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Foodborne Pathogens Detection: Persevering Worldwide Challenge

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Abstract

According to Health Canada, foodborne disease is responsible of more than 4 million cases per year. In United States, more than 48 million people get sick, 128,000 are hospitalized and 3000 die every year in United States due to foodborne diseases according to the Center for Disease Control and Prevention. Cross-contamination from the raw materials, during the process or on working surface has to be rapidly detected. Good manufacturing practices (GMP) and hazard analysis critical control point (HACCP) can help to reduce the incidence of contamination. However, the development of sensitive and rapid methods of detection is still an important need. Standard culture-based methods request the consumption of large amounts of media, are time-consuming and interferences can occur when samplings are done in complex food matrices. The polymerase chain reaction (PCR)-based methods are new technologies. These methods show high level of specificity and sensitivity because they can detect nucleic acid sequences of target bacteria. However, they require an expensive instrumentation and trained scientific technicians. This review is focusing on the development of new simple, sensitive, specific, and time-saving technologies in order to detect quickly foodborne pathogens for application in food industries.

Keywords: foodborne pathogens, rapid technologies, food industries, food safety

1. Introduction

Large-scale of foodborne outbreaks is still an ever-present threat to public health, particularly, for very young and elderly people as well as pregnant women, and people susceptible to a weakened immune system [1]. The global incidence of foodborne disease is difficult to estimate, but it has been reported that every year, foodborne pathogens cause millions of infections and

intoxications as well as thousands of deceases. Moreover, outbreaks generate billions of dollars in worth of damage, public health problems, and agricultural product losses [2].

The etiology was determined in the United States in the period from 1993 to 1997 and reported outbreaks showing that bacteria caused 75% of outbreaks and 86% of cases [3]. Furthermore, among the 31 pathogens identified as causing foodborne illnesses, *Salmonella*, *Campylobacter*, *Staphylococcus aureus*, *Listeria monocytogenes*, *Clostridium perfringens*, and *Escherichia coli* O157:H7 have been incriminated for the large majority of illnesses, hospitalizations, and deaths [4]. Indeed, *Salmonella* spp., *L. monocytogenes*, *E. coli* O157:H7, and *S. aureus* are on the top of list for the largest number of outbreaks, cases, and deaths [5, 6].

The frequent occurrence of foodborne diseases in previous years is mainly based on five factors, inter-related, and difficult to control to a large degree involving environmental conditions, health system including infrastructure social situation, behavior and lifestyles, health and demographic situation, and food supply system [7]. Although pathogen detection is a growing concern for three main application areas including water, environment quality control [8, 9], and clinical diagnosis, food industry still remains the major area concerned with 38% of the relative number of works appeared in the literature about the detection of pathogenic bacteria [10].

In industrialized countries, the public health authorities set up strict measures and regulations for food control systems such as hazard analysis critical control point system (HACCP) and good manufacturing practice (GMP) in order to overpower the spread of these diseases at the level of the food processing and the food supply system. HACCP is a method of food safety assurance based on the application of good hygiene practices. The HACCP system identifies any additional or more specific control measures necessary in food operations, places an additional emphasis on those points of good hygienic practices, foresees corrective measures if monitoring results indicate a loss of control, and finally provides more training and responsibility to operators [7]. Thus, the detection of foodborne pathogenic bacteria is an important key to the prevention and the control of some hazardous points in food processing or supply systems. Traditional detection methods may take up to a week to yield a confirmed result, challenging many researchers to gear their efforts toward the development of rapid methods for obtaining analytical results in the shortest time. The present chapter attempts to compare the different methods of pathogens detection currently used in food industry as measures of prevention from foodborne diseases. Certainly, it is essential to be well informed about the different methods of pathogens detection but this is as much interesting to find out the possible sources of contamination.

2. Sources of contamination

Foodborne diseases are induced by the consumption of foods or water contaminated by pathogens [11]. **Figure 1** shows most of the pathways leading to the presence of foodborne pathogens in daily food products for nowadays consumers. These food products include fresh produce such as fruits, vegetables, herbs, seeds and nuts, milk and dairy products, meat products as well as poultry and eggs. From the preharvest phase, most of these products go

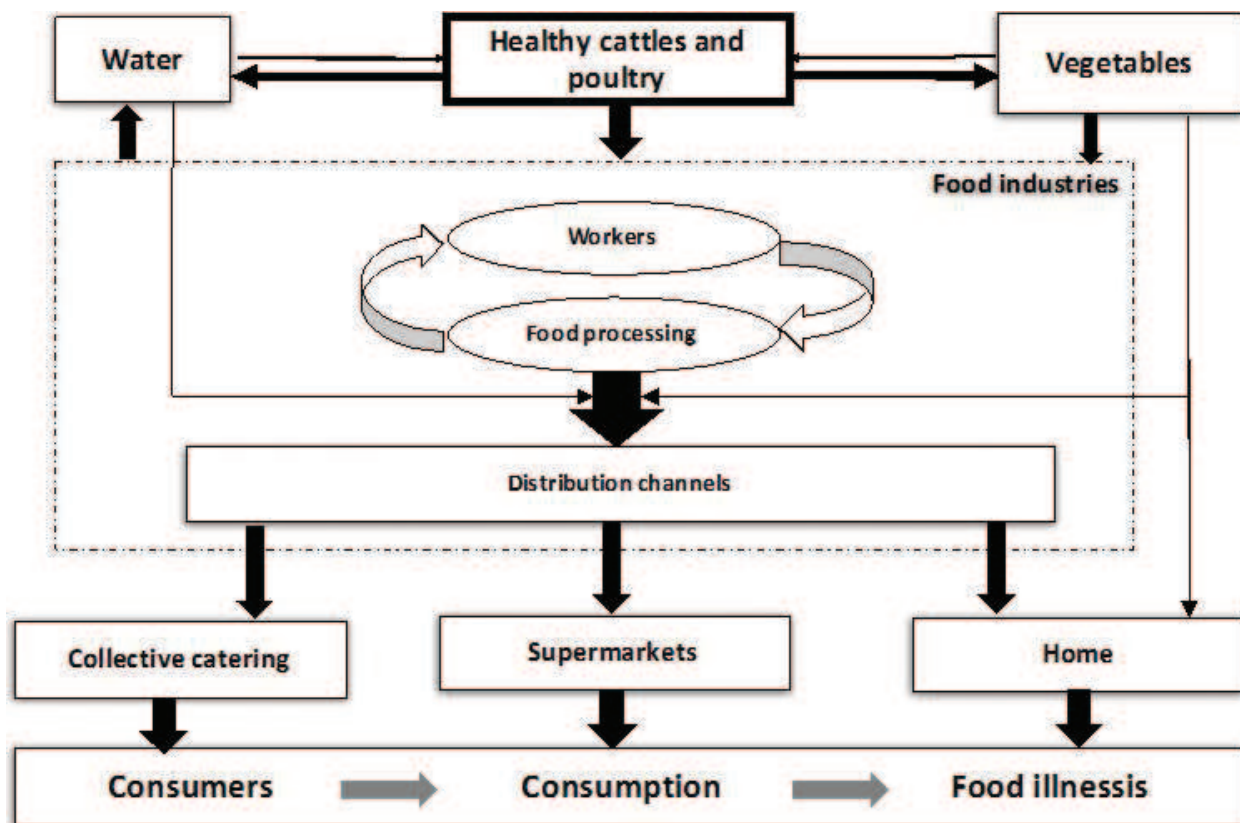


Figure 1. Potential flow of food contamination (adapted from [61]).

