

Chapter 2

Detection, Identification, and Analysis of Foodborne Pathogens

Abstract The detection and enumeration of microorganisms in food are an essential part of any quality control or food safety plan. Traditional methods of detecting foodborne pathogenic bacteria are often time-consuming because of the need for growth in culture media, followed by isolation, biochemical and/or serological identification, and in some cases, subspecific characterization. Advances in technology have made detection and identification faster, more sensitive, more specific, and more convenient than traditional assays. These new methods include for the most part antibody- and DNA-based tests, and modifications of conventional tests made to speed up analysis and reduce handling. With few exceptions, almost all assays used to detect specific pathogens in foods are qualitative assays, as they still lack sufficient sensitivity for direct testing and require some growth in an enrichment medium before analysis. One of the most challenging problems to circumvent with these assays is sample preparation. The possibilities of combining different rapid methods, including improved technologies for separation and concentration of specific bacteria, and for DNA extraction and purification, will facilitate the direct detection of pathogens in food. The goal is to avoid the enrichment, providing rapid alternatives to conventional quantitative culture methods. Further improvements, especially in genetic methods, can be expected, including the use of DNA microarray technology.

Keywords Detecting foodborne pathogens • Traditional culture methods • Rapid methods • Immunoassays • Molecular methods • Qualitative assays • Enrichment • Sample preparation

2.1 Introduction

The detection and enumeration of pathogens in food and on surfaces that come into contact with food are an important component of any integrated program to ensure the safety of foods throughout the food supply chain. Both government authorities and

food companies use microbiological analysis to monitor the state of contamination at all times and analyze its trends so as to detect emerging risks. Microbiological analysis is also an essential tool for carrying out tests in accordance with the microbiological criteria established for each food type, as well as being essential for evaluating the actions of different management strategies based on the Hazard Analysis and Critical Control Points (HACCP) system (Stannard 1997; Jasson et al. 2010). The implementation of preventive systems such as the HACCP has greatly improved food safety, but it will not be fully effective until better methods of analysis are developed. These new detection methods are the necessary technologies that will substantially improve our food safety once integrated in the HACCP (Bhunja 2008).

Microbiological analysis of foods is based on the detection of microorganisms by visual, biochemical, immunological, or genetic means, either before enrichment (quantitative or enumerative methods) or after enrichment (qualitative methods, also known as presence/absence tests).

Traditional culture methods for detecting microorganisms in food are based on the incorporation of the food sample into a nutrient medium in which the microorganisms can multiply, thus providing visual confirmation of their growth. These conventional test methods are simple, easily adaptable, very practical, and generally inexpensive. Although not lacking in sensitivity, they can be laborious and depend on the growth of the microorganisms in different culture media (pre-enrichment, selective enrichment, selective plating, identification), which may require several days before results are known. Products that are minimally processed have an inherently short shelf life, which prevents the use of many of these conventional methods. Therefore, extensive research has been carried out over the years to reduce assay time through the use of alternative methods for detecting foodborne microorganisms and reduce the amount of manual labor by automating methods whenever possible (Jantzen et al. 2006a; Feng 2007; Betts and Blackburn 2009; Jasson et al. 2010).

In spite of its importance, the microbiological analysis of food has many limitations. Uncertainty of the analytical result must be considered when establishing microbiological criteria, including the variance associated with the sampling plan, method of analysis, and laboratory performance (Betts and Blackburn 2009). The microbiological analysis of food remains a challenging task for virtually all assays and technologies, especially for particular pathogenic species (Feng 2007). The problems may be due to

- The complexity of food matrices and composition.
- The heterogeneous distribution of low levels of pathogens.
- The stress suffered by the microorganisms during the processing of foods.
- The presence of bacteria from the normal microbiota, especially in raw foods.

The complexity of food matrices remains the major obstacle to the development of effective sampling and rapid testing methods (Feng 2007). Long duration enrichments are often used due to the low number of pathogenic microorganisms that tend to be present in food samples. Although previous enrichment is a limitation in terms of assay speed and precludes quantification of the original contaminant, it provides

essential benefits, such as diluting the effects of inhibitors, allowing the differentiation of viable from nonviable cells, and allowing for the repair of cell stress or injury that may have resulted during food processing (Jantzen et al. 2006b; Wu 2008). Hence, it would be difficult to completely eliminate enrichment culture from the process of pathogen detection in foods (Feng 2007).

