majority of cases resulting in hospitalizations and death (CDC 2011). Among foodborne bacterial pathogens, *Campylobacter* spp., *Listeria monocytogenes*, *Salmonella* spp., *Staphylococcus aureus*, *Clostridium perfringens*, *Escherichia coli* O157:H7 and other shiga toxin-producing *E. coli* strains (STEC), and *Vibrio* spp. are the leading causes of foodborne illnesses (CDC 2011). In some cases, foodborne illnesses can be caused by the ingestion of preformed toxins produced by bacteria, such as *Clostridium botulinum*, *C. perfringens*, *Staphylococcus aureus*, *Bacillus cereus*, STEC, and *V. parahemolyticus* (Thatcher 1966; Sharma and Mutharasan 2013a).

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Food products can be exposed to pathogens through water, air, and contact with soil, fertilizer, and the food processing environment, from raw material production to final consumption. Food safety has always been a challenging field and has attracted more and more attention, especially with the increasing demand for minimally processed products (Law and others 2015). Fruits, vegetables, dairy, seafood, meat, poultry, and ready-to-eat (RTE) products are mostly involved in multistate foodborne outbreaks according to the Foodborne Outbreak Online Database (CDC 2015). In order to ensure food safety and minimize the occurrence of foodborne illness, it is critical to test food for the presence of foodborne pathogens. This highlights the need for rapid, sensitive, and selective methods to detect pathogens in food products. There has been huge progress in both detection and separation/concentration techniques in recent years. This review focuses on the development of rapid culture-independent detection methods for the detection of bacterial pathogens and toxins in food matrices, with a brief discussion of aspects closely related to and affecting detection. These aspects include the influences of different food matrices on the detection and various noncultural separation/concentration techniques.

Culture-based and culture-independent detection methods

Traditional culture methods for detecting bacterial pathogens in food are based on the growth of viable bacteria on nutrient media. A general procedure includes homogenizing a food

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sample, enriching viable pathogens if the concentration of pathogens is low, and then separating the target pathogen on a selective medium followed by biochemical tests to ensure the presence of the target; further subtyping studies are necessary to identify certain targets (Sharma and Mutharasan 2013a). The enrichment is useful for resuscitating injured cells (due to heat, cold, acid, or osmotic shock during food processing), increasing the level of target pathogens and diluting inhibitory compounds in processed food products (Gracias and McKillip 2004; Dwivedi and Jaykus 2011). The culture methods are sensitive, generally inexpensive, and simple. However, they are laborious and may require several days depending on the growth of bacteria. When 1 or more enrichment steps are required, 8 to 24 h may be added to the detection (Gracias and McKillip 2004). In addition, some bacterial species may enter a viable but nonculturable state where they are still viable but not culturable on routine agar, which impairs their detection by culture-based techniques (Li and others 2014). Immunoassays such as enzyme-linked immunosorbent assay (ELISA) and nucleic acid-based approaches, such as polymerase chain reaction (PCR), have been used as alternative methods to reduce the detection time from days to hours. The heterogeneous distribution and low concentration of pathogens are still challenges to these methods (López-Campos and others 2012). The limit of detection (LOD) of immunoassays is usually 10^4 to 10^5 colony forming units per milliliter or gram (CFU/mL or CFU/g; López-Campos and others 2012). Due to the insufficient sensitivity of common immunoassays for direct measurement in original samples, cultural enrichment is essential to obtain an appropriate cell density to confirm the presence of pathogens. For PCR, sufficient cell numbers are critical to obtain high yield and good quality DNA in nucleic acid extraction procedures and to achieve the typical sensitivity between 10^3 and 10^4 CFU/mL (Gracias and McKillip 2004; López-Campos and others 2012). Therefore, cultural enrichment has been commonly involved in bacterial pathogen detection in food matrices (Kimura and others 1999; Gilbert and others 2003; Arora and others 2006; Navas and others 2006; Kim and others 2007; Kumar and others 2008, 2011). Although these methods replace the selective and differential plating steps with more rapid assays, the lengthy cultural enrichment still delays the detection. In addition, detection methods following cultural enrichment steps may not be quantitative and cannot determine the original contamination level.

While conventional culture methods and cultural enrichment are sometimes still necessary (Taskila and others 2012), emerging methods are targeted on developing more rapid and less laborious methods. Rapid methods are very important for the food industry, as the presence of pathogens in raw and processed products can be detected immediately and the proper control of the contaminated products can be conducted accordingly. Reduction in detection time is also critical for foodborne illness control, as the contaminated food products and outbreak sources can be identified quickly to prevent further spread of the illness. With the development of techniques with higher sensitivity, direct detection of low levels of foodborne pathogens in food samples which no longer require enrichment has become available. In addition, detection techniques based on noncultural methods for effective separation, concentration, and purification of pathogens significantly reduce the overall time of testing (Stevens and Jaykus 2004). Figure 1 provides a comparison of culture-based and culture-independent detection methods.

The Effect of Food Matrices on Detection

Although enrichment cultures have generally been used as reference methods for pathogen detection studies in foods, many molecular and other novel techniques do not require cultural enrichment. However, there are many matrix-associated intrinsic characteristics that interfere with assays involving enzymes, such as PCR. Pathogen detection from a complex food matrix thus provides a critical challenge. The food matrix is a heterogeneous assortment of various components, such as inorganic particles, biochemical compounds, and indigenous microflora. Fat and various other particulates can interfere with antibody-binding, and carbohydrates can interfere with nucleic acid amplification methods (Dwivedi and Jaykus 2011). PCR has been shown to be inhibited by certain quantities of sodium chloride, sucrose, and lysine; it is thought that these components may bind to nucleic acid templates or interfere with DNA polymerase activity (Rossen and others 1992; Wilson 1997). According to the results of other published studies, some polyphenolic compounds in apple juice interfere with PCR assays (Siebert and others 1996). Milk is also well known for its inhibitory effect on both DNA extraction techniques and PCR (Rossen and others 1992; Ongol and others 2009). Studies have shown that complex food matrices, such as ground beef (Thomas and others 1991) and chicken (Fluit and others 1993), can also inhibit PCR assays. Some pretreatments, such as homogenization or blending, of fruits, vegetables, and meats may release enzymes or antimicrobial components which interfere with downstream analysis and detection (Bhunia 2014).

In addition to PCR and enzymatic reactions, food matrix components and intrinsic properties can interfere with other types of foodborne pathogen detection assays. For example, when studying *E. coli* O157:H7 detection in milk, spinach, and ground beef matrices using a capacitive immunosensor, it was discovered that the pathogens adhere differently to each of the matrices (Li and others 2011a). A matrix-dependent variability measurement contributed to a drop in capacitance signal in all 3 food items. Another study utilizing a piezoelectric cantilever sensor assay to detect *L. monocytogenes* in a milk matrix discovered that the high density of milk, attributed to the dissolved sugars, proteins and fats, caused a decrease in the sensor resonance frequency (Sharma and Mutharasan 2013b). When using a surface plasmon resonance (SPR) sensor detection technique, the low pH of 3.7 of apple juice interfered with antibody–antigen binding during the assay (Taylor and others 2006). Changing the pH to 7.4 was capable of rectifying this interference. Another study using SPR sensing determined that the lipid and protein components of broiler meat interfered with the detection of foodborne pathogens including *Campylobacter jejuni* (Wei and others 2007). Also, when working with a cucumber matrix, SPR detection of *E. coli* O157:H7 was depressed, most likely due to the nonspecific adsorption of carbohydrates, vitamins, and dietary fiber (Wang and others 2013a). In this study, cucumber and ground beef were homogenized in buffer, and the filtered solution was used as the matrix. *E. coli* O157:H7 was also detected with less sensitivity in ground beef samples due to the interference of large numbers of nonpathogenic *E. coli* cells, which blocked the transport of the target pathogen to the sensor surface. Raw food samples often have high levels of native microflora, which are capable of interfering with the detection of specific foodborne pathogens. For example, in a raw milk matrix environment, high levels of native microflora, such as *Enterococcus* and *Lactobacillus* species, interfered with *L. monocytogenes* and

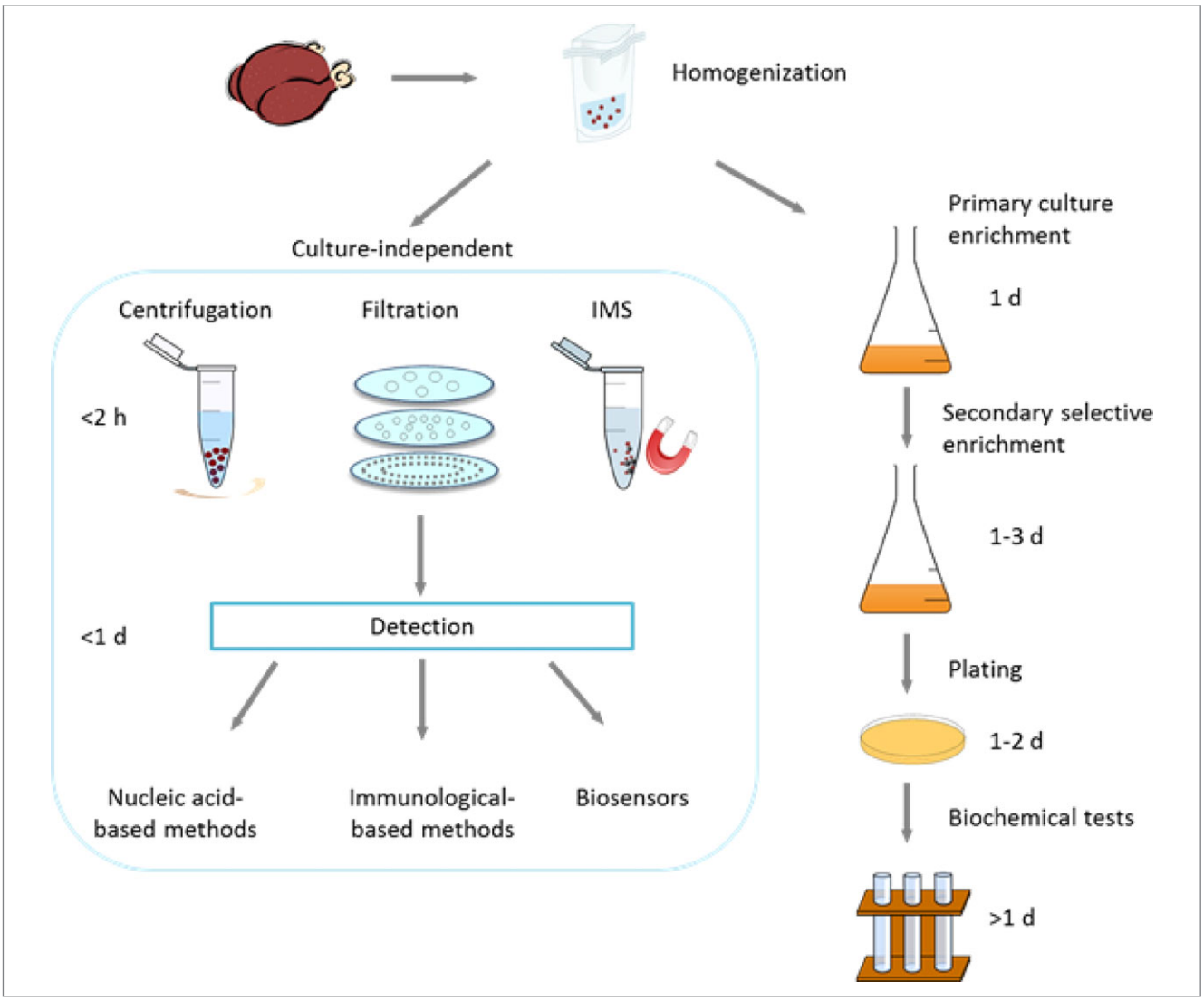

Figure 1–Comparison of culture-independent detection (left), including preprocessing techniques of centrifugation, filtration, and immunomagnetic separation (IMS), to conventional culture-based method (right). Culture-based detection may take place after enrichment or plating steps, which prolongs the total detection time.