through either a local distribution directly from the farmer to the consumer, or a wider distribution to the industry. In industrialized countries, consumers get these raw materials for home use through the supermarkets. In all cases, food is an excellent source of energy and nutrition, not only for human and animals but also for the proliferation of microorganisms.

The contamination by the fresh produce has been well discussed by [2]. Food manufacturing mostly relies on fresh produce, as raw materials that offer to consumers a wide range of benefits such as nutrients, vitamins, and fibers. From farm to fork, the contamination of fresh produce by pathogens may occur at any stage during transformation process from the preharvest to the postharvest phase. In the field, contamination can occur through some elements of nature (water, soil, seeds, insects, dust, etc.) whereas the central part of contamination during the postharvest phase is related to handlers and equipment during processing, transportation, and preparation [12]. The risk for this kind of products is that they are usually consumed in raw state or not heat-treated, avoiding the elimination of pathogens before consumption [13]. *Salmonella* spp., pathogenic *E. coli*, *L. monocytogenes*, *S. aureus*, *Shigella* spp., *Yersinia* spp., and *Clostridium* spp. are the main pathogens contaminating fresh produce.

In another side, as described by [14], healthy cattle may hideaway in their liver, kidneys, lymph nodes, and spleen human pathogenic microorganisms. From slaughtering, the first step in meat processing, carcasses are exposed to microorganisms present in animal intestinal tracts and consequently contaminate other cut surfaces and carcasses. Thus, carcass contact surfaces,

water, air, and staff during processing and distribution channels are potential sources of contamination in meat and meat products. Concerning poultry products, critical steps that may lead to contamination are defeathering and evisceration with higher probability in case of contaminated hands and toll workers. The pathogens that threaten these products are *Salmonella* and *Campylobacter*. *L. monocytogenes* is the most incriminated pathogen in the contamination of dairy products, which are vulnerable to the risks from udders of cows and milk equipment.

It is obvious that the high volume of food production may lead to a greater likelihood of a cross-contamination as previously described and consequently a high spread of the disease. This finding was also supported by [15] mentioning that in industrialized countries, the amounts of outside food consumption including international travels as well as the increasing demand for minimally processed ready-to-eat (RTE) products increase the risk of foodborne diseases. In a large case-control, 20% of infections with *E. coli* O157:H7 was associated to eating at a table-service restaurant, 35% of infections with *S. enteritidis* with egg consumption in a restaurant, and 35% were attributed to eating chicken prepared out of home.

Although fresh produce, red meat, poultry and milk are the raw materials not only for food industry and restaurants, but also for supermarkets. However, supermarket RTE food products themselves are the raw materials for consumers' homemade meals [16]. To avoid cross-contamination from raw materials, it is essential to wash hands, tools, and prepare surfaces before and after processing. Also, food products that are already prepared/cooked have to be refrigerated at 4°C. However, hot foods should be kept above 60°C. Besides, it is recommended to split large volumes of food into small portions for rapid cooling in the refrigerator as well as heating whole canned foods before tasting. Otherwise, there is a high increase in the consumption of street food and consequently in the need of more food service establishments [7].

The large number of interconnected factors increases the risks of cross-contaminations. To control the spread of these pathogens, first there is a need for monitoring the contamination of raw materials from suspected sources to the end of the supply chain by applying hygiene and sanitation practices and also the advent of new rapid technologies of detection.

3. Conventional methods

According to [17], conventional microbiological methods are usually performed for the isolation and enumeration of pathogens in food samples. Nowadays, these standard culture methods are still considered as the "gold standard" as they are sensitive, inexpensive, and give both qualitative and quantitative information on the number and the nature of microorganisms present in food samples.

On the other side, conventional methods are time-consuming considering all basic pre-enrichment, enrichment, and plating steps needed. They mainly rely on specific media to enumerate and isolate viable bacterial cells in food. The pre-enrichment of the food samples, in a non-selective or selective broth culture, can be used to increase the number of injured

but viable bacteria that can be a potential threat to human health, to a detectable level [18]. Pre-enrichment recover a larger proportion of bacteria from food matrices and is usually followed by sublethal stressors such as heating, cooling, acids, or osmotic shocks [19]. In addition to that, the occurrence of toxin production in food requires that the cell pathogen concentration reaches a specific level as much as 5 log CFU/g of *Staphylococcus aureus* and *Bacillus cereus*, 3 log CFU/g of *Clostridium botulinum* (CFU referring to colony-forming unit). Thus, all existing detection technologies have to be preceded by an enrichment step [20].

Enrichment steps (selective enrichment and selective plating) may require an additional period of 8–24 h before the enumeration or the detection can be completed and mostly they will be followed by biochemical screening and serological confirmation [21]. A variety of chromogenic and fluorogenic culture media are available for selective isolation and differentiation of food-associated spoilage bacteria by incorporation of enzyme substrates. As no single microbiological test, among these standard culture methods, provides a confirmed identification of any unknown microorganism, there is a need for several additional series of analysis [22].

Conventional methods can be laborious too as they usually require the preparation of culture media and colony counting with the most probable number (MPN) method [23]. The duration of these methods depends on the ability of the microorganisms to grow in pre-enrichment, selective enrichment, and selective plating media. This process is often slow and takes 48–72 hours for preliminary identification and more than a week for the confirmation of the pathogen species [4].

Qualitative culture methods are only used to determine the absence or presence of microorganisms in food samples. However, the quantitative ones are preferred for enumeration. The limit of detection (LOD) or sensitivity, the minimum amount of detectable cells, is defined by the presence of microorganisms in 25 g of food examined for qualitative methods and a concentration of <10–100 MPN of bacteria per gram or >10–100 viable counts for quantitative methods [24] considering that the LOD for plating methods is 1 CFU/g.

Regarding the high spread of foodborne pathogens illness, the inspection regulations are very strict with the requirements for process control. The LOD for food pathogens is restricted to 1 cell per unit of food sample [25]. Depending on the target pathogen and the food sample, the analytical unit may be considered from 25 to 325 g.