2.2 Separation and Concentration of Microorganisms Present in Food

The problem of low cell numbers present in food samples can also be solved by separating and concentrating microorganisms in food in order to discriminate the target pathogen from other cells and to use them at an appropriate concentration for the sensitivity level of the detection method. Food matrix materials can be simultaneously eliminated in order to avoid false-negative results. Several strategies including antibody-based as well as physical- and chemical-based methods have been developed for the separation and concentration of pathogens from various sample matrices (Stevens and Jaykus 2004; Bhunia 2008). In the case of beverages and liquid food, the concentration can be easily achieved through filtration or ultrafiltration (Chen et al. 2005; Hunter et al. 2011). However, these techniques do not allow the selective isolation of the organisms from a mixed population.

For solid foods, the main system present in the market is immunomagnetic separation and concentration (IMS) (Olsvik et al. 1994). With this technology, superparamagnetic particles or polystyrene beads are coated with iron oxide and antibodies that allow for the specific capture and isolation of intact pathogen cells present in suspensions of complex mixtures such as pre-enrichment media (Aminul Islam et al. 2006). The application of a magnetic field retains the particles along with the cells attached to the particles, allowing the rest of the organic and liquid material to be removed by washing. Captured antigens can be plated or further tested using other assays. IMS coupled with different rapid and automated assays has been used for the detection of pathogens such as *E. coli* O157:H7 (Seo et al. 1998; Fu et al. 2005; Aminul Islam et al. 2006; Hunter et al. 2011). In fact, IMS does not yield a pure culture of the target microbe, needing to be coupled to other tests for more definitive detection and identification results (Feng 2007).

There is an IMS technique that uses samples 500 times larger than most common assays by recirculating the sample during the capture phase in order to increase sensitivity and reduce detection times (Fedio et al. 2011). Specific bacteriophage tail-associated proteins can be attached to paramagnetic beads instead of antibodies as a way of capturing bacteria in suspension. There are bacteriophage-based capture kits that can be integrated into rapid detection methods in a similar way as in IMS (Favrin et al. 2003). The IMS may be employed either directly or after enrichment, but at present the major drawback of all these IMS-based assays is the susceptibility to inhibitors or interference by food components and the requirement for enrichment (Feng 2007).

2.3 Traditional Culture Methods

Standardized methods (e.g., ISO methods) are usually considered the reference analytical methods for official controls. In most cases, they are traditional culture methods that use selective liquid or solid culture media, to grow, isolate, and enumerate the target microorganism and simultaneously prevent the growth of other microorganisms present in the food (Jasson et al. 2010).

2.3.1 *Quantitative Culture Methods*

Enumeration of the microorganisms present in a sample is normally performed by plate count method or the most probable number (MPN) method. The plate count method is based on culturing dilutions of sample suspensions in the interior or on the surface of an agar layer in a Petri dish. Individual microorganisms or small groups of microorganisms will grow to form individual colonies that can be counted visually. The MPN method calculates the number of viable microorganisms in a sample by preparing decimal dilutions of the sample, and transferring subsamples of 3 serial dilutions to 9 or 15 tubes containing liquid culture medium, to carry out the method on 3 or 5 tubes, respectively. The tubes are incubated, and those that show growth (turbidity) are counted. Taking into account the dilution factor, the final result is compared to a standard MPN table, which will indicate the MPN of bacteria in the product (Blodgett 2010). This method is more labor-intensive and expensive than plate counting. The confidence limits are also quite large, even when studying many replica samples of each dilution level. The method is therefore usually less accurate than plate count methods but has the advantage of being more sensitive. Thus, it is widely used for estimations of levels of bacteria below 10 per gram of food (Table 2.1) (Stannard 1997; Betts and Blackburn 2009).

2.3.2 *Qualitative Culture Methods*

Qualitative procedures are used when it is not necessary to know the amount of a microorganism present in a sample but only its presence or absence. The technique requires an accurately weighed sample (usually 25 g). The typical colonies of the target microorganism on a selective/differential solid medium plate are often called *presumptive*. To confirm the identity of the desired microorganism, various biochemical and/or serological tests need to be carried out with pure cultures obtained from these presumptive colonies (Betts and Blackburn 2009) (see Sect. 2.6).