S. enterica detection via conventional methods (Suh and Knabel 2001; Nero and others 2009). Other studies report unknown assay interference in the detection of pathogenic organisms (D'Souza and Jaykus 2003).

Noncultural Target Separation and Concentration Methods

While the purpose of cultural enrichment is to increase the number of the target bacterial pathogens to reach the sensitivity level of a detection method, many noncultural methods address the prerequisites of sensitive and accurate detection by separating target bacterial pathogens from food samples, removing inhibitors in the matrices and concentrating the pathogens to a detectable level, preferably without affecting the properties of bacterial cells. For practical applications, techniques which require simple manipulation, minimal amount of equipment and low cost receive much interest. For food matrices with different properties, various strategies have been developed (Stevens and Jaykus 2004; Bhunia 2008). Here we will briefly highlight a few commonly used methods from recent studies.

Centrifugation and filtration are still among the most commonly used physical separation methods due to the simplicity of these techniques. Beverage and liquid food samples can be easily treated with filtration-based separation (Wolffs and others 2006; López-Campos and others 2012). Filtration methods have also been developed for homogenized high-solid-content food samples. Some new innovations include concentration systems with capability to deal with large-volume samples and automated systems. Brewster (2009) reported a 3-stage system using different filter materials at each stage to concentrate *E. coli* O157:H7 from large-volume stomached beef samples, which may allow detection at very low numbers of pathogens in naturally contaminated foods. Li and others (2013) developed an automated cross-flow microfiltration instrument. With biochemical pretreatment and prefiltration of chicken homogenates, the instrument achieved 500- to 1000-fold concentration of microorganisms. Commercial products such as InnovaPrep concentrating Pipette (InnovaPrep LLC, Drexel, Mo., USA) are also available. When using centrifugation and filtration, as series of concentration steps are usually used, the recovery may decrease during multiple separations and repeated

washings. For example, less than 20% recovery of some bacterial strains was reported in a study using several steps of filtration and centrifugation, which detected 10^1 CFU/g of target organisms in food samples (Fukushima and others 2007).

While many chemical methods and physicochemical methods have been investigated in foodborne separation and concentration (Stevens and Jaykus 2004), methods based on biological interactions such as immunomagnetic separation (IMS) has become one major approach for separating foodborne bacteria followed by various detection methods (Uyttendaele and others 2000; Gehring and others 2004; Ravindranath and others 2009; Zhao and others 2009; Yang and others 2013; Wang and others 2015). Besides antibody–antigen interaction, ligands such as aptamers (Joshi and others 2009; Wu and others 2014a), bacteriophage proteins (Poshtiban and others 2013), and other binding molecules (El-Boubbou and others 2007) have been attached to magnetic beads for bacteria or bacterial nucleic acid (Zhang and others 2010; Li and others 2011b) separation in a similar way as IMS. The magnetic-based separations have the advantages of specific removal of the target from food particles, inhibitors, and other organisms using rapid and easy manipulation with a common separation time within 1 h (Joshi and others 2009; Wang and others 2011; Poshtiban and others 2013; Cloutier and others 2015; Wang and others 2015), good recovery of the target (Uyttendaele and others 2000; El-Boubbou and others 2007; Ravindranath and others 2009; Yang and others 2013; Wang and others 2015), no dilution of the sample or loss of carrier during washing, and possible automation and scale-up separation (Horák and others 2007; Lau and others 2013; Chen and others 2014). The efficiency and selectivity of magnetic separation greatly depends on the ligands, as sometimes it is hard to obtain a ligand with high affinity and specificity to the target. The size, surface area, and surface coating of magnetic particles also affect molecular attachment, properties of the particles, and therefore the efficacy of the separation (Irwin and others 2002; Tu and others 2003; Stevens and Jaykus 2004; Horák and others 2007; Jiang and others 2011). Magnetic nanoparticles (MNPs) have attracted considerable interest in separation due to their super-paramagnetic property and large surface-to-volume ratio as excellent ligand attachment platforms. In addition, the size of MNPs is 1 to 2 orders of magnitude smaller than bacterial pathogens, which allows multiple MNPs to attach to 1 bacterial cell and improve the separation (Wang and others 2011). Different compounds and procedures have been used to stabilize the magnetic core, improve surface functionalization, and minimize nonspecific interactions (Wang and others 2011; Li and others 2011b; Cloutier and others 2015). The longevity of the biological material attached to magnetic particles, interferences in food matrices, the strength of the magnetic field, and sufficient mixing to maximize the interactions between the functionalized particles and targets need to be considered when using the magnetic separation technique. In most cases, IMS cannot differentiate dead cells from viable cells, which are the most critical for food contamination. Usually, the pathogens attached to the magnetic beads are not detached during the detection. Therefore, the interference of magnetic beads to detection signals needs to be considered.

Instead of functionalized magnetic beads to remove the target pathogens from food samples, immobilized molecules on solid surfaces or materials are used to directly capture the target to the detector surface for analysis, and unbound substances in the samples are removed by washing steps (Taylor and others 2006; Waswa and others 2007; Chen and others 2008; Wang and others 2010). Different surface modification techniques such as using chemical linkers, self-assembled monolayer (SAM), protein A/G for antibody-orientated immobilization, and biomolecule labels (such as streptavidin-biotin binding) are used to immobilize the ligands (Varshney and Li 2009; Kausaite-Minkstimiene and others 2010). Similar factors when using bead-based separation play roles here as well. In addition, since the food matrices are introduced and directly contact with the detection system, such as a chip surface of a biosensor, the washing step is critical and the regeneration of the surface is needed if the chip is not disposable.

The pretreatment methods applied to bacteria affect the detection. Taylor and others (2005a) compared three methods of preparing *E. coli* O157:H7 samples for detection with a SPR immunosensor: untreated (viable cells), heat-killed then ethanol-soaked, and detergent-lysed. The treatment changed the shape or the size of the bacteria by breaking the bacterial cells into small pieces, which further affected the number, uniformity, and the diffusion of the analytes. It was found that detergent-lysed samples achieve the lowest detection limit in the SPR-based detection. Overall, the effect on cell viability should be considered according to the sample requirement of a detection method (Stevens and Jaykus 2004).

Nucleic Acid-Based Detection Methods

Nucleic acid-based detection methods consist of 2 main methodologies: amplification of nucleic acids by PCR or a related technique, and hybridization of nucleic acids using probes (Zhao and others 2014). Species, strain, or serotype-specific DNA and RNA are detected using synthetic oligonucleotides, which are complementary to the target (Salazar and others 2015). Some foodborne pathogens, including *C. botulinum*, *V. cholera*, *S. aureus*, and *E. coli* O157:H7 produce toxins capable of causing foodborne disease. Toxin-producing genes can also be assayed using nucleic-acid based techniques (Zhao and others 2014). These techniques are advantageous in that they are time-efficient, not labor-intensive, capable of reducing human errors, and eliminate the subjectivity of assay interpretation (Mandal and others 2011; Lee and others 2014). Results of these assays are easy to interpret due to the specificity of this type of detection. A summary of nucleic acid-based detection methods that have been applied without cultural enrichment and have been reported in the literature is given in Table 1.

PCR-based detection

PCR-based detection is one of the most commonly used methods to detect foodborne pathogens and toxins in foods. PCR was developed in 1986 as a method to amplify DNA *in vitro* (Mullis and others 1986; Mullis 1990). Many PCR-based assays for the detection of genes and genomic regions of foodborne pathogens have been developed and applied with food samples, including those that require no cultural enrichment steps.

General PCR. PCR assays for the detection of foodborne pathogens and toxins in a variety of food matrices have been developed and validated. Since components of the food matrix generally interfere with enzymatic reactions, food samples are often preprocessed prior to PCR with centrifugation or bead-based techniques to separate and concentrate the pathogens (Rossen and others 1992; Lantz and others 1998; Yang and others 2007). DNA is extracted from the concentrated cells and used as template in the PCR. A study looking at the detection of *S. enterica* Enteritidis and *L. monocytogenes* in a raw milk matrix used amino-modified silica-coated MNPs to concentrate the target DNA from

Table 1–Summary of nucleic acid-based techniques used for the detection of foodborne bacterial pathogens in food matrices without cultural enrichment

Method	Targets	Food matrices	Pretreatment/ separation/ concentration	Limit of detection (LOD)	Detectable range in food	Detection time	Reference
PCR	*S. enterica* Enteritidis *L. monocytogenes*	Raw milk	Nanoparticles	8 CFU/mL 13 CFU/mL	Not stated	Not stated	Bai and others (2013)
	C. jejuni	Whole milk Chicken rinse	Immunomagnetic separation (IMS)	1 CFU/mL	Not stated	8 h	Waage and others (1999)
	E. coli O157:H7	Ground beef	Metal hydroxides	10^3 CFU/mL meat homogenate	Not stated	Not stated	Taylor and others (2005b)
	L. monocytogenes	Chicken salad Macaroni salad Potato salad Seafood salad	Centrifugation	10^5 CFU/g 10^4 CFU/g 10^3 CFU/g	10^3-10^6 CFU/g	Not stated	Isonhood and others (2006)
	E. coli O157:H7	Ground beef	Filtration Centrifugation	10^3 CFU/g	Not stated	12 h	Cui and others (2003)
Multiplex PCR	*S. aureus* *E. sakazakii* *E. coli* O157:H7 *V. parahaemolyticus* *Salmonella* spp.	Milk Chicken	None	10^2-10^4 CFU/mL food homogenate	Not stated	Not stated	Chiang and others (2012)
	Salmonella spp. *L. monocytogenes*	Whole milk Cheese Ice cream Beef	IMS	10-10^2 CFU per reaction	Not stated	Not stated	Hsih and Tsen (2001)
	S. enterica Typhimurium and Saintpaul *E. coli* O157:H7	Tomato and jalapeño pepper washes	None	10-10^2 CFU/mL	Not stated	Not stated	Timmons and others (2013)
Quantitative PCR	*S. enterica*	Chicken rinse	Filtration	7×10^2 CFU/100mL	Not stated	3 h	Wolffs and others (2006)
	E. coli O157:H7	Apple juice Raw milk Beef	None	10^4 CFU/mL 10^3 CFU/mL 10^5 CFU/g	10^4-10^9 CFU/mL 10^3–10^9 CFU/mL 10^5–10^9 CFU/g	Not stated	Hsu and others (2005)
	E. coli O157:H7 *L. monocytogenes*	Ground beef Hot dog	None	10^3-10^4 CFU/g	Not stated	Not stated	Nguyen and others (2004)
	S. aureus	Raw milk	None	10 CFU per reaction	Not stated	Not stated	Fusco and others (2011)
	V. cholera	Raw oysters	None	6-8 CFU/g	Not stated	3 h	Lyon (2001)
Multiplex-quantitative PCR	*E. coli* O157:H7 *Salmonella* spp. *S. aureus*	Spinach Lettuce	None	10^3 CFU/g	Not stated	Not stated	Elizaquível and others (2008)
	E. coli O157:H7 *S. enterica* Typhimurium *L. monocytogenes*	Lettuce Tomato Ground beef	Nanoparticles	10^3 CFU/g	Not stated	Not stated	Yang and others (2013)
Quantitative reverse transcription RT-PCR	*L. monocytogenes* *S. enterica* Enteritidis	Whole milk Ice cream	Metal hydroxides	$\geq 10^2$ CFU/mL $\geq$10 CFU/mL	Not stated	Not stated	Lucore and others (2000)
	L. monocytogenes	Soft cheese Fermented sausage Cured ham Minced meat Milk	None	10^3-10^4 CFU/mL or g	Not stated	Not stated	Rantsiou and others (2008)
Loop-mediated isothermal amplification	STEC *E. coli*	Lettuce (iceberg and romaine) Sprouts (alfalfa, clover, mung bean) Spinach (baby, savoy, semi-savoy)	None	10^5 CFU/25g in lettuce, spinach, and clover and mung bean sprouts 10^6 CFU/25g for alfalfa sprouts	Not stated	Not stated	Wang and others (2014)