These methods are recognized for their low cost and ease of use that are relatively interesting compared to alternative methods [21]. Despite these traditional methods are still used due to their high selectivity [10], they are laborious, time-consuming, and may be limited by their low sensitivity [26] compared to other rapid methods. In addition, there is a probability that false negative results may occur due to viable but nonculturable (VBNC) cells.

The challenge of pathogen detection in food matrix, as reported by [23, 17], resides in the presence of pathogens in low numbers and uniformly distributed in a food heterogenic matrix with the presence of non-pathogenic microorganisms that may interfere with the identification step. Food matrices can be found in different physical states (powder, liquid, gel, or semi-solid) and contain a wide range of ingredients that may interfere with the detection.

4. Alternative methods for the detection of foodborne pathogens

To overcome the limitations of conventional methods, various rapid methods have been developed and are commercially available to meet the needs of food industry. Considering that commercialized rapid detection methods should be validated from a recognized organization such as the Association Française de Normalisation (AFNOR) in the European Union or the Association of Analytical Communities (AOAC International) in the United States, most kits of detection are validated according to their the sensitivity and specificity [27]. Ideally for industrial applications, rapid methods should be characterized by their specificity, high sensitivity, and fast performance. Nowadays, current rapid methods are able to detect pathogens in raw and processed foods in low numbers to avoid the risk of infection, which are more time-efficient, labor-saving, and prevent human errors [28]. Currently, the range of detection time for available rapid methods is estimated from a few minutes to a few hours. Nevertheless, the sensitivity and specificity still have to be improved for testing foods samples without the needs to be pre-enriched before analysis [29]. Indeed, the enrichment step is considered as the main limitation in most of the methods but remains essential for the revival of stressed or injured cells, the differentiation of viable from nonculturable cells and the dilution of inhibitors present in the food sample [30].

Rapid detection methods can be categorized into biosensors, immunological methods, and nucleic acid-based methods (Figure 2). Simple polymerase chain reaction (PCR), multiplex PCR, real-time PCR, nucleic acid sequence-based amplification (NASBA), loop-mediated isothermal amplification (LAMP), and oligonucleotide DNA microarray are classified as nucleic-based methods. Biosensors-based methods include optical, electrochemical, and mass-based biosensors. Finally, enzyme-linked immunosorbent assay (ELISA) and lateral flow immunoassay are

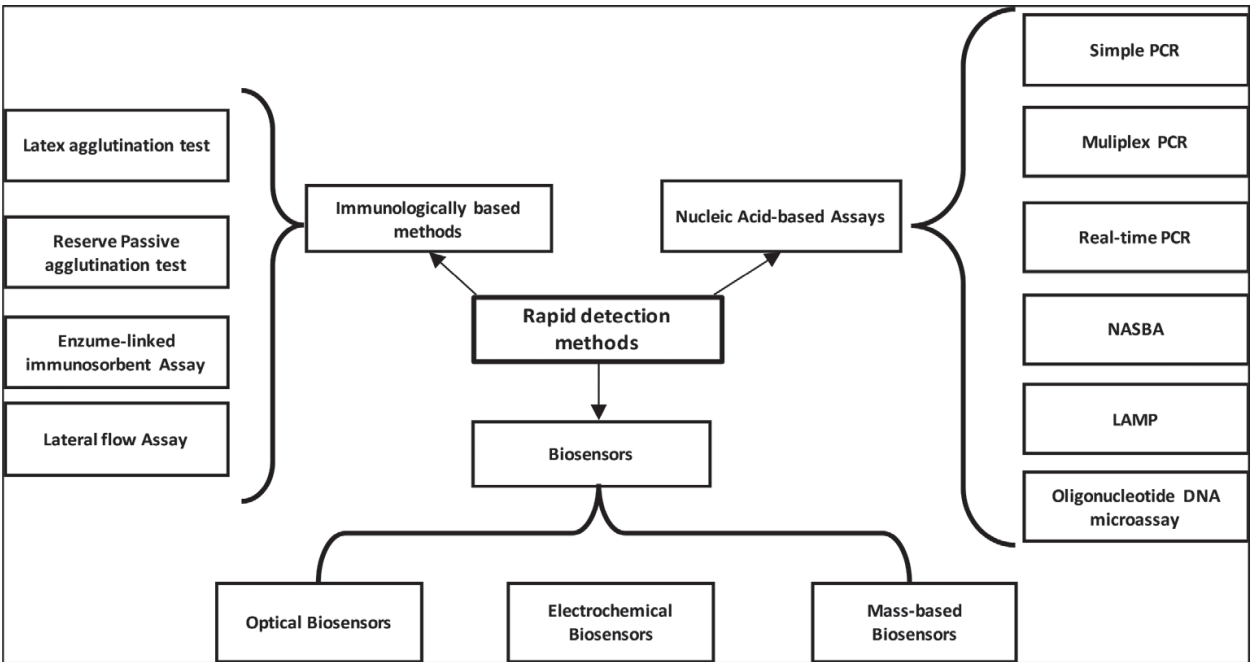


Figure 2. Mapping of rapid detection technologies for foodborne pathogens [32].

recognized as immunology-based methods [31]. Several publications have already detailed the principle of each of these methods [4, 28, 31–33]. However, the aim of this work is to focus on the advantages and limitations of these methods for application in food industry. With the development of new methods, immunology-based methods and PCR become categorized as conventional techniques for the detection of pathogens [34].

4.1. Nucleic acid-based methods

Nucleic acid-based methods prevent ambiguous or wrongly interpreted results. They operate by detecting specific deoxyribonucleic acid (DNA) or ribonucleic acid (RNA) sequences in the target pathogen and hybridizing the target nucleic acid sequence to a synthetic oligonucleotide, which is complementary to the target sequence [4]. Invented 20 years ago, simple PCR [35] is widely used for the detection of *L. monocytogenes* [36], *E. coli* O157:H7 [37], *S. aureus* [38], *Campylobacter jejuni* [39], *Salmonella* spp. [40], and *Shigella* spp. [41]. The presence of sufficient numbers of target molecules, the purity of the target template, the complexity of food matrices containing potential inhibitory compounds may affect the reliability of PCR amplification [42].

Through the years, PCR techniques have undergone significant improvements for faster detection with the development of real-time PCR for monitoring PCR amplification products, in addition to the methods of simultaneous detection such as multiplex PCR and oligonucleotide DNA microarray that can detect up to five or more pathogens simultaneously [43] such as *Salmonella enteritidis*, *S. aureus*, *Shigella flexneri*, *L. monocytogenes*, and *E. coli* O157:H7 [44].