Table 2.1 Main characteristics of some culture-based and rapid detection methods (Adapted from Mandal et al. 2011)

Test method		Sensitivity ^a	Specificity	Duration of the assay (h)
Qualitative culture	Presence/absence	Defined by the quantity of food examined, e.g., presence in 25 g	Good	>72
Qualitative rapid detection	Presence/absence	Defined by the quantity of food examined, e.g., presence in 25 g	Variable ^b	Variable ^b
Quantitative culture	MPN	<10–100 MPN of bacteria per gram	Good	24–48
	Viable counts	>10–100	Good	24–72
	Impedance	100	Moderate/good	6–24
Quantitative rapid detection	Bioluminescence	10 ⁴	No ^c	<1–3
	DEFT, SPC	10 ³ –10 ⁴	No ^c	<1
	Flow cytometry	10 ⁵ –10 ⁷	Good	<1
	Immunological methods (LFD, ELISA, ELFA)	10 ⁴ –10 ⁵	Moderate/good	<1–3
	Nucleic acid–based assays (FISH, Q-PCR)	10 ³ –10 ⁴	Excellent	<1–3

^aCFU per g or mL, unless otherwise stated^bDepends on the rapid method used^cCan be made specific with specific separation and concentration steps (IMS- or phage-based) and/or selective labeling of cells

2.4 Rapid and Automated Methods

The fast pace at which rapid methods are being developed precludes a discussion of all available methods. In this section, the breadth of rapid methods available and the scientific principles of the methods used for detection of pathogenic bacteria in foods are revised. Existing methods are presented in various formats and continue to be modified or adapted so that current methods have to be validated or evaluated using traditional standardized culture methods as reference (Feng 2007; Jasson et al. 2010; AOAC International 2011). Some rapid methods may also be considered as reference methods if they are shown to provide more accurate results than culture methods, such as current methods for detecting enterohemorrhagic strains of *E. coli* other than O157:H7, for example (Inset 2.1).

2.4.1 Changes to Culture Methods

Automation may be very useful in reducing the time required to prepare culture media, perform serial dilutions, count colonies, etc. (Fung et al. 1988; Jasson et al. 2010). There are a wide variety of rapid culture methods that have been designed to

replace the standard agar plate, reducing the workload, facilitate rapid implementation, simplify handling, and/or reduce the need for a complete laboratory infrastructure, which do not necessarily shorten assay times. Some of these modified culture methods are based on the colony counting method, using, for instance, disposable cardboards containing dehydrated media (Chain and Fung 1991). Others are based on the MPN method (Torlak et al. 2008). In recent years a staggering number of chromogenic and fluorogenic culture media have been developed for the detection and enumeration of specific bacteria. The addition of these media to culturing protocols facilitates the rapid identification of presumptive colonies of the target microorganism (Manafi 2000). This has led to its incorporation in some official methods (Jasson et al. 2010).

The production of positively or negatively charged end products by bacteria during active growth (in initial stages of nutrient degradation) results in a variation in impedance of the culture medium. This can be measured at regular intervals over a period of 24 h after inoculation in specific media. This variation is proportional to the change in the number of bacteria in the culture. Thus, bacterial growth can be quantified (Wawerla et al. 1999; Jasson et al. 2010). This system is capable of analyzing hundreds of samples at a time since the instrument is computer-driven and automated to enable continuous monitoring. This technique is suited for testing samples with a low number of microorganisms. The limit of detection can be as low as 100 colony-forming units (CFU) per mL (Bosilevac et al. 2005) (Table 2.1). Methods based on the measure of impedance may be used in both quantitative and qualitative applications to detect all microorganisms or specific pathogens (Yang and Bashir 2008).

2.4.2 *ATP Bioluminescence*

This technique measures the emission of light produced by an enzymatic reaction between luciferin and luciferase that requires the presence of ATP (bioluminescence). The amount of light produced (measured by means of a luminometer) is proportional to the concentration of ATP, and therefore the number of microorganisms in the original sample. Bioluminescence produced by ATP may be used to enumerate the total microorganisms in a sample but is only applicable if the number of bacteria present is high (more than 10^4 CFU/g) (Samkutty et al. 2001; Jasson et al. 2010) (Table 2.1). The technique is widely used to measure the cleanliness of surfaces that come into contact with food, including the presence of organic residues and microbial contaminants. It provides results in less than 5 min (Cunningham et al. 2011). The system lacks specificity, however. An alternative and more specific approach includes an IMS step for capturing the target bacteria, which is then detected by bioluminescence (Hunter et al. 2011); species-specific bacteriophages instead of chemicals can be used to lyse cells to release ATP, thereby providing additional assay specificity (Kannan et al. 2010).