(*Continued*)

Table 1–Continued

Method	Targets	Food matrices	Pretreatment/ separation/ concentration	Limit of detection (LOD)	Detectable range in food	Detection time	Reference
	Salmonella spp.	Cantaloupe Spinach Tomato	Centrifugation Propidium monoazide	6.1×10^3–6.1×10^4 CFU/g	Not stated	3 h	Chen and others (2011)
	V. parahaemolyticus	Shrimp	Centrifugation	5.3×10^2 CFU/g	Not stated	1 h	Yamazaki and others (2008)
	ETEC *E. coli*	Raw milk	None	547 CFU/mL	Not stated	1 h	Yang and others 2014
Nucleic acid sequence-based amplification	*E. coli*	Drinking water	None	40 CFU/mL	Not stated	40 min	Min and others (2002)
	S. aureus	Milk	None	1-10 CFU/mL	Not stated	3–4 h	O'Grady and others (2009)
Microarray	*Y. pestis*	1% Skim milk	None	10^6 CFU/mL	Not stated	Not stated	Goji and others (2012)
	L. monocytogenes	Milk	None	10^8 CFU/mL	Not stated	Not stated	Bang and others (2013)
Microfluidic lab-on-a-chip	*S. enterica* *E. coli* O157:H7 *L. monocytogenes*	Banana Milk Hotdog	None	3.72×10^4 DNA copies 3.58×10^4 DNA copies 1.79×10^4 DNA copies	Not stated	14 min	Zhang and others (2011)

the milk extracts (Bai and others 2013). The nanoparticles were incubated with the extracted DNA for 30 min, followed by collection with a magnet. The particles were able to directly bind to a trace amount of genomic DNA under optimized conditions. PCR using primers to genomic regions specific to both *S.* Enteritidis and *L. monocytogenes* detected both pathogens at a contamination level of 8 and 13 CFU/mL, respectively. *C. jejuni* could be detected in whole milk using pretreatment of the sample matrix with immunomagnetic particles and subsequent PCR (Waller and Ogata 2000). The detection limit was 1 CFU/mL in milk, and the assay was also tested with chicken skin rinses resulting in the same detection level of the pathogen. PCR was also used to develop a detection method of *C. jejuni* in minced beef, chicken, and pork matrices (Waage and others 1999). Contaminated food matrices were pretreated with sedimentation and centrifugation techniques to remove coarse particles and solidified fat. Following DNA extraction, PCR was conducted with primers specific to genomic regions of the pathogen, followed by electrophoretic detection. Also using beef as a matrix, a study looked at the detection of *E. coli* O157:H7 in ground beef using metal hydroxides, specifically double-strength titanous hydroxides, as a pretreatment step to concentrate the pathogen prior to DNA extraction and PCR (Taylor and others 2005b). Metal hydroxides are charged particles with high molecular weights that are used as affinity agents to bacterial cells (Dwivedi and Jaykus 2011). The PCR assay detected the pathogen using specific primers to a portion of the Shiga toxin II gene at the LOD of 10^3 CFU/mL meat homogenate. A similar study, using both filtration and centrifugation as preprocessing steps with ground beef, used PCR and primers specific to the Shiga toxin I gene to detect 10^3 CFU/g of *E. coli* O157:H7 (Cui and others 2003). *L. monocytogenes* detection via PCR was studied in mayonnaise-based RTE salads including chicken, macaroni, potato, and seafood salads (Isonhood and others 2006). Food matrix samples were homogenized and underwent a centrifugation process prior to DNA extraction. PCR yielded detection limits of 10^5 CFU/g for chicken, 10^4 CFU/g for macaroni, and 10^3 CFU/g for both potato and seafood salads.

Multiplex PCR. Multiplex PCR (mPCR) is a variant of traditional PCR in which multiple targets can be detected simultaneously in 1 reaction. Instead of only 1 set of primers, mPCR uses multiple sets of primers capable of each detecting a gene, a gene variant, or a genomic marker of a specific organism. Therefore, mPCR is advantageous in that less time and effort is required to produce the same detection results. Since implemented in 1988 (Chamberlain and others 1988), mPCR has been widely used to study foodborne pathogen detection. An mPCR study used milk and chicken meat matrices to test the detection of *S. aureus*, *Cronobacter sakazakii*, *E. coli* O157:H7, *V. parahaemolyticus*, and *Salmonella* spp., among others (Chiang and others 2012). Without enrichment procedures, the detection limit of all pathogens ranged from 10^2 to 10^4 CFU/mL of food homogenate. An mPCR assay to simultaneously detect *E. coli* O157:H7, *B. cereus*, *V. parahaemolyticus*, *Salmonella* spp., *L. monocytogenes*, and *S. aureus* in Korean RTE food products including chicken, steamed pork hocks, oysters, fresh tomato juice, sushi, and lettuce (Lee and others 2014) was also developed. Detection of all 6 pathogens was achieved at 10^4 CFU/mL or greater in homogenized food samples. Another mPCR assay was developed for the detection of *S. enterica* Typhimurium and Saintpaul and *E. coli* O157:H7 in fresh tomato and jalapeño pepper washes (Timmons and others 2013). From the washes, the assay was capable of detecting between 10 and 10^2 CFU/mL of the pathogens.

Although a robust and specific assay for detecting foodborne pathogens and toxins in foods, a limitation of the mPCR technique is that the primers used need to be carefully designed with similar annealing temperatures. Since the mPCR assay is a single reaction, all primers will need to anneal during the same temperature to be efficient. The concentration of each primer also needs to be validated, due to the possibility of interactions between primer sets that may result in primer dimers or amplification of unwanted target sequences (Zhao and others 2014). Other limitations to the assay include the need for optimizing buffer concentrations, magnesium chloride concentrations, the amount of dNTPs, the amount of template DNA, and the proper cycling temperatures (Markoulatos and others 2002). Generally, primers should not be much higher than a concentration of 0.5 μM and should be in a 10^7 molar excess with respect to the template DNA (Markoulatos and others 2002).

Quantitative real-time PCR. Quantitative real-time PCR (qPCR) is an approach like general PCR, which monitors the formation and quantity of the amplified DNA products (Zhao and others 2014). The assay offers simultaneous amplification and detection of target DNA. Amplified DNA is detected using a fluorescently labeled moiety which is detected by the thermocycler at every cycle (Dwivedi and Jaykus 2011). An increase of fluorescence is compared with the cycle number to generate an amplification curve. From this curve, a quantification cycle (Cq or Ct) can be determined. The Cq value represents the amount of fluorescence which is significantly higher than the background (Postollec and others 2011). Since amplification is monitored and quantified during the assay, there is no need for post-assay detection with gel electrophoresis or any other method. One of the earliest qPCR assays used SYBR Green, an intercalating dye which binds to the minor grooves of double-stranded DNA. An increase in DNA was directly proportional to the fluorescence intensity. The amplified DNA is quantified by measuring the fluorescence intensity of the intercalating dye. Studies have used SYBR Green qPCR methods to detect foodborne pathogens in food matrices. For example, a study looking at *Salmonella* spp. in chicken carcass rinse was able to detect the pathogen at 7.5×10^2 CFU per 100 mL after 2 filtration steps (Wolffs and others 2006). However, since SYBR Green can also bind nonspecific amplified PCR products generated in the assay, the method often lacks adequate specificity. To determine if amplified PCR products are the correct size, a melting curve analysis can be conducted; a single peak indicates one melting temperature and thus one amplicon. Other qPCR methods using probes have also been developed including Scorpions Probes (Sigma-Aldrich Co., St. Louis, Mo., USA), molecular beacons (MBs), and TaqMan. TaqMan uses a probe with a reporter fluorophore dye at the 5′ end and a quencher molecule at the 3′ end (Applied Biosystems Inc., Foster City, Cafil., USA). Probe-based assays are more reproducible and provide a higher degree of sensitivity than SYBR Green assays. During qPCR with a TaqMan probe, as the DNA polymerase amplifies the DNA product and extends the primer, it reaches the probe and cleaves off the reporter (Dwivedi and Jaykus 2011). This results in an increased fluorescence which can be read by the thermocycler. Therefore, amplification is quantified based on the fluorescence of the nucleotide which is released from the internal probe. A study using a TaqMan qPCR assay looking at the detection of *E. coli* O157:H7 was capable of detecting 10^4 CFU/mL in an apple juice matrix, 10^3 CFU/mL in a raw milk matrix, and 10^5 CFU/mL in a beef sample matrix (Hsu and others 2005). The assay targeted 2 genes specific to the pathogen including the Shiga toxin II gene. Another TaqMan probe-based assay was developed to detect *V. cholera* in raw oysters (Lyon 2001). The assay could detect 6 to 8 CFU/g and could be completed in as little as 3 h. A qPCR assay developed with both SYBR Green and TaqMan chemistry looked at detecting *S. aureus* with associated enterotoxin in raw milk (Fusco and others 2011). Both assays were capable of detecting *S. aureus* with the LOD of 10 CFU per reaction. A study using an alternative probe-based qPCR was able to detect between 10^3 and 10^4 CFU/g of *E. coli* O157:H7 and *L. monocytogenes* in raw ground beef and hot dog meat (Nguyen and others 2004). In addition to qPCR assay methods, many multiplex qPCR (m-qPCR) assays have also been developed for pathogen detection. An m-qPCR assay was developed for the detection of *E. coli* O157:H7, *Salmonella* spp., and *S. aureus* on minimally processed vegetables including spinach and lettuce (Elizaquível and Aznar 2008). The LOD achieved in this assay was 10^3 CFU/g.

A general disadvantage of PCR assays used for detection of foodborne pathogens is that the PCR amplification process will target DNA from both live and dead cells. Some studies have developed PCR assays for the detection of only live bacterial DNA. For example, a study used qPCR coupled with a propidium monoazide (PMA) pretreatment which inhibits DNA amplification from dead cells (Li and Chen 2013). This technique was capable of detecting 10 to 10^3 CFU/g of live cells in a spinach matrix. Another assay using PMA pretreatment studied the simultaneous detection of *E. coli* O157:H7, *S. enterica* Typhimurium, and *L. monocytogenes* in lettuce, tomato, and ground beef food matrices (Yang and others 2013). The m-qPCR assay, coupled with a MNP-based concentration step, could detect 10^3 CFU/g of live cells of each foodborne pathogen.

Quantitative reverse transcription PCR. Like qPCR, quantitative reverse transcription PCR (RT-qPCR) can simultaneously detect and measure the quantity of target-amplified DNA. However, RT-qPCR uses RNA instead of DNA as starting material in the reaction. The RNA is reverse transcribed into complementary DNA (cDNA) using a reverse transcriptase and specific primers (Bustin 2000). The cDNA is then used as template in a general PCR assay. RT-qPCR can be performed in 1 step in a single reaction or in 2 sequential steps. Single-step protocols are preferred due to the minimized risk for contamination (Wong and Medrano 2005). RT-qPCR is also highly sensitive, so small differences in sample preparation and amplification conditions can impact the results. Since RNA degrades faster when outside of a cell, an advantage of RT-qPCR is that generally only RNA from live cells is extracted (Yaron and Matthews 2002). Another advantage of this technique is that the relative or quantitative mRNA levels of an organism under certain conditions can be deduced. This could apply to the food matrix composition, the temperature, the pH or water activity, or the amount of oxygen (Zhao and others 2014). A RT-qPCR assay for the detection of *L. monocytogenes* and *S. enterica* Enteritidis was developed for whole milk and ice cream matrices (Lucore and others 2000). The method coupled the use of metal hydroxides to immobilize bacterial cells before RNA extraction. The LODs for both pathogens were $\geq 10^2$ CFU/mL in whole milk and ≥ 10 CFU/mL for ice cream. Another RT-qPCR method for the detection of *L. monocytogenes* was developed for soft cheese, fermented sausage, cured ham, minced meat, and milk food matrices (Rantsiou and others 2008). The detection limit of this assay was 10^3 to 10^4 CFU/mL or g food sample.