Presently, as shown in **Table 1**, there is an important selection of commercially available kits based on nucleic acid methods for the detection of foodborne pathogens. However, although these techniques are automated for reliable results and characterized with high sensitivity and specificity, they induce some disadvantages such as difficulties to differentiate viable from non-culturable cells and the design of the primers. In some case, they require trained staff in order to minimize the occurrence of cross-contamination. According to [45], the isothermal amplification method for nucleic acids, NASBA, and an amplification system for RNA analytes (e.g., viral genomic RNA, mRNA, or rRNA) could be extended from viral diagnostics to the gene expression and cell viability. Despite, the low cost of these methods and the non-requirement of thermal cycling system, post-NASBA product detection is still considered labor-intensive.

Otherwise, the LAMP method, can provide a large amount, usually 10^3 higher to simple PCR, of DNA with rapidity under isothermal conditions [4], lower detection limits compared to conventional PCR [46, 47] and higher specificity due to the use of four primers targeting six specific regions [48].

4.2. Immunology-based methods

The most successful and popular technology in the field of the detection of bacterial cells, spores, viruses, and toxins is represented by immunological methods. This technology is faster, more robust, and has the ability to detect contaminating organisms as well as their biotoxins. However, they are less specific and less sensitive than nucleic acid-based detection [49]. Compared to traditional counting methods, antibody-based methods generate less assay time

| Pathogen | Method | Commercially available kits | Sensitivity | Catalog number | Sample matrix | Company |
|---|------------------------|--|--|---------------------|--|---------|
| <i>Staphylococcus</i> | PCR | BAX® System Real-time PCR assay | 10 ⁴ CFU/mL, after enrichment | D12762689 | Powdered infant formula, ground beef, soy protein isolate | HYGIENA |
| <i>Salmonella</i> spp. | PCR | BAX® System Standard PCR assays for <i>Salmonella</i> | 10 ⁴ CFU/mL, after enrichment | D11000133–D14368501 | Poultry, dairy, fruits, vegetables, bakery products, pet food and environmentals | HYGIENA |
| <i>Salmonella</i> spp. | Real-Time PCR | BAX® System Real-time PCR assay for <i>Salmonella</i> | 10 ⁴ CFU/mL, after enrichment | D14306040 | Meat, poultry, dairy, fruits, vegetables, bakery products, pet food and environmentals | HYGIENA |
| <i>E. coli</i> O157:H7 | Multiplex PCR | BAX® System PCR assay for <i>E. coli</i> O157:H7 MP | 10 ⁴ CFU/mL, after enrichment | D12404903 | Raw ground beef, beef trim, produce | HYGIENA |
| <i>Salmonella</i> | DNA hybridization test | GeneQuence® for <i>Salmonella</i> | 1–5 CFU/25 g | 6700 - | Food and environmental samples | NEOGEN |
| stx and eae genes – STEC Screening | Real-time PCR assay | BAX® System Real-Time PCR STEC Assay | 10 ⁴ CFU/mL, after enrichment | D14642964 | Raw ground beef, beef trim, produce | HYGIENA |
| <i>E. coli</i> O26, O111, O121 - | Real-time PCR assay | | 10 ⁴ CFU/mL, after enrichment | D14642970 | Raw ground beef, beef trim, produce | HYGIENA |
| <i>E. coli</i> O45, O103, O145 | Real-time PCR assay | | 10 ⁴ CFU/mL, after enrichment | D14642987 | Raw ground beef, beef trim, produce | HYGIENA |
| <i>E. coli</i> O157:H7 | Real-time PCR assay | BAX® System Real-Time PCR Assay for <i>E. coli</i> O157:H7 | 10 ⁴ CFU/mL, after enrichment | D14203648 | Raw ground beef, beef trim, produce | HYGIENA |
| <i>Listeria</i> spp. | PCR | BAX® System <i>Listeria</i> spp | 10 ⁵ CFU/mL, after enrichment | D11000147 | Food and environmentals | HYGIENA |
| <i>Listeria</i> spp. (except <i>L. grayii</i>) | PCR | BAX® System PCR Assay for Genus <i>Listeria</i> 24E | 10 ⁴ CFU/mL, after enrichment | D13608135 | Dairy, meat, fish, vegetables, environmentals | HYGIENA |
| <i>Listeria</i> species | Real-time PCR assay | BAX® System Real-Time PCR Assay for Genus <i>Listeria</i> | 10 ⁴ CFU/mL, after enrichment | D15131113 | Dairy, ready-to-eat meat, seafood, vegetables, environmentals | HYGIENA |
| <i>Listeria monocytogenes</i> | PCR | BAX® System PCR Assay for <i>L. monocytogenes</i> | 10 ⁵ CFU/mL, after enrichment | D11000157 | Variety of food types | HYGIENA |

| Pathogen | Method | Commercially available kits | Sensitivity | Catalog number | Sample matrix | Company |
|-------------------------------|------------------------|---|--|----------------|---|---------|
| <i>Listeria monocytogenes</i> | PCR | BAX® System PCR Assay for <i>L. monocytogenes</i> 24E | 10 ⁴ CFU/mL, after enrichment | D13608125 | Dairy, meat, fish, vegetables, environmentals | HYGIENA |
| <i>Listeria monocytogenes</i> | Real-time PCR assay | BAX® System Real-Time PCR Assay for <i>L. monocytogenes</i> | 10 ⁴ CFU/mL, after enrichment | D15134303 | Dairy, ready-to-eat meat, seafood, vegetables, environmentals | HYGIENA |
| <i>Listeria</i> spp. | DNA hybridization test | GeneQuence® for <i>Listeria</i> | 1–5 CFU/25 g | 6708 | Food and environmental samples | NEOGEN |
| <i>Listeria monocytogenes</i> | DNA hybridization test | GeneQuence® for <i>L. monocytogenes</i> | 1–5 CFU/26 g | 6709 | Food and environmental samples | NEOGEN |

Table 1. Commercially available nucleic acid-based methods for the detection of foodborne pathogens (adapted from [32]).

but present a lack of ability to detect microorganisms in “real-time” mode if the quantity of pathogens is not high enough to provide real-time information. As reported by [50], problems that may emerge are the low sensitivity of the assays, low affinity of the antibody to the pathogen or other analytes being measured, and potential interference from contaminants.