2.4.3 Microscopic Methods

2.4.3.1 Direct Epifluorescent Filter Technique

The direct epifluorescent filter technique (DEFT) is a microscopic method for the enumeration of viable cells in a sample based on the binding properties of the fluorochrome acridine orange. Once treated with detergents and proteolytic enzymes, the samples are filtered through a polycarbonate membrane. The cells are stained on this same filter and examined under an epifluorescent microscope (Pettipher et al. 1992), a process that can be carried out semi-automatically by connecting the microscope to an image analysis system (Hermida et al. 2000). The number of viable cells can be obtained in 10 min. However, DEFT is a very labor-intensive technique that does not have the capability of processing a large number of samples and is only applicable if the number of bacteria present is high (10^3 – 10^4 CFU/g) (Table 2.1). Additionally, fluorescent food material can be trapped on the filter, and the technique can only be used with raw food and usually for enumerating total viable microorganisms (Hermida et al. 2000). Nevertheless, DEFT may be used for the detection and enumeration of specific bacteria in food samples provided they can be isolated from the unfilterable matrix. IMS followed by DEFT and solid-phase cytometry (see Sect. 2.4.3.3) have given results that compared favorably with IMS followed by plating (see below).

2.4.3.2 Flow Cytometry

Flow cytometry quantitatively measures optical characteristics of cells when they are forced to pass individually through a beam of light. Fluorescent dyes can be used to test the viability and metabolic state of microorganisms (Veal et al. 2000). Samples are injected into a fluid (dye), which passes through a sensing medium in a flow cell. The cells are carried by the laminar flow of water through a focus of light, each cell emits a pulse of fluorescence, and the scattered light is collected by lenses and directed onto selective detectors (photomultiplier tubes). This technique is fast, automatic, and potentially very specific, as long as appropriate dyes are available for selectively labeling specific types of microorganisms and appropriate methods for separating cells from food are utilized so as not to interfere with detection (Seo et al. 1998). The sensitivity of flow cytometry, however, is low (Table 2.1); the detection limit with food samples is around 10^5 – 10^7 CFU/g (Betts and Blackburn 2009). Currently, there are various flow cytometry methods developed for foods, especially for liquid samples such as dairy products, water, and other beverages (Comas-Riu and Rius 2009).

2.4.3.3 Solid-Phase Cytometry

Solid-phase cytometry (SPC) is a technique that combines aspects of flow cytometry and epifluorescence microscopy (D’Haese and Nelis 2002). After filtration of

the sample, the retained microorganisms are fluorescently labeled with argon laser excitable dyes on the membrane filter and automatically counted by a laser scanning device. Each fluorescent spot can be visually inspected with an epifluorescence microscope connected to a scanning device by a computer-driven moving stage. Depending on the fluorogenic labels used, information on the identity and the physiological status of the microorganisms can be obtained within a few hours. SPC, like DEFT (see Sect. 2.4.3.1), is only applicable if the number of bacteria present is high (10^3 – 10^4 CFU/g). Although both techniques were originally recommended for the determination of the total viable microbial count in liquid samples, they may also be utilized for the rapid detection and enumeration of pathogens in food samples, provided they can be specifically isolated. The efficiency of viable bacteria detection from foods by IMS followed by SPC and DEFT was assessed using the pathogen *E. coli* O157:H7 (Pyle et al. 1999). Within 5–7 h of enrichment, the IMS-SPC method detected higher numbers of cells than were detected by plating. SPC in conjunction with fluorescent viability staining has also been reported as a tool to detect viable but nonculturable *Campylobacter jejuni* (Cools et al. 2005).

2.4.4 Immunological Detection Methods

The antibody-based system has facilitated the design of a variety of assays and formats. In some cases, the antigen–antibody complex formed is directly measurable or even visible. Incubation times are usually very short for methods such as agglutination reactions commonly used for the rapid identification of microorganisms (see Sect. 2.6). Normally, the antibody is labeled with a fluorescent reagent or with an enzyme so that the antigen–antibody interaction may be visualized more easily when it occurs.

2.4.4.1 Lateral Flow Devices

Lateral flow devices (LFD) are typically comprised of a simple dipstick made of a porous membrane that contains colored latex beads or colloidal gold particles coated with detection antibodies targeted toward a specific microorganism. The particles are found on the base of the dipstick, which is put in contact with