Non-PCR-based nucleic acid detection

Loop-mediated isothermal amplification. Loop-mediated isothermal amplification (LAMP) is a relatively novel method developed in 2000 (Notomi and others 2000) and has been used for the specific detection of foodborne pathogens. LAMP uses the *Bst* DNA polymerase large fragment under isothermal conditions to amplify target DNA by an autocycling strand displacement DNA synthesis method. In a LAMP reaction, there are 4 primers, 2 inner and 2 outer, which target 6 to 8 specific sequences. This allows for higher specificity than general PCR (Xu and others 2012). Instead of cycling through temperatures like general PCR, LAMP requires an isothermal temperature approximately 59 °C to 65 °C; a water bath at a constant temperature would suffice in place of a thermocycler. The LAMP reaction also produces roughly 10^3-fold higher levels of amplified product in 1 h compared with conventional PCR (Zhao and others 2014). Like PCR amplicons, LAMP amplicons can be detected by fluorescence in real time or by gel electrophoresis and appropriate staining methods. The first application of the LAMP procedure with a foodborne pathogen was the detection of Shiga toxin genes in *E. coli* O157:H7 (Maruyama and others 2003). A LAMP assay for the detection of Shiga toxin-producing *E. coli* in iceberg lettuce, romaine lettuce, alfalfa sprouts, clover sprouts, mung bean sprouts, baby spinach, savoy spinach, and semi-savoy spinach matrices was developed (Wang and others 2014). The technique utilized primers detecting Shiga toxin genes, other virulence genes, and O-antigen gene clusters. The assay was compared with previously developed qPCR assays and found to be equally sensitive. A LAMP assay was developed for the detection of *Salmonella* spp. in cantaloupe, spinach, and tomato matrices (Chen and others 2011). The assay used centrifugation and PMA pretreatment and could detect 6.1×10^3 to 6.1×10^4 CFU/g in 3 h. A study used LAMP to detect *V. parahaemolyticus* in shrimp utilizing centrifugation as a pretreatment (Yamazaki and others 2008). The assay could detect 5.3×10^2 CFU/g in 1 h. Another study used a LAMP assay to detect enterotoxigenic *E. coli* from raw milk (Yang and others 2014). The assay was capable of detecting 547 CFU/mL of the pathogen. Variations of the LAMP assay, such as reverse transcription LAMP, multiplex LAMP, and *in situ* LAMP, have also been developed for use in detecting foodborne bacterial pathogens.

Nucleic acid sequence-based amplification. Nucleic acid sequence-based amplification (NASBA) is a primer-dependent technology which amplifies nucleic acids in a single reaction at isothermal temperature (Compton 1991; Malek and others 1994). The method is used for the detection of specific RNA sequences *in vitro*. Three enzymes are used in the reaction: avian myeloblastosis virus reverse transcriptase (AMV-RT), T7 DNA-dependent RNA polymerase, and RNase H (Compton 1991; Malek and others 1994; Dwivedi and Jaykus 2011). Two primers are used: the forward primer contains a 5′ promoter region which is recognized by the T7 RNA polymerase, followed by a complementary sequence to the target RNA, and the reverse primer is identical to a sequence on the target RNA molecule. In the first stage of the reaction, the forward primer binds to its target sequence, followed by extension by the AMV-RT. This extension creates an RNA-cDNA hybrid molecule. The RNase H hydrolyzes the RNA component of the hybrid molecule, leaving only single-stranded cDNA. The reverse primer then anneals, followed by extension by AMV-RT, creating a double-stranded cDNA molecule. T7 RNA polymerase then binds to its promoter region on the double-stranded cDNA, resulting in the transcription of many copies of the target RNA. These transcribed copies become the template for another round of reactions as with general PCR, the assay results in exponential amplification of the target product, in this case RNA. The amplified products can be visualized using gel electrophoresis or enzyme-linked gel assays (Law and others 2015). This technique has been used for foodborne pathogen detection, including the detection of viable cells (Churruca and others 2007). A study using NASBA was capable of detecting 1 to 10 CFU/mL of *S. aureus* in milk (O'Grady and others 2009). Also using NASBA, an assay for the detection of *E. coli* in drinking water was developed which could detect 40 CFU/mL (Min and Baeumner 2002).

DNA microarray. A microarray is a small device consisting of short single-stranded DNA oligonucleotide probes attached to glass slides or chips (Call and others 2001; Rasooly and Herold 2008; McLoughlin 2011). The probes on the device are generally short sequences ranging from 25 to 80 bp and are complementary to genes or genomic markers of different target pathogenic organisms (Severgnini and others 2011). During a microarray assay, the DNA (or RNA) from a target organism is extracted and labeled with a fluorescent dye. The DNA is then denatured to generate single-stranded molecules which bind to their corresponding complementary probes on the array. Results are obtained through visualization of the fluorescence signal when double-stranded DNA is formed. The fluorescence signal intensity is proportional to the concentration of the target DNA sequence (Lauri and Mariani 2009). Related to foodborne pathogenic bacteria, one of the first studies using microarray technology used the assay for the simultaneous detection of 22 pathogens from broth cultures (Wang and others 2007). Some of the pathogens that this assay was able to detect were *L. monocytogenes*, *V. cholera*, *C. jejuni*, and *Shigella* spp. A study using microarray technology developed an assay for the detection of *Y. pestis* in 1% skim milk (Goji and others 2012). The assay detected 10^6 CFU/mL of the pathogen using a microarray composed of 533 probes specific to target DNA sequences. Another assay using milk as a food matrix was developed to detect *L. monocytogenes* using microarray technology (Bang and others 2013). The assay could detect 10^8 CFU/mL of the pathogen by using random probes specific to the *L. monocytogenes* genome. It is noted that the 2 aforementioned studies were able to detect their targets only at very high concentrations. In a real-life scenario, pathogens and/or toxins would be present in food at generally low concentrations, making these specific methods inadequate under the conditions tested.

Oligonucleotide microarray technology is generally considered advantageous in that it is highly sensitive and specific to target sequences, it can detect multiple pathogenic organisms at once, and is not labor intensive. However, the assay requires the production of the oligonucleotide probes and fluorescent labeling of input DNA sequences, which can be expensive. Various commercial kits are available for detection studies; however, there exist few arrays kits specific for food applications (Rasooly and Herold 2008).

Microfluidic chip

The term microfluidics refers to the technology of moving nanoliter and picoliter volumes of liquid through micro-sized channels in a device (Dutse and Yusof 2011). The programmable devices, called micro-total analysis systems or lab-on-a-chip (LOC) are a portable diagnostic tool which can be applied to pathogen detection studies (Mairhofer and others 2009). Various steps, such as sample preparation, enrichment, labeling, signal

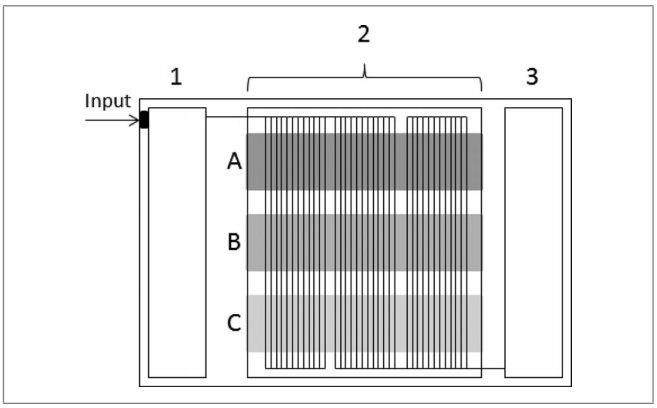

Figure 2–Top-view of a hand-held microfluidic lab-on-a-chip device (LOC) which utilizes PCR for foodborne pathogen detection. (1) A food sample homogenate or extracted DNA is pipetted into the first chamber of the device where preprocessing of the sample occurs. (2) DNA flows into the second chamber for PCR which contains 3 temperature zones for cycling: (A) melting, (B) extension, and (C) annealing. (3) After the specified number of cycles (determined by vertical lines), the PCR products flow into the third chamber where the DNA fragments are purified and detected.

amplification, and detection, are all conducted within channels and wells that are etched into the chip. LOC has been developed for nucleic-acid-based assays in recent years. See Figure 2 for a general layout of a PCR LOC. Since food samples are often complex in nature and may only contain trace amounts of pathogenic organisms of concern, the organisms or associated DNA should be extracted from the food matrix before sample insertion into the LOC (Lui and others 2009). Usually small volumes are introduced into microfluidic systems; therefore, techniques should be utilized to concentrate the organism or DNA of concern. In some LOCs, organisms can be purified from a heterogeneous sample using methods employing magnetic particles. This procedure can also be used with food matrices in which target organisms are not homogeneously distributed. Once cells are purified, DNA is extracted and purified, followed by amplification via a technique such as PCR or NASBA. DNA, instead of cells, can also be directly used as a starting material in the LOCs. The amplified DNA can be visualized by optical methods or by fluorescence signal detection (Yoon and Kim 2012). Results are often possible within minutes with single-cell sensitivity levels (Liu and others 2002; Dutse and Yusof 2011). Microfluidic LOC devices have been applied to the detection of pathogens such as *S.* Enteritidis, *S. aureus*, *E. coli*, and *C. jejuni* (Ishii and others 2013; Kim and others 2014). A microfluidic LOC device utilizing mPCR was developed to detect *S. enterica*, *E. coli* O157:H7, and *L. monocytogenes* in hotdog, milk, and banana food matrices (Zhang and others 2011). This assay could detect 3.72×10^4, 3.58×10^4, and 1.79×10^4 genome copies of each pathogen, respectively, in 1μL of purified DNA in only 14 min.

Immunological-Based Methods

The 2 major categories of immunological methods reported for the culture-independent detection of foodborne pathogens are ELISA and lateral flow immunoassay (LFI). Many biosensors are also based on antibody–antigen interactions, but those techniques involving signal transduction and processing will be discussed in a later section. A summary of recent immunological-based detection methods without cultural enrichment from the literature is given in Table 2.

ELISA

ELISA is one of the most widely used assays for foodborne pathogen detection. Recent ELISA developments have focused on new approaches that increase antibody density for more sensitive detection, coupling ELISE with IMS or other capture–concentration methods, and new antibody development. Nanomaterials have been involved as antibody binding platforms due to their large surface-to-volume ratio, which provides enhanced antibody density. Chunglok and others (2011) reported using single-walled carbon nanotubes (SWCNTs) for antibody and enzyme immobilization. Direct and sandwich assays based on the functionalized SWCNTs were used to detect *S. enterica* serovar Typhimurium in milk samples, which resulted in at least a 2 logs lower LOD than other immobilization methods. Cho and Irudayaraj (2013) developed an ELISA assay using 3 antibody-functionalized gold nanoparticles (AuNPs), which formed a network structure to allow more enzyme-labeled antibodies to connect to the target, as shown in Figure 3, and therefore enhanced the signal. Coupled with IMS, this method detected 3 CFU/mL of *E. coli* O157:H7 and 15 CFU/mL of *S.* Typhimurium in spiked food samples within 2 h of inoculation. Shen and others (2014) also used AuNPs for signal enhancement, which were functionalized with detection antibody and single-stranded DNA to link multiple enzyme molecules for each binding event. Combined with MNPs for target separation, they detected *E. coli* O157:H7 at a concentration of 6.8×10^2 CFU/mL in vegetable and milk matrices, and 6.8×10^3 CFU/mL in ground beef. Antibodies are the critical factor for determining the sensitivity and specificity of ELISA. There have been various reports on the selection and development of antibodies for foodborne pathogens (Sunwoo and others 2006; Charlermroj and others 2012; Meyer and others 2012). With the generation of new monoclonal antibodies to botulinum neurotoxin serotypes B (BoNT/B), Scotcher and others (2010) reported a sandwich ELISA with better sensitivity than traditional mouse bioassay and low detection limit in milk samples. An automated ELISA-LOC system was developed for potential in-field or remote screening, which could be used for foodborne pathogen detection (Yang and others 2011).