Among other immunological methods, both of ELISA and lateral flow immunoassay are mainly used for the detection of foodborne pathogens. ELISA is specific and labor-saving as it allows the detection of bacterial toxins and can handle large number of samples. However, this technology presents several disadvantages such as the need of trained staff and the possibility of false negative results due to the cross-reactivity with closely related antigens. As immunoassays rely on the specific binding of an antibody to an antigen, the response of the test depends on the amount of the antigen in the sample and the availability of the binding sites. Thus, the low sensitivity of this technology, in the field of the detection of foodborne pathogens, requires a pre-enrichment step to reach a detectable level of antigen in the sample as well as a labeling of antigens and antibodies [51, 52]. On the other hand, lateral flow assay is low cost, reliable, easy-to-operate, sensitive, specific, and allows the detection of bacterial toxins but still requires labeling of antigens and antibodies [4]. Commercialized kits of these two techniques are summarized in **Table 2**. Toward the progress of rapid methods, new antibody-based methods have been coupled with other methods for pathogen detection, such as immunomagnetic separation on magnetic beads coupled with matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF) for detection of staphylococcal enterotoxin B [53] and combination of immunomagnetic separation with flow cytometry for the detection of *L. monocytogenes* [54].

4.3. Biosensors

Nowadays, the use of biosensors is increasing in the field of food pathogen detection using nucleic acid- and immunology-based methods considered as conventional ones. In recent years, there has been much research activity in the area of biosensors development for detecting pathogenic microorganisms. Compared to standard methods, biosensors are more favorable for checking food safety, throughout the production process, due to their real-time response [55]. Biosensors are powerful analysis tools covering a wide range of applications particularly food quality monitoring, disease detection, toxins of defense interest, environmental monitoring, soil quality monitoring, drug discovery, and prosthetic devices [56].

As defined by [35], biosensor devices are constituted with two main parts: the bioreceptor (biological material recognizing the analyte) and the transducer (converting the bio-recognition energy into optical or electrical signals). A bioreceptor can be a microorganism, cell, enzyme, antibody, nucleic acid, aptamers, or biomimic. However, the transduction may be optical, electrochemical, thermometric, piezoelectric, magnetic and micromechanical, or combinations of the above techniques.

The classification of the several types of biosensors is based on their bioreceptors or transducers, as described by [35]. Electrochemical, mass-based, and optical biosensors are the mainly used biosensors for the detection of foodborne pathogens [51], especially surface plasmon

| Pathogen | Method | Commercially available kits | Sensitivity | Catalog number | Sample matrix | Company |
|--|------------------------------------|--|--|--|---|----------------------|
| Shiga Toxin-producing <i>E. coli</i> (STEC) including <i>E. coli</i> O157:H7 and Verotoxin | Lateral flow Assay | Food check <i>E. coli</i> O157 test kit, Carcass Sponge Kit, Assay Cassettes | 1 CFU/375 g of ground beef | FCEC-001, FCEC-005, FCEC-006 | Raw ground beef, beef trims and carcass | Foodchek Systems Inc |
| | | RapidChekO <i>E. coli</i> O157 (including H7) Test Kit | 1 CFU/25 g of food. | 7,000,157, 7,000,158, 7,000,161, 7,000,165 | Boneless beef trim and ground beef | Romer Labs |
| | | Transia Card <i>E. coli</i> O157 | — | — | Raw ground beef Raw beef product | Raisio Diagnostics |
| | | Reveal® for <i>E. coli</i> O157:H7 | 1 CFU/25 g; 1 CFU/375 g | 9714 | | NEOGEN |
| | Enzyme-Linked Immuno Sorbent Assay | 3MTM TecraTM <i>E. coli</i> O157 VIA | 1–5 CFU/25 g sample | ECOVIA48 ECOVIA96 | NR | 3 M Canada |
| | | Assurance® EIA EHEC | — | 4000 01 | Meat, dairy, poultry, fruit, nuts, and more | BioControl |
| <i>Listeria</i> | Enzyme-Linked Immuno Sorbent Assay | 3MTM TecraTM <i>Listeria</i> VIA | 1–5 CFU/25 g sample or 1–5 CFU/swab | LISVIA48 | NR | 3 M Canada |
| | | Assurance <i>Listeria</i> EIA | — | 67,000–96 | Environmental surfaces and food samples. | BioControl |
| | Lateral flow Assay | Reveal®2.0 for <i>Listeria</i> | 1 CFU/analytical unit | 9707 | Food and environmental samples | NEOGEN |

| Pathogen | Method | Commercially available kits | Sensitivity | Catalog number | Sample matrix | Company |
|-----------------------|------------------------------------|---|---|---------------------------------|--|-----------------|
| <i>Salmonella</i> spp | Enzyme-Linked Immuno Sorbent Assay | 3MTM TecraTM <i>Salmonella</i> Visual Immunoassay (VIA) | 1–5 CFU/25 g sample | SALVIA48 | All Foods | 3 M Canada |
| | | 3MTM TecraTM <i>Salmonella</i> ULTIMA VIA | 1–5 CFU/25 g sample | SALULT96 | All Foods | 3 M Canada |
| | | MaxSignal® <i>Salmonella</i> Test Strip Kit | 1x10 ⁵ CFU - 1x10 ⁶ CFU/mL | BO_1063–01 | Food and Feed Products | Bioo Scientific |
| | Lateral flow Assay | RapidChek® <i>Salmonella</i> | — | 7,000,183–7,000,167 | Raw ground beef (25 g, 375 g), raw ground chicken, chicken carcass rinsates, liquid eggs, sliced cooked turkey, environmental samples and peanut butter. | SDIX |
| | | RapidChek® SELECT™ <i>Salmonella</i> | — | 7,000,190–7,000,195 - 7,000,198 | | SDIX |
| | | RapidChek® SELECT™ <i>Salmonella enteritidis</i> | — | 7,000,220–7,000,222 | Food samples | SDIX |
| | | TRANSIA™ PLATE <i>Salmonella</i> gold | — | SA0180 | All foods | BioControl |
| | | Reveal® 2.0 | 1 CFU/analytical unit 10 ⁶ CFU/mL post enrichment | 9706 | Chicken carcass rinse, raw ground turkey, raw ground beef, hot dogs, raw shrimp, ready-to-eat meal products, dry pet food, ice cream, fresh spinach, cantaloupe, peanut butter, swabs from stainless steel surfaces, and sprout irrigation water | NEOGEN |

| Pathogen | Method | Commercially available kits | Sensitivity | Catalog number | Sample matrix | Company |
|------------------------------|------------------------------------|---|--|----------------|---|------------|
| <i>Staphylococcus aureus</i> | Enzyme-Linked Immuno Sorbent Assay | 3MTM Tecra™ S. <i>aureus</i> VIA (3 M) | 1–5 CFU/25 g sample | STAVIA96 | Food samples | 3 M Canada |
| | | 3MTM Tecra™ Staph Enterotoxin VIA (3 M) | 1 ng/mL of sample extract | SETVIA48 | Food samples | 3 M Canada |
| | Lateral flow Assay | TRANSIA® PLATe Staphylococcal Enterotoxins | 0.25 ng S. enterotoxins/g sample | ST0796 | Milk and dairy products | BioControl |
| | | TRANSIA™ PLATE Staphylococcal Enterotoxins Plus | 0.25 ng S. enterotoxins/g sample | ST0777 | Milk and dairy products | BioControl |
| | | TRANSIA™ PLATE Staphylococcal Enterotoxins ID | 20–60 pg./mL of each serological group (A-E) | ST0712 | Milk and dairy products, Meat, poultry and eggs, Seafood and other foods, Feed products | BioControl |
| | | TRANSIA® IAc Staphylococcal Enterotoxins | 0.1 ng S. enterotoxins/g sample | ST0705 | Milk and dairy products | BioControl |
| | | TRANSIA® TUBE Staphylococcal Enterotoxins | 0.5 ng S. enterotoxins/g | ST724B | Milk and dairy products | BioControl |
| NR: not reported. | | | | | | |