Based on the configuration of ELISA, variants for better sensitivity, shorter detection time, and capability of multiplex detection have been developed. Instead of traditional color change indication, fluorescent and chemiluminescent labels are frequently reported. Karoonuthaisiri and others (2009) reported a sandwich chemiluminescent immunoassay based on enzyme catalysis for simultaneous detection of *E. coli* O157:H7 and *S.* Typhimurium, which was evaluated in spiked milk samples. A multiplex sandwich immunoassay combined with IMS and fluorescent detection was reported by Cho and others (2014) for the highly sensitive detection of *E. coli* O157:H7 and *S.* Typhimurium within 2 h. The detection was achieved by enzymatic digestion to release fluorophores which attached to the magnetically captured target. Wu and others (2014b) developed a method using aptamer-functionalized magnetic beads for target separation, forming a sandwich structure with secondary antibody and labeling the sandwich with AuNPs conjugated with a detection antibody and enzyme. *S.* Typhimurium spiked in milk at 10^3 CFU/mL was detected using this method.

Lateral flow assays

Lateral flow along the membrane strip provides a simple and rapid form of detection assay. Usually, a test result is indicated by color change, which is provided by AuNPs or enzymatic reactions

Table 2–Summary of immunological-based techniques used for the detection of foodborne bacterial pathogens in food matrices without cultural enrichment

Targets	Food matrices	Pretreatment/ separation/ concentration	Method	Limit of detection (LOD)	Detectable range in food	Detection time	Specificity	Reference
S. Typhimurium	Milk and juice	None	Polyacrylonitrile fiber-based sandwich enzyme-linked immunosorbent assay (ELISA)	10^7 cells/mL in milk and 10^6 cells/mL in juice	Not stated	~2 h	Low cross-reactivity with *E. coli*	Chattopadhyay and others (2013)
S. Typhimurium	Fat-free and 9% fat milk	None	Single-walled carbon nanotubes enhanced direct and sandwich ELISA	Fat free milk: 10^3 (direct) and 10^4 (sandwich) CFU/mL; 9% milk: 10^4 CFU/mL	Not stated	Direct: overnight; Sandwich: ~3 h	Not stated	Chunglok and others (2011)
E. coli O157:H7 and *S.* Typhimurium	Reduced-fat milk, ground beef, and pineapple juice	Immunomagnetic separation (IMS)	Gold nanoparticle (AuNP) network ELISA	*E. coli* O157:H7: 3 CFU/mL in milk and beef; *S.* Typhimurium: 15 CFU/mL in juice	Not stated	~4 h	No cross-reactivity with *S.* Enteritidis, *L. monocytogenes* and the other target bacterium	Cho and Irudayaraj (2013)
E. coli O157:H7	Vegetable and milk	IMS	AuNP-enhanced ELISA	6.8×10^2 CFU/mL in vegetable and milk, and 6.8×10^3 CFU/mL in ground beef	Not stated	~2 h	Low interference from *S.* Senftenberg, *Shigella sonnei* and *E. coli* K12	Shen and others (2014)
E. coli O157:H7 and *S.* Typhimurium	Milk	None	Sandwich chemiluminescent immunoassay	*E. coli:* 7.5×10^6 CFU/mL *S.* Typhimurium: 10^7 CFU/mL	Not stated	~4 h	Cross-reactivity with *Salmonella* strains	Karoonuthaisiri and others (2009)
E. coli O157:H7, *S.* Typhimurium and *L. monocytogenes*	Spinach, chicken, and milk	None	Sandwich fluorescent immunoassay	5 CFU/mL	Not stated	2 h	No cross-reactivity with the other two target bacteria	Cho and others (2014)
S. Typhimurium	Milk	None	Enzyme-linked antibody aptamer sandwich assay	10^3 CFU/mL	$10^3 \sim 10^6$ CFU/mL	~2 h	No cross-reactivity with serovars of *Salmonella*, species of *Enterobacteriaceae*, and Gram-positive bacteria	Wu and others (2014b)
Botulinum neurotoxin serotypes B (BoNT/B)	Milk	Centrifugation	Sandwich ELISA	39 pg/mL	LOD to 20000 pg/mL	hours	Low cross-reactivity with other BoNT serotypes	Scotcher and others (2010)

(*Continued*)

Table 2–Continued

Targets	Food matrices	Pretreatment/ separation/ concentration	Method	Limit of detection (LOD)	Detectable range in food	Detection time	Specificity	Reference
V. cholerae	Seafood	None	Lateral flow immunoassay (LFI)	5×10^5 to 10^6 CFU/mL in various seafood	Not stated	15 min	No cross-reactivity with *V. mimicus*, *Vibrio* spp. and various Gram-negative bacteria	Chaivisuthangkura and others (2013)
E. coli O157:H7 and *E. coli* BL21	Milk, orange juice and lettuce	pH adjustment of juice; IMS and cell lysis	Multiplex LFI	*E. coli* BL21: 10^2 CFU/mL in milk and juice and 10^6 CFU/mL in lettuce; *E. coli* O157:H7: 10^1 CFU/mL in milk and juice and 10^5 CFU/mL in lettuce	LOD to 10^7 CFU/mL	~30 min	No cross-reactivity with *B. subtilis* and *S. enterica*	Hossain and others (2012)
BoNT/A and /B	Milk, apple and orange juice	Centrifugation to remove fatty content, pH adjustment	Multiplex LFI	5 and 10 ng/mL of BoNT/A and BoNT/B in milk, 25 and 10 ng/mL of BoNT/A and BoNT/B in apple juice	Not stated	~15 min	Specific to each target serotype	Ching and others (2012)

(Shim and others 2007; Park and others 2008). The assays have advantages of low cost, long-term stability, minimum skill required, short detection time, and are suitable for in-field screening of large numbers of samples, even though they are mostly for qualitative detection (Shim and others 2007; Park and others 2008; Shan and others 2015). However, for food sample analysis, IFIs have higher false-positive rates than with ELISA and PCR (Bohaychuk and others 2005), and their low sensitivity sometimes delays the results due to cultural enrichment necessity (Shim and others 2007; Sithigorngul and others 2007). A strip test using AuNPs as indicators for the detection of *V. cholerae* O1 was developed and evaluated in spiked seafood samples (Chaivisuthangkura and others 2013). Without cultural enrichment, bacterial concentrations of 5×10^5 to 10^6 CFU/mL in seafood samples were detected. Recent developments in lateral flow assays include the development of novel labels, new formats of assays, new supporting materials, and multiplex detection (Shan and others 2015). Some assays combine the test strip with devices to measure the color intensities or biosensor technology to be correlated with analyte concentration. A multiplex test strip was used for the detection of BoNT/A and /B in spiked milk, apple juice, and orange juice. The 2 serotypes of the toxin were detected and differentiated on the strip at a ng/mL level (Ching and others 2012). Hossain and others (2012) reported a test strip on paper for multiplex detection of *E. coli* O157:H7 and *E. coli* BL21, and evaluated the test strip in spiked food samples. Two enzymes and their substrates were used to indicate the presence of the 2 bacteria, respectively, and a hydrophobic barrier was used to prevent the interference between the 2 detection zones. This assay was also combined with IMS to improve sensitivity and was used for quantitative detection with the measurement of color intensity. Chai and others (2011) developed a portable detection system to detect the reflecting light from the reacting area on a test strip. Without incubation, false-positive results were still high (23 out of 25 samples). Therefore, LFIs may have limited applications in the detection of low-level contamination without enrichment techniques.

Biosensors

Compared to conventional culture methods, biosensors have the advantages of less detection time and relatively simple manipulation which does not require highly trained personnel. Biosensors can achieve the similar, or even better, sensitivity than other detection methods. Real-time biosensors, portable biosensors, and LOC biosensors (Yoon and Kim 2012) have also become available in recent years, which show great potential in food safety applications. There are many biosensors which have been evaluated for culture-independent detection of foodborne bacterial pathogens and bacterial toxins in food matrices, mainly by using spiked food samples. In this section, biosensors without cultural enrichment after inoculation are reviewed, especially those which have seen development in recent years. The biosensors are categorized based on their transducing mechanisms. Key performance characteristics such as detection time, LOD, detection range, and specificity are summarized in Table 3.

Optical biosensors

Optical biosensors are the most widely reported biosensors in foodborne pathogen detection, and there are now commercialized optical biosensors available. These sensors are based on various well-founded techniques including light absorbance, chemiluminescence, fluorescence, light polarization and rotation, SPR, and total internal reflectance (Terry and others 2005). Nanomaterials

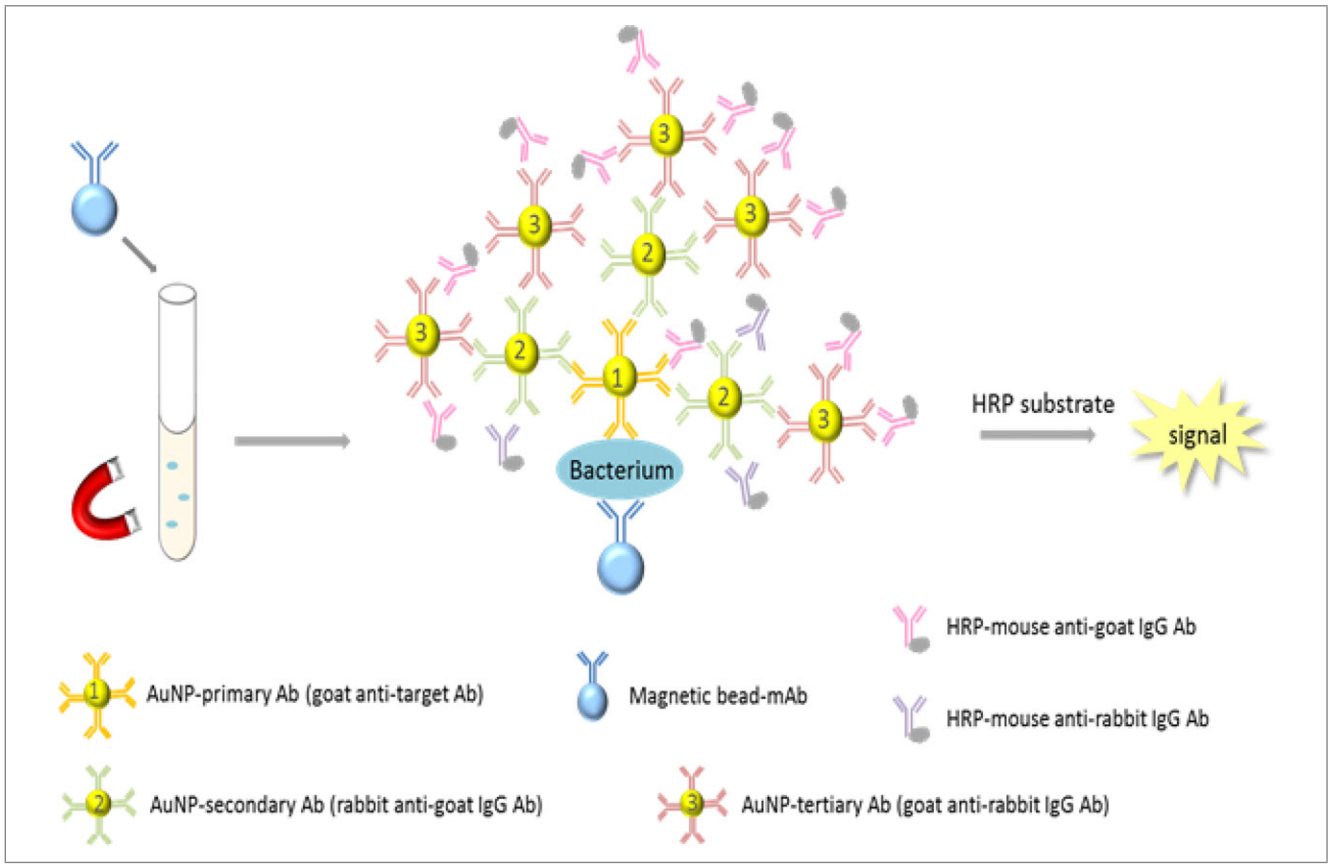

Figure 3–Schematic of an enzyme-linked immunosorbent assay (ELISA) based on immune-gold nanoparticle (AuNP) network. Monoclonal antibody (mAb)-functionalized magnetic beads are used to separate the target bacterium from food samples. Three kinds of AuNPs coupled with antibody (Ab) components (primary, secondary, and tertiary) and horseradish peroxidase (HRP)-labeled antibodies formed a network with captured target for signal enhancement (Cho and Irudayaraj 2013).

such as nanofibers, nanoparticles, and quantum dots have attracted much interest due to their unique optical properties, and their applications in biosensors have been reported (Leung and others 2007; Vinayaka and Thakur 2010).

Commercial SPR platforms such as Biacore (GE, previously Biacore AB) and Spreeta (Texas Instruments) are currently available. SPR sensors have advantages such as label-free detection, real-time monitoring of biomolecular interactions, reusable sensor chip, and feasible miniaturization for which optical fibers and waveguide structures are used for light transmission instead of a traditional prism (Hoa and others 2007; Shankaran and others 2007). Because SPR sensors monitor the interactions in very close vicinity of the transducer surface, it has the potential to study the interactions without rinsing out unreacted or excess reactants (Shankaran and others 2007). Due to the same reason, sensing elements such as antibody, aptamer, and DNA are usually immobilized on the SPR sensor surface to ensure high signal-to-noise ratio and rapid detection. Wei and others (2007) reported a SPR biosensor for the detection of *C. jejuni* and tested the biosensor on contaminated chicken rinse. Antibody against *C. jejuni* was immobilized on the Spreeta sensor surface. A detection limit of 10^3 CFU/mL of *C. jejuni* ATCC 35918 was reached with a detection time of about 60 min. It is noted that there was significant interference from the matrices, such as the proteins and lipid compounds. Waswa and others (2007) described an SPR immunosensor for *E. coli* O157:H7 detection in spiked milk, apple juice, and ground beef samples by immobilizing antibody on a Spreeta gold sensor surface, which had a detection limit of 10^2 to 10^3 CFU/mL. A sandwich assay was employed for the detection of *E. coli* inoculated on spinach leaves (Linman and others 2010). A secondary anti-*E. coli* antibody labeled with horseradish peroxidase was bound to the captured cells; the enzymatic reaction produced an insoluble dark blue product to enhance the SPR measurement. This biosensor could detect 10^4 CFU/mL *E. coli* with a dynamic range of 10^4 to 10^6 CFU/mL. *C. sakazakii* was detected in spiked milk and dissolved milk powder samples using SPR biosensor (Rodriguez-Emmenegger and others 2011). Antibodies against *C. sakazakii* were covalently attached to polymer-forming SAM on the chip surface in order to suppress the interference of fouling from the milk samples. The biosensor is capable of detecting 10^6 cells/mL in the milk samples. Miniaturized SPR biosensor, multiplex detection, and nanomaterials have attracted much interest in recent years. Miniature SPR biosensor using a Spreeta platform was reported to detect *S.* Enteritidis in spiked chicken samples, and the biosensor detected 10^6 CFU/mL of the target pathogen (Son and others 2007). Biacore launched several SPR sensors which comprised multiple flow cells enabling serial or parallel injections of samples in 2005 (Situ and others 2010). Taylor and others (2006) used an 8-channel custom-built SPR sensor to detect *E. coli* O157:H7, *S.* Typhimurium, *L. monocytogenes*, and *C. jejuni*, individually and in combination, in apple juice. An antibody-based sandwich assay was performed to amplify the response, which took around 1 h to detect the 4 species of bacteria in the matrices ranging from 3.4×10^3 to 1.2×10^5 CFU/mL. Instead

Table 3–Summary of biosensors used for the detection of foodborne bacterial pathogens in food matrices without cultural enrichment

Targets	Food matrices	Pretreatment/ separation/ concentration	Method	Limit of detection (LOD)	Detectable range in food	Detection time	Specificity	Reference
C. jejuni	Chicken rinse	None	Surface plasmon resonance (SPR) immunosensor	10^3 CFU/mL	$10^3 \sim 10^7$ CFU/mL	~60 min	Low cross-reactivity with *S.* Typhimurium	Wei and others (2007)
E. coli O157:H7	Milk, apple juice, and ground beef	None	SPR immunosensor	$10^2 \sim 10^3$ CFU/mL	LOD to 10^4 CFU/mL	~30 min	No cross-reactivity with *E. coli* K12 and *Shigella boydii*	Waswa and others (2007)
E. coli	Spinach leaves	Centrifugation	Sandwich SPR immunosensor (enzymatic signal enhancement)	10^4 CFU/mL	$10^4 \sim 10^6$ CFU/mL	~2.5 h	Not stated	Linman and others (2010)
C. sakazakii	Milk and dissolved milk powder	None	SPR immunosensor	10^6 cells/mL	Not stated	30 min	No cross-reactivity with *Yersinia enterocolitica*	Rodriguez-Emmenegger and others (2011)
S. Enteritidis	Chicken extract	Filtration of food sample and sonification of *Salmonella* cells	SPR immunosensor	10^6 cells/mL	Not stated	~20 min	Not stated	Son and others (2007)
E. coli O157:H7, *S.* Typhimurium, *L. monocytogenes* and *C. jejuni*	Apple juice (pH 3.7 and pH 7.4)	Heat-kill and ultrasonication of cells, and pH adjustment of apple juice pH 7.4	Sandwich multi-channel SPR immunosensor	3.4×10^3 to 1.2×10^5 CFU/mL of four targets	LOD ~ 10^7 CFU/mL	~1 h	Secondary antibodies specific to their target	Taylor and others (2006)
E. coli O157:H7	Cucumber and ground beef	Filtration of food	SPR biosensor	3.0×10^4 CFU/mL in cucumber and 3.0×10^5 CFU/mL in beef sample	LOD to 10^8 CFU/mL	~5 h	Some cross-reactivity with *E. coli* DH5α and *L. monocytogenes*	Wang and others (2013a)
Staphylococcal enterotoxin A (SEA)	Milk	None	SPR immunosensor	0.1 μg/g	0.1 ~1.0 μg/g	~1 h	Some cross-reactivity with SEB at high concentration	Tsai and Li (2009)
Mixture of botulinum neurotoxin serotypes A, B and F (BoNT/A, /B and /F)	20 % Honey	None	Sandwich SPR immunosensor	5 ng/mL	5 ~ 10 ng/mL	~20 min	May have some cross-reactivity with non-target serotypes	Ladd and others (2008)
BoNT/B	Apple juice, carrot juice, 3% powdered milk, and fresh nonfat milk	None	SPR immunosensor	0.3 ± 0.06, 0.3 ± 0.1, 2 ± 1, and 5 ± 3 pM in each food sample, respectively	LOD to 10^{-7} M	~4 h	May have cross-reactivity with other tetanus toxins	Ferracci and others (2011)
S. aureus and *E. coli*	Apple juice	None	Colorimetric biosensor	$10^{7.9}$ (*E. coli*) and 10^8 CFU/mL (*S. aureus*)	Not stated	Not stated	Not stated	Pires and others (2011)
E. coli O157:H7	Iceberg lettuce	Filtration of lettuce	Light scattering immunosensor	10 CFU/mL	10 to 10^7 CFU/mL	<6 min	No cross-reactivity with *E. coli* K12	You and others (2011)
S. Typhimurium	Pork	None	FRET-based optical fiber biosensor	10^5 CFU/g	$10^5 \sim 10^7$ CFU/g	<20 min	No cross-reactivity with *L. monocytogenes*	Ko and Grant (2006)
S. Enteritidis	Milk	Centrifugation of spiked sample and lysis of cells	Fluorescence nucleic acid biosensor	1.5×10^2 CFU/mL	1.5×10^2 to 3×10^3 CFU/mL	~5.5 h	No cross-reactivity with *S.* Typhimurium and *E. coli* K88	Song and others (2014)
Staphylococcal enterotoxin B (SEB)	Milk	None	Sandwich aptamer SPR biosensor and surface-enhanced raman scattering	3.6×10^{-15} M	$3.6 \times 10^{-15} \sim 3.6 \times 10^{-9}$ M	~2 h	Low cross-reactivity with bovine serum albumin (BSA) and avidin	Temur and others (2012)

(*Continued*)

Table 3–Continued

Targets	Food matrices	Pretreatment/ separation/ concentration	Method	Limit of detection (LOD)	Detectable range in food	Detection time	Specificity	Reference
E. coli O157:H7	Milk	Removal of lipids and proteins	Amperometric immunosensing strip	5×10^3 CFU/mL	5×10^3 ~5×10^5 CFU/mL	~35 min	No cross-reactivity with *E. coli*. K12, *L. monocytogens, S. choleraesuis* and *V. parahaemolyticus*	Lin and others (2008)
E. coli O157:H7	Apple juice	pH adjustment and IMS	Voltammetric sandwich immunosensor	5×10^3 CFU/mL	Not stated	80 min	Not stated	Gehring and Tu (2005)
E. coli O157:H7, *Campylobacter* and *Salmonella*	Milk	None	Voltammetric sandwich immunosensor	10^4 CFU/mL	Not stated	Not stated	Not stated	Viswanathan and others (2012)
Salmonella	Milk		Voltammetric sandwich immunosensor	10^3 CFU/mL	Not stated	~60 min	No cross-reactivity with *E. coli*	Afonso and others (2013)
SEB	Watermelon juice, soymilk, apple juice, and pork	Centrifugation and sonication (pork)	Voltammetric sandwich immunosensor (enzymatic signal enhancement)	ng/mL level	Not stated	~40 min	No cross-reactivity with AFB1, AFB2 and IL-6. High cross-reactivity with SEA and SEC	Tang and others (2010)
E. coli CECT 675 (surrogate for *E.coli* O157:H7)	Milk and apple juice	Filtration	Potentiometric aptamer biosensor	6 CFU/mL in milk and 26 CFU/mL in apple juice	Not stated	<10 min	No cross-reactivity with *S. enterica*, *Lactobacillus casei*, *E. coli* (CECT 4558)	Zelada-Guillén and others (2010)
E. coli O157:H7	Ground beef	Centrifugation	Impedance immunosensor	8×10^5 CFU/mL	8×10^5 to 8×10^7 CFU/mL	35 min	Not stated	Varshney and Li 2007
Bacillus cereus	Alfalfa sprouts, strawberries, lettuce and tomatoes	Filtration	Sandwich conductometric immunosensor	35 CFU/mL	35–88 CFU/mL	6 min	No cross-reactivity with *Bacillus megaterium* and generic *E. coli*	Pal and others (2008)
SEB	Milk and baby food	Centrifugation	Conductometric immunosensor	5 ng/mL	5 to 100 ng/mL	A few min	No cross-reactivity with BSA, lysozyme and IgG	Yang and others (2010)
E. coli O157:H7	Pasteurized milk, ground beef, and spinach	Filtration of solid food	Capacitive immunosensor	Milk, spinach and beef was 10^3, 10^3, and 10^4 CFU/mL, respectively	LOD to 10^5 CFU/mL	~1 h	Low cross-reactivity with non-O157:H7 *E. coli*	Li and others (2011a)
Listeria innocua	2% milk	None	Impedimetric biosensor	10^5 CFU/mL	LOD to 10^8 CFU/mL	~25 min	No cross-reactivity with *E. coli*	Tolba and others (2012)
S. Typhimurium	Fat-free milk	None	Impedimetric immunosensor	10^3 CFU/mL	LOD to 10^7 CFU/mL	~1 h	Low cross-reactivity with *E. coli* and *S. aureus*	Dong and others (2013)
E. coli O157:H7	Whole milk	None	Impedimetric immunosensor	83.7 CFU/mL	Not stated	Not stated	Low cross-reactivity with *B. cereus, S. aureus*, and *E. coli* DH5a	Joung and others (2013)
E. coli O157:H7	Ground beef and cucumber	Filtration of food samples	Impedimetric immunosensor	1.5×10^4 and 1.5×10^3 CFU/mL in ground beef and cucumber, respectively	LOD to 1.5×10^7 CFU/mL	~30 min	Low cross-reactivity with *S. aureus, L. monocytogenes* and *E. coli* DH5α	Wang and others (2013b)
E. coli O157:H7	Apple juice, milk and ground beef	Centrifugation, DNA extraction and PCR amplification	Quartz crystal microbalance (QCM) DNA biosensor	5.3×10^2 CFU/mL or CFU/g	Not stated	A few hours including PCR	No cross-reactivity with *L. monocytogenes, S. choleraesuis, S. aureus* and *E. coli* K12	Chen and others (2008)