Table 2. Commercially available immunology-based methods for the detection of foodborne pathogens (adapted from [32]).

| Pathogen | Method | Commercially available kits | Sensitivity | Sample matrix | Company | References |
|--|---|---|--|---|---------------------------------------|------------|
| <i>Escherichia coli</i> O157:H7 | Optical immunosensor based on selective antibody expressed by human cell line | CANARY™ system | 500 CFU/g | Lettuce | Massachusetts Institute of Technology | [62] |
| <i>Escherichia coli</i> O157:H7 and <i>Salmonella</i> | Electrochemical immunosensor based on the assembly of three nanoparticle | Michigan State Electrochemical Biosensor | 10 ¹ to 10 ⁶ CFU/mL | Fresh produce and meat products | Michigan State University | [63] |
| Detection of <i>Salmonella</i> and <i>Campylobacter</i> | Interferometric biosensor | Georgia Tech Interferometric Biosensor | 5000 CFU/mL for <i>Salmonella</i> 500 CFU/mL for <i>Campylobacter</i> | Poultry products | Georgia Research Tech Institute | [62] |
| Staphylococcal enterotoxin B and Botulinum toxin A | Fluorescent immunoassay biosensor | Naval Research Laboratory array biosensor | From 20 to 500 ng/mL for Botulinum toxin A From 0.1 to 0.5 ng/ml for Staphylococcal enterotoxin B | Tomatoes, sweet corn, beans and mushrooms | Naval Research Laboratory | [64] |
| <i>Escherichia coli</i> O157, <i>Salmonella</i> , <i>Listeria</i> and <i>Campylobacter</i> | Electro-immunoassay biosensor | Detex Pathogen Detection System | NR | Chicken breast | Molecular Circuitry Inc. | [65] |
| CANARY™: Cellular Analysis and Notification of Antigen Risks and Yields. | | | | | | |

Table 3. Commercially available biosensor devices for the detection of foodborne pathogens (adapted from [22]).

resonance (SPR) biosensors due to their high sensitivity [35]. Few commercial biosensors for the detection of foodborne pathogens are nowadays available. **Table 3** presents the rare commercially available devices of biosensors for food analysis [57]. Unlike nucleic-acid based methods and immunological methods, biosensors are easy-to-operate and they do not require any pre-enrichment step [58].

Optical biosensors are very suitable for the detection of pathogens substances in the food as they detect analytes with no need of special sample treatment even in complex matrices, in addition to the less interference and the low loss of signal. As described by [59], optical biosensors are based on the measurement of the change in amplitude, phase, frequency, or polarization of light. Also, optical devices are more specific and more sensitive than the other biosensors, with a compact design minimally invasive. However, the enhancement of stability of immobilized biocomponents is still a challenge. The main inconvenient of these biosensors is that their commercialization is slower than other rapid methods due to several factors such as their high cost in quality assurance, stability, sensitivity issues, and instrumentation design [60].

Electrochemical biosensors, the second type of biosensors, can handle large numbers of samples and are label-free detection devices but they are low sensitive, and analysis may be interfered by food matrices in addition to many required washing steps, which is not suitable for analyzing samples containing low amount of microorganisms. Finally, mass-based biosensors are cost-effective, easy-to-operate, label-free, and real-time detection devices but low specific and low sensitive with long incubation time of bacteria and many required washing/drying steps, in addition to the regeneration of crystal surface that may be problematic [22].

5. Conclusion

The first step to ensure food safety resides in the prevention by raising industry and consumer awareness. Few primary daily actions can prevent food diseases. Despite conventional methods are often regarded as the “Gold standard” for their specificity and reliability, in addition to their low cost and simplicity, they remain time-consuming and laborious. Over the years, many rapid methods for the detection and identification of foodborne pathogens have been developed to overcome the limitations of their conventional counterparts. Several different types of nucleic-based methods, immunology-based methods and biosensors have been developed and discussed in a large number of publications. Each one offers advantages depending on the target pathogen and the food sample. But also, several disadvantages have to be solved for practical applications in the food industry.

Compared to conventional microbiological methods, rapid commercially available technologies are sensitive enough to detect pathogens, which are expected to be more time-efficient, labor-saving, and able to reduce human errors significantly. Although they are expensive and require a trained technical staff, they are not practical for daily industrial uses.

Nowadays, novel detection methods are released regularly but their acceptance by the industry depends not only on speed but also on initial investment, cost, technical support, and usability.

Indeed, advanced researches have converged to rise to the challenge of developing new simple, sensitive, specific, and time-saving technologies of foodborne pathogens detection that could be mostly practical in food industry.

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References

- [1] Scallan E, Hoekstra RM, Angulo FJ, Tauxe RV, Widdowson M-A, Roy SL, et al. Foodborne illness acquired in the United States—Major pathogens. *Emerging Infectious Diseases*. 2011;**17**(1):7
- [2] Yeni F, Yavaş S, Alpas H, Soyer Y. Most common foodborne pathogens and mycotoxins on fresh produce: A review of recent outbreaks. *Critical Reviews in Food Science and Nutrition*. 2016;**56**(9):1532-1544
- [3] Olsen SJ, MacKinnon LC, Goulding JS, Bean NH, Slutsker L. Surveillance for foodborne-disease outbreaks—United States, 1993-1997. *MMWR. CDC Surveillance Summaries*. 2000;**49**(1):1-62
- [4] Zhao X, Lin C-W, Wang J, Oh DH. Advances in rapid detection methods for foodborne pathogens. *Journal of Microbiology and Biotechnology*. 2014;**24**(3):297-312
- [5] Oussalah M, Caillet S, Saucier L, Lacroix M. Inhibitory effects of selected plant essential oils on the growth of four pathogenic bacteria: *E. coli* O157: H7, *Salmonella typhimurium*, *Staphylococcus aureus* and *Listeria monocytogenes*. *Food Control*. 2007;**18**(5):414-420
- [6] Wu S, Duan N, Gu H, Hao L, Ye H, Gong W, et al. A review of the methods for detection of staphylococcus aureus enterotoxins. *Toxins*. 2016;**8**(7):176
- [7] Motarjemi Y, Käferstein F. Food safety, hazard analysis and critical control point and the increase in foodborne diseases: A paradox? *Food Control*. 1999;**10**(4):325-333
- [8] Leonard P, Hearty S, Brennan J, Dunne L, Quinn J, Chakraborty T, et al. Advances in biosensors for detection of pathogens in food and water. *Enzyme and Microbial Technology*. 2003;**32**(1):3-13
- [9] O’Kennedy R, Leonard P, Hearty S, Daly S, Dillon P, Brennan J, et al. Advances in biosensors for detection of pathogens in food and water. In: van Amerongen A, Barug D,