(*Continued*)

Table 3–Continued

Targets	Food matrices	Pretreatment/ separation/ concentration	Method	Limit of detection (LOD)	Detectable range in food	Detection time	Specificity	Reference
Pseudomonas aeruginosa	Apple juice	None	Molecular imprinting polymers (MIP)-based QCM sensor	10^7 CFU/mL	$10^7 \sim 10^9$ CFU/mL	3 min	Low cross-reactivity with *Acinetobacter calcoaceticus, E. coli,* and *Serratia marcescens*	Tokonami and others (2013)
E. coli	Apple juice	None	MIP-based QCM sensor	10^8 CFU/mL	$10^8 \sim 10^9$ CFU/mL	~7 min	Low cross-reactivity with *Streptococcus* and *Bacillus*	Yilmaz and others 2015
SEA	Milk	None	QCM immunosensor	0.194 mg/L	LOD to 0.97 mg/L	10 min	Not stated	Salmain and others (2012)
E. coli O157:H7	Ground beef	None	Cantilever immunosensor	10 cells/mL	10 to 10^4 cells/mL	10 min	Not stated	Maraldo and Mutharasan (2007a,b)
L. monocytogenes	Milk	None	Cantilever immunosensor	10^3 CFU/mL (label-free) and 10^2 CFU/mL (sandwich)	Not stated	<1 h	Not stated	Sharma and Mutharasan (2013b)
SEB	Apple juice and milk	None	Cantilever immunosensor	25 and 2.5 fg/mL in apple juice and milk, respectively	LOD to 2.5 pg/mL	~20 min	Low cross-reactivity with food components	Maraldo and Mutharasan (2007a,b)
S. Typhimurium	Tomato, cantaloupe and spinach leaf surface	None	Magnetoelastic (ME) phage-based biosensors	10^2 CFU/mL level	$10^2 \sim 10^8$ CFU/mL	30 min	No cross-reactivity with 74 bacteria	Li and others (2010); Park and others (2013a,b)

of using antibodies, other sensing elements have been explored for SPR biosensors. For example, lectin (wheat germ agglutinin from *Triticum vulgaris*) was used as a bioreceptor for the detection of *E. coli* O157:H7 (Wang and others 2013a). The LOD of *E. coli* O157:H7 in contaminated cucumber samples and ground beef samples was 3.0×10^4 and 3.0×10^5 CFU/mL, respectively. Several SPR immunosensors have been developed to detect bacterial toxins in food: staphylococcal enterotoxin A (SEA) in milk (Tsai and Li 2009), a mixture of BoNT/A, /B, and /F in honey (Ladd and others 2008), and BoNT/B in milk, apple juice, and carrot juice (Ferracci and others 2011). It should be noted that this biosensor detected the BoNT/B substrate instead of the toxin itself, and therefore aimed at detecting the endoproteolytic cleavage activity of the toxin. The coupling of SPR and other technologies also led to diverse possibilities to improve the detection performance, such as SPR coupled with mass spectrometry (Zhukov and others 2004; Chen and others 2007; Nedelkov 2007), and SPR combined with fluorescence imaging (Zordan and others 2009). Although these systems have not been evaluated for the detection of pathogens in food, they can potentially be used for this purpose.

Fiber-optic biosensors utilize optical fibers as the transducing materials. These sensors have advantages, including compatibility with various surface modification approaches, potential of remote control, real-time monitoring, and low cost (Leung and others 2007; Narsaiah and others 2012). This is another relatively mature technology with commercialized detection platforms such as RAPTOR (Research International, Inc., Monroe, Wash., USA; Jung and others 2003). Fluorescence resonance energy transfer (FRET) is a phenomenon of nonradiative energy transfer from a fluorescent donor molecule to an acceptor molecule due to dipole–dipole interactions when in close proximity (Lakowicz 2006). Ko and Grant (2006) developed a FRET immunosensor using a silica fiber core for fluorophore-labeled antibody-protein G complexes immobilization and fluorophore excitation by the evanescent wave. This biosensor was used to detect *S.* Typhimurium spiked in pork samples, and a detection limit of 10^5 CFU/mL was achieved. There are many studies using fiber-optic biosensors to detect bacterial pathogens in food samples, and some enabled multiplex detection. However, due to the limited sensitivity of these methods, cultural enrichment is conducted (Morgan and others 2006; Nanduri and others 2006; Mendonça and others 2012; Ohk and Bhunia 2013).

Other optical methods have been implemented for biosensors for foodborne pathogen detection in food matrices without enrichment. Functionalized polymer vesicles, which respond to bacterial metabolites, were synthesized and used for colorimetric detection of *S. aureus* and *E. coli* spiked in apple juice (Pires and others 2011). The color response was analyzed using a colorimeter, and a spectrometer was used to obtain spectra of the vesicles. You and others (2011) developed a handheld device based on light scattering in latex immunoagglutination assays. Filtered lettuce solution spiked with *E. coli* O157:H7 was prepared for this sensor which can be potentially used directly for fresh produce sample detection. Song and others (2014) reported a FRET-based biosensor using MBs with carbon nanoparticles and a quencher, carbon nanotubes with ssDNA, and a nicking enzyme to detect *S.* Enteritidis 16S rRNA. *S.* Enteritidis cells spiked in the milk samples were lysed and detected at the level of 10^2 CFU/mL. For staphylococcal enterotoxin B (SEB) detection, Temur and others (2012) developed a sandwich assay using aptamer-modified magnetic and gold nanorods. The binding between the target

and sensing elements were investigated using a SPR biosensor, and the toxin in milk was detected using surface-enhanced Raman scattering at attomole level, which indicated the potential of the probes to be used in sensitive biosensing.

Electrochemical biosensors

Electrochemical biosensors are classified into amperometric/voltammetric, potentiometric, and conductometric/impedimetric according to the parameters measured. An electrochemical measurement can be applied to liquid, solid, or gaseous analytes, but the latter 2 are not common for food sample analysis. Electrochemical biosensors have good sensitivity, fast response, and simplicity (Sharma and Mutharasan 2013a). They can be used in turbid media, and can be miniaturized and are disposable such as screen-printed carbon electrodes (SPCEs). Compared to SPR in a study for *E. coli* detection using the same antibody immobilization method, an impedimetric biosensor reached a detection limit 4 logs lower than the SPR biosensor (Maalouf and others 2007). The electrochemical biosensors have been the most commonly used and have received the most interest during the past 2 decades, and many studies have successfully detected foodborne bacterial pathogens in food samples.

Enzymatic reactions and electroactive labels, including nanomaterials are commonly used in culture-independent amperometric/voltammetric biosensors. Lin and others (2008) fabricated a disposable amperometric sensing strip, based on an indirect ELISA with the primary antibody immobilized on AuNP-modified SPCEs. An enzyme-linked sandwich immunoassay was also combined with magnetic separation in a voltammetric biosensor to detect *E. coli* O157:H7 in spiked apple juice (Gehring and Tu 2005). *E. coli* O157:H7 spiked apple juice samples were treated to eliminate lipids and proteins, and the target pathogen was detected at 10^3 CFU/mL. Viswanathan and others (2012) reported a disposable voltammetric sandwich immunosensor for multiplex detection of *E. coli* O157:H7, *Campylobacter*, and *Salmonella* using multiwalled carbon nanotube-polyallylamine-modified screen-printed electrode for antibody immobilization and antibody-functionalized nanocrystal (CdS, CuS, and PbS) for target labeling. Milk spiked with the pathogens was used as the food matrix for evaluation. The 3 different species of foodborne pathogens were each detected at 10^4 CFU/mL. Afonso and others (2013) reported a SPCE-based voltammetric sandwich immunosensor for the detection of *Salmonella*, using magnetic separation and AuNPs as labels for electrochemical detection. Tang and others (2010) developed a voltammetric sandwich immunosensor using horseradish peroxidase-nanosilica-doped multiwalled carbon nanotubes for signal amplification. This immunosensor detected SEB in spiked liquid and solid food samples and had a good correlation with ELISA. Because of the saturation kinetics of enzymes, a small dynamic range may be found for amperometric/voltammetric biosensors based on enzymes. Potential interferences from the oxidation/reduction of interfering compounds also need to be considered when using these biosensors (Palchetti and Mascini 2008). Selective membranes are often used to minimize the interferences (Wahono and others 2012).

Detection strategies involving the changes in pH or ion concentration can be coupled with potentiometric measurements which are able to detect ions in a wide concentration range (Palchetti and Mascini 2008). A real-time aptamer-based potentiometric biosensor was reported for the detection of *E. coli* CECT 675 as a nonpathogenic surrogate for *E. coli* O157:H7 in milk and apple juice (Zelada-Guillén and others 2010). SWCNTs were used as ion-to-electron transducers and for aptamer immobilization. A filtration system was used to remove the charged species in food samples. Ion-sensitive field-effect transistor (ISFET) and light-addressable potentiometric sensor (LAPS) are 2 major approaches based on potentiometric measurement. There have not been many applications of these biosensors for bacterial pathogen detection during the last decade, mainly due to the incompatibility of biomolecule immobilization methods with ISFET fabrication and inadequate device stability (Palchetti and Mascini 2008; Sharma and Mutharasan 2013a). The Threshold® Immunoassay System is a commercially available LAPS device, which was reported for *E. coli* O157:H7 detection in food samples but with cultural enrichment (Tu and others 2000).