- Lauwaars M, editors. Rapid Methods for Biological and Chemical Contaminants in Food and Feed. Wageningen, The Netherlands: Wageningen Academic Publishers; 2005. pp. 85-104
- [10] Lazcka O, Del Campo FJ, Munoz FX. Pathogen detection: A perspective of traditional methods and biosensors. *Biosensors & Bioelectronics*. 2007;**22**(7):1205-1217
- [11] Dwivedi HP, Jaykus L-A. Detection of pathogens in foods: The current state-of-the-art and future directions. *Critical Reviews in Microbiology*. 2011;**37**(1):40-63
- [12] Gorny J. Microbial contamination of fresh fruits and vegetables. In: *Microbiology of Fruits and Vegetables*. Boca Raton, FL: CRC Press; 2006. pp. 3-32
- [13] Ribot E, Hyytia-Trees E, Cooper K. PulseNet and emerging foodborne pathogens. In: *Microbial Food Contamination*. 2nd ed. Boca Raton: CRC Press; 2008
- [14] Marriott NG, Gravani RB. Food Contamination Sources. *Principles of Food Sanitation*; 2006. pp. 76-82
- [15] Angulo FJ, Jones TF, Angulo FJ. Eating in restaurants: A risk factor for foodborne disease? *Clinical Infectious Diseases*. 2006;**43**(10):1324-1328
- [16] Goldburn K, editor. Management of *E. coli* O157 in fresh fruit and vegetables: how can we be safe. Chilled Food Association Conference an *E. coli*: cases, controls and common sense. London, UK; 2009
- [17] Doyle MP, Buchanan RL. *Food Microbiology: Fundamentals and Frontiers*. Washington DC, USA: American Society for Microbiology Press; 2012
- [18] Zhao T, Doyle MP. Evaluation of universal preenrichment broth for growth of heat-injured pathogens. *Journal of Food Protection*. 2001;**64**(11):1751-1755
- [19] Harrigan WF. *Laboratory Methods in Food Microbiology*. London, UK: Gulf Professional Publishing; 1998
- [20] Wang Y, Salazar JK. Culture-independent rapid detection methods for bacterial pathogens and toxins in food matrices. *Comprehensive Reviews in Food Science and Food Safety*. 2016;**15**(1):183-205
- [21] Gracias KS, McKillip JL. A review of conventional detection and enumeration methods for pathogenic bacteria in food. *Canadian Journal of Microbiology*. 2004;**50**(11):883-890
- [22] Ivnitski D, Abdel-Hamid I, Atanasov P, Wilkins E. Biosensors for detection of pathogenic bacteria. *Biosensors & Bioelectronics*. 1999;**14**(7):599-624
- [23] Mandal PK, Biswas AK, Choi K, Pal UK. Methods for rapid detection of foodborne pathogens: An overview. *American Journal of Food Technology*. 2011;**6**(2):87-102
- [24] López-Campos G, Martínez-Suárez JV, Aguado-Urda M, López-Alonso V. Detection, identification, and analysis of foodborne pathogens. In: *Microarray Detection and Characterization of Bacterial Foodborne Pathogens*. New York, USA: Springer; 2012. pp. 13-32
- [25] Gill A. The importance of bacterial culture to food microbiology in the age of genomics. *Frontiers in Microbiology*. 2017;**8**

- [26] Lee N, Kwon KY, Oh SK, Chang H-J, Chun HS, Choi S-W. A multiplex PCR assay for simultaneous detection of *Escherichia coli* O157: H7, *Bacillus cereus*, *Vibrio Parahaemolyticus*, *Salmonella* spp., *Listeria monocytogenes*, and *Staphylococcus aureus* in Korean ready-to-eat food. *Foodborne Pathogens and Disease*. 2014;**11**(7):574-580
- [27] Beumer R, Hazeleger W. Rapid methods: The detection of foodborne pathogens. *Food Engineering & Ingredients*. 2009:15-17
- [28] Mandal P, Biswas A, Choi K, Pal U. Methods for rapid detection of foodborne pathogens: An overview. *American Journal Of Food Technology*. 2011;**6**(2):87-102
- [29] Feng P. Impact of molecular biology on the detection of foodborne pathogens. *Molecular Biotechnology*. 1997;**7**(3):267-278
- [30] Feng P. Rapid methods for detecting foodborne pathogens. FDA, editor *Bacteriological Analytical Manual Online*. 2001
- [31] Law JW-F, Ab Mutalib N-S, Chan K-G, Lee L-H. Rapid methods for the detection of foodborne bacterial pathogens: Principles, applications, advantages and limitations. *Frontiers in Microbiology*. 2015;**5**:770
- [32] Valderrama WB, Dudley EG, Doores S, Cutter CN. Commercially available rapid methods for detection of selected food-borne pathogens. *Critical Reviews in Food Science and Nutrition*. 2016;**56**(9):1519-1531
- [33] Gopinath SC, Tang T-H, Chen Y, Citartan M, Lakshmipriya T. Bacterial detection: From microscope to smartphone. *Biosensors and Bioelectronics*. 2014;**60**:332-342
- [34] Alahi MEE, Mukhopadhyay SC. Detection methodologies for pathogen and toxins: A review. *Sensors*. 2017;**17**(8):1885
- [35] Velusamy V, Arshak K, Korostynska O, Oliwa K, Adley C. An overview of foodborne pathogen detection: In the perspective of biosensors. *Biotechnology Advances*. 2010;**28**(2):232-254
- [36] Manzano M, Cocolin L, Ferroni P, Cantoni C, Comi G. A simple and fast PCR protocol to detect *listeria monocytogenes* from meat. *Journal of the Science of Food and Agriculture*. 1997;**74**(1):25-30
- [37] Lindqvist R. Preparation of PCR samples from food by a rapid and simple centrifugation technique evaluated by detection of *Escherichia coli* O157: H7. *International Journal of Food Microbiology*. 1997;**37**(1):73-82
- [38] Johnson W, Tyler S, Ewan E, Ashton F, Pollard D, Rozee K. Detection of genes for enterotoxins, exfoliative toxins, and toxic shock syndrome toxin 1 in *Staphylococcus Aureus* by the polymerase chain reaction. *Journal of Clinical Microbiology*. 1991;**29**(3):426-430
- [39] Wang H, Farber J, Malik N, Sanders G. Improved PCR detection of *campylobacter jejuni* from chicken rinses by a simple sample preparation procedure. *International Journal of Food Microbiology*. 1999;**52**(1):39-45