Conductometric biosensors were reported for culture-independent detection, yet even more studies used impedimetric measurement. Pal and others (2008) developed a sandwich conductometric immunosensor on membrane pads to detect *B. cereus* in food matrices. The liquid sample was moved through the pad by lateral flow, and the changes in resistance due to the formation of the sandwich structure involving polyaniline when the target presented was measured. Yang and others (2010) reported a biosensor constructed by a network of SWCNT-antibody complexes between 2 electrodes for the detection of SEB. Changes in the resistance were measured, and the biosensor was evaluated with spiked milk and baby food. Impedimetric biosensors are commonly run using an electrochemical impedance spectroscopy technique, which analyzes both the resistive and capacitive properties of biological components. The advantages of impedimetric biosensors include their rapid response and the capability of both labeled and label-free bacterial detection, since cell membranes and microbial metabolism can result in changes in electrical impedance. The sensitivity of impedimetric biosensors has been improved by using high-density electrodes (such as interdigitated array micro/nano-electrodes) for larger active areas and enhanced capability to monitor the changes in the immediate proximity of electrode surface (Van Gerwen and others 1998; Yang and others 2004; Wang and others 2009; Wang and others 2010), and nanomaterials for efficient immobilization of biomolecules and signal amplification (Wang and others 2006; Suni 2008; Dong and others 2013). An interdigitated array microelectrode-based impedance immunosensor was reported for the detection of *E. coli* O157:H7 in ground beef with magnetic separation (Varshney and Li 2007). *E. coli* O157:H7 at 8×10^5 CFU/mL was detected in 35 min. Li and others (2011a) reported a label-free capacitive immunosensor with antibody immobilized on a gold electrode through SAM to detect *E. coli* O157:H7 in spiked milk, ground beef, and spinach samples. *Listeria innocua*, which is a surrogate for *L. monocytogenes*, was detected using an impedimetric biosensor based on the recognition of the target by bacteriophage-encoded peptidoglycan hydrolases (endolysin) immobilized on a gold screen-printed electrode (Tolba and others 2012). The biosensor was evaluated with 2% milk spiked with the target organism. Dong and others (2013) described label-free detection of *S.* Typhimurium in spiked milk by immobilizing anti-*Salmonella* antibodies onto the gold nanoparticles and poly(amidoamine)-multiwalled carbon nanotubes-chitosan nanocomposite film-modified glassy carbon electrode using an impedance measurement. Joung and others (2013) used an alumina nanoporous membrane with hyaluronic acid as the transducer and immobilized antibody on the membrane for *E. coli* O157:H7 detection in whole milk, as shown in Figure 4. Graphene has become an attractive material for biosensing due to its unique nanostructure and electrical properties,

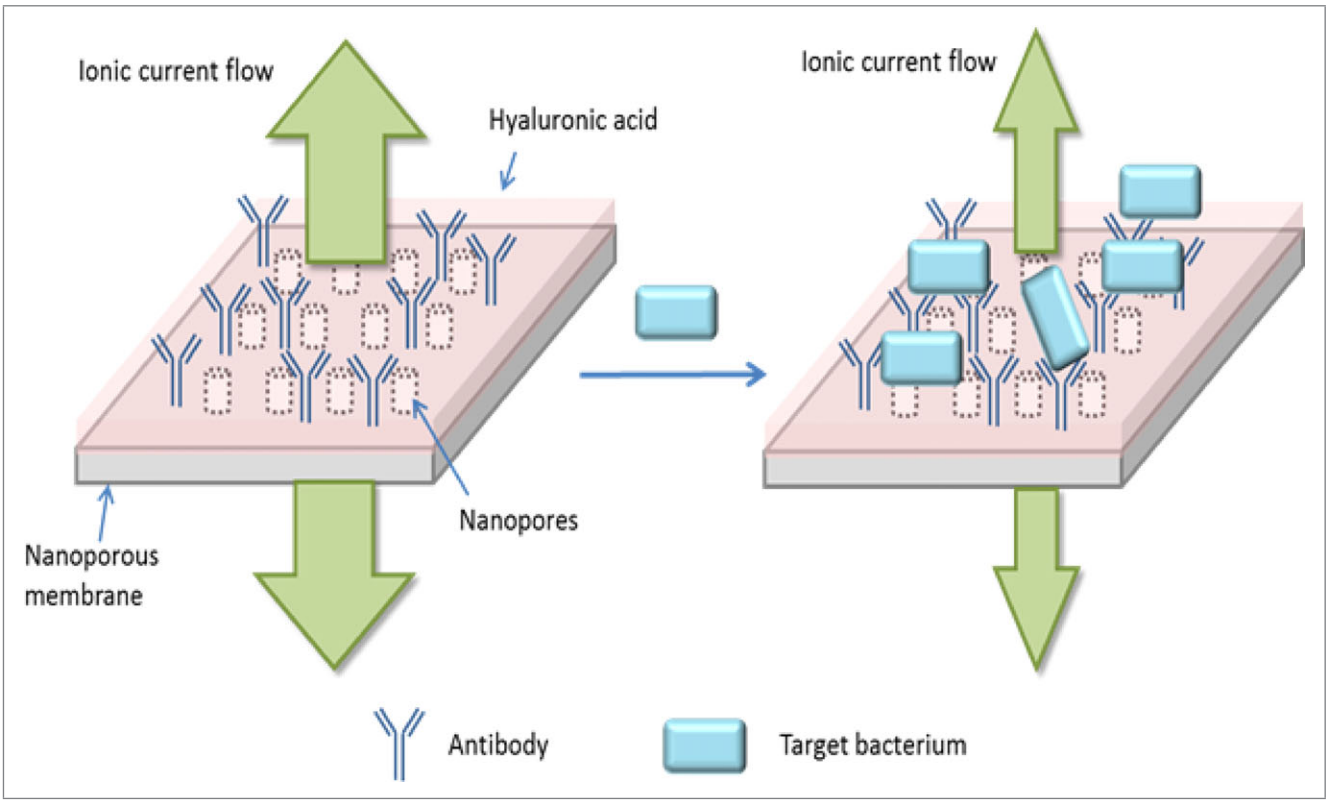

Figure 4–Schematic of a nanoporous membrane-based impedimetric immunosensor for the detection of food pathogens (Joung and others 2013). The alumina nanoporous membrane is coated with hyaluronic acid (HA) and antibodies. When target bacteria are bound to the HA/antibody-coated membrane surface, the flow of ions through the nanopores is reduced proportionally to the target concentration. Changes in impedance can then be measured.

which has also been employed in conductometric biosensors for bacterial detection at 10^1 CFU/mL (Huang and others 2011; Wan and others 2011), although these studies have not evaluated the biosensors in food matrices. Wang and others (2013b) tested a gold nanoparticle-modified graphene paper electrode for label-free detection of *E. coli* O157:H7 using an impedimetric immunosensor, which detected 1.5×10^4 and 1.5×10^3 CFU/mL of target bacteria in spiked ground beef and cucumber samples, respectively. Many different configurations of impedimetric biosensors are available due to the advantages of the measurement methods, and they are expected to continue attracting research interest as portable and rapid detection devices.

Piezoelectric biosensors

Piezoelectric quartz crystal microbalance (QCM) is one major type of piezoelectric biosensor which has been investigated for pathogen detection. QCM biosensors have the advantage of real-time monitoring, ease of use, biocompatible electrodes (such as Au) for ligand immobilization and label-free detection. Salmain and others (2012) used a QCM immunosensor for label-free detection of SEA, and they optimized a strategy to immobilize antibody on the gold transducer. They reported a detection limit of 0.194 mg/L in milk. Nanogram levels of mass changes can be detected by QCM sensors, which is usually not low enough for sensitive detection. Therefore, mass amplification is a commonly used approach for improving the sensitivity. Chen and others (2008) reported a DNA QCM biosensor using AuNPs capped with a DNA probe for signal amplification to detect the *E. coli* O157:H7 *eaeA* gene. A detection level of 10^2 CFU/mL (or CFU/g) was achieved in apple juice, milk, and ground beef. New sensing elements and effective ligand immobilization approaches are also under development. Tokonami and others (2013) and Yilmaz and others (2015) described QCM biosensors using molecular imprinting-based synthetic receptors for bacterial detection and evaluated the biosensor in apple juice samples. The polymeric structure for target recognition was developed via contact imprinting of whole cells. Although the detection limit is not satisfactory, the studies using molecular imprinting polymers indicate a potential new detection strategy.

Cantilever sensors based on piezoelectric materials for mass sensing have also been reported for pathogen detection. *E. coli* O157:H7 at a level of 10 cells/mL in spiked ground beef supernatant samples was detected in 10 min using a piezoelectric-excited millimeter-sized cantilever (PEMC) immunosensor (Maraldo and Mutharasan 2007a). The PEMC sensor comprises a piezoelectric layer which provides a sensitive response to weak stresses. Sharma and Mutharasan (2013b) also reported use of a PEMC immunosensor, which detected *L. monocytogenes* in milk with a detection limit of 10^3 CFU/mL in a label-free format and 10^2 CFU/mL in a sandwich format. The same group evaluated the PEMC immunosensor for the detection of SEB in apple juice and milk (Maraldo and Mutharasan 2007b).

Magnetoelastic biosensors

Magnetoelastic (ME) sensors are made of amorphous ferromagnetic alloys. When excited by an external time-varying magnetic field, the materials exhibit a ME resonance which can be detected by using a noncontacting pickup coil (Ruan and others 2003).

When a target pathogen comes in contact with the alloy sensor surface, the added mass causes a change in resonance frequency and the signal can be remotely detected by the pick-up coil. Therefore, ME sensors are remote and wireless devices which may become very useful tools for in-field monitoring. Several studies reported the detection of foodborne pathogens on the surface of fresh produce with minimal or no sample preparation. Filamentous E2 phages were immobilized on ME sensors and the sensors were placed on tomatoes for 30 min to bind *S.* Typhimurium on the surface. The resonance frequency, which changes before and after contact with tomatoes, was measured (Li and others 2010). ME biosensors also showed comparable sensitivity and repeatability as qPCR but required less time and skill to manipulate, for detecting *S.* Typhimurium on spinach leaves and cantaloupes (Park and others 2013a, b). For these ME biosensor studies, *S.* Typhimurium was detected at 10^2 CFU/mL on the surface of the produce items (Li and others 2010; Park and others 2013a, b).

As sensitivity, specificity, and rapidity continue improving with the development of biosensors, there are always challenges with reproducibility and in-field performance. Nanotechnology has been introduced in biosensors due to the special properties which facilitate detection. The reduced probability of nano-sized transducers to interact with an analyte, manipulation of individual nano-sized objects (Farahi and others 2012), and the unclear toxicity of nanomaterials still need further investigation. Developments in new transducing materials, new recognition approaches, multiplex detection configurations, microfluidic techniques, integrated biosensing systems (for example, LOC), and wireless devices would promote biosensors to become mature products for direct application in the food industry.

Conclusions

Since foodborne pathogens are generally present in food samples at low concentrations, novel preprocessing and rapid detection methodologies are required to work with these low numbers. Many methods to detect foodborne pathogens have been developed in recent years to address the attention of food safety and public health concerns, especially with the increasing demands of fresh and minimally processed food products. Instead of conventional culture-based methods which require time-consuming processes, nucleic-acid-based methods including widely used PCR methods, antibody-based assays including ELISA, and biosensors have been reported for the detection of foodborne pathogens without cultural enrichment. Although the cost of using these methods is variable depending on reagents and equipment, generally they are more cost-effective than conventional culture-based methods due to the reduced detection time. Nucleic acid-based detection methods are rapid, highly target-specific, reproducible, and only require small amounts of target DNA. qPCR and isothermal amplification techniques, including LAMP and NASBA, are some important improvements in the field of detection. In the future, LOC devices will be a portable option for not only nucleic acid-based detection, but also other types of detection assays. ELISA and LFI do not require a DNA extraction step. However, the sensitivity of the assays is one of the major concerns of using these methods. Many studies have improved the sensitivity and enabled multiplex detection. Automation of these assays is also under investigation. Biosensors are rapid, sensitive, and low-cost detection devices which are designed for simple manipulation. Different sensing elements, transducing technologies and configurations of biosensors have been reported in recent years for direct detection of foodborne pathogens. They are potential alternatives to conventional methods, although much work still needs to be done to ensure their reliability, stability of biomaterials, and compatibility to in-field tests. Evaluation of the culture-independent detection methods in food matrices usually shows less sensitive detection than in buffer solutions due to the interference from food components and nontarget microorganisms. Effective and rapid separation methods, highly specific detection probes, and comprehensive validation of new assays will aid in the development of future detection technologies.

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Author Contributions

YW provided the idea of the manuscript. YW and JKS developed and wrote the manuscript. Both authors read, edited, and approved the final manuscript.

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