- [40] Cheah Y-K, Salleh NA, Lee L-H, Radu S, Sukardi S, Sim J-H. Comparison of PCR fingerprinting techniques for the discrimination of *Salmonella enterica* subsp. *enterica* serovar Weltevreden isolated from indigenous vegetables in Malaysia. *World Journal of Microbiology and Biotechnology*. 2008;**24**(3):327
- [41] Lindqvist R. Detection of *Shigella* spp. in food with a nested PCR method—sensitivity and performance compared with a conventional culture method. *Journal of Applied Microbiology*. 1999;**86**(6):971-978
- [42] Lampel KA, Orlandi PA, Kornegay L. Improved template preparation for PCR-based assays for detection of food-borne bacterial pathogens. *Applied and Environmental Microbiology*. 2000;**66**(10):4539-4542
- [43] Chen J, Tang J, Liu J, Cai Z, Bai X. Development and evaluation of a multiplex PCR for simultaneous detection of five foodborne pathogens. *Journal of Applied Microbiology*. 2012;**112**(4):823-830
- [44] Kim JS, Lee GG, Park JS, Jung YH, Kwak HS, Kim SB, et al. A novel multiplex PCR assay for rapid and simultaneous detection of five pathogenic bacteria: *Escherichia coli* O157: H7, *Salmonella*, *Staphylococcus aureus*, *Listeria monocytogenes*, and *Vibrio parahaemolyticus*. *Journal of Food Protection*. 2007;**70**(7):1656-1662
- [45] Leone G, van Gemen B, Schoen CD, van Schijndel H, Kramer FR. Molecular beacon probes combined with amplification by NASBA enable homogeneous, real-time detection of RNA. *Nucleic Acids Research* 1998;**26**(9):2150-2155
- [46] Xu Z, Li L, Chu J, Peters BM, Harris ML, Li B, et al. Development and application of loop-mediated isothermal amplification assays on rapid detection of various types of staphylococci strains. *Food Research International*. 2012;**47**(2):166-173
- [47] Zhao X, Li Y, Wang L, You L, Xu Z, Li L, et al. Development and application of a loop-mediated isothermal amplification method on rapid detection *Escherichia coli* O157 strains from food samples. *Molecular Biology Reports*. 2010;**37**(5):2183-2188
- [48] Hara-Kudo Y, Yoshino M, Kojima T, Ikeda M. Loop-mediated isothermal amplification for the rapid detection of *Salmonella*. *FEMS Microbiology Letters*. 2005;**253**(1):155-161
- [49] Iqbal SS, Mayo MW, Bruno JG, Bronk BV, Batt CA, Chambers JP. A review of molecular recognition technologies for detection of biological threat agents. *Biosensors & Bioelectronics*. 2000;**15**(11):549-578
- [50] Meng J, Doyle MP. Introduction. Microbiological food safety. *Microbes and Infection*. 2002;**4**(4):395-397
- [51] Zhang G. Foodborne pathogenic bacteria detection: An evaluation of current and developing methods. *The Meducator*. 2013;**1**(24)
- [52] Park SH, Aydin M, Khatiwara A, Dolan MC, Gilmore DF, Bouldin JL, et al. Current and emerging technologies for rapid detection and characterization of *Salmonella* in poultry and poultry products. *Food Microbiology*. 2014;**38**:250-262

- [53] Schlosser G, Kačer P, Kuzma M, Szilágyi Z, Sorrentino A, Manzo C, et al. Coupling immunomagnetic separation on magnetic beads with matrix-assisted laser desorption ionization-time of flight mass spectrometry for detection of staphylococcal enterotoxin B. *Applied and Environmental Microbiology*. 2007;**73**(21):6945-6952
- [54] Hibi K, Abe A, Ohashi E, Mitsubayashi K, Ushio H, Hayashi T, et al. Combination of immunomagnetic separation with flow cytometry for detection of listeria monocytogenes. *Analytica Chimica Acta* 2006;**573**:158-63
- [55] Bahadır EB, Sezgintürk MK. Applications of commercial biosensors in clinical, food, environmental, and biothreat/biowarfare analyses. *Analytical Biochemistry*. 2015;**478**:107-120
- [56] Bhalla N, Jolly P, Formisano N, Estrela P. Introduction to biosensors. *Essays in Biochemistry*. 2016;**60**(1):1-8
- [57] da Costa Silva LM, dos Santos VPS, Salgado AM, Pereira KS. Biosensors for contaminants monitoring in food and environment for human and environmental health. *State of the Art in Biosensors-Environmental and Medical Applications: InTech; Rijeka*. 2013
- [58] Singh A, Poshtiban S, Evoy S. Recent advances in bacteriophage based biosensors for food-borne pathogen detection. *Sensors*. 2013;**13**(2):1763-1786
- [59] Narsaiah K, Jha SN, Bhardwaj R, Sharma R, Kumar R. Optical biosensors for food quality and safety assurance—A review. *Journal of Food Science and Technology*. 2012;**49**(4):383-406
- [60] Velasco-Garcia MN, Mottram T. Biosensor technology addressing agricultural problems. *Biosystems Engineering*. 2003;**84**(1):1-12
- [61] Andral B, Bouve J, Brugere H, Espié E, Leclerc V, Roze S, et al. Epidémiologie environnementale et alimentaire des STEC. Agence française de sécurité sanitaire des aliments Bilan des connaissances relatives aux *Escherichia coli* producteurs de Shiga-toxines (STEC) Maisons-Alfort; 2003. pp. 81-103
- [62] Innovative Biosensors Are Opening New Frontiers 2004. Available from: http://www.atrp.gatech.edu/pt16-3/16-3_p1.html
- [63] Wang Y, Fewins PA, Alocilja EC. Electrochemical immunosensor using nanoparticle-based signal enhancement for *Escherichia coli* O157: H7 detection. *IEEE Sensors Journal*. 2015;**15**(8):4692-4699
- [64] Sapsford KE, Taitt CR, Loo N, Ligler FS. Biosensor detection of botulinum toxoid a and staphylococcal enterotoxin B in food. *Applied and Environmental Microbiology*. 2005;**71**(9):5590-5592
- [65] Detex Pathogen Detection Technology Ready to Roll; VWR to Distribute 2000 [01/22/2018]. Available from: <https://www.dairynetwork.com/doc/detex-pathogen-detection-technology-ready-to-0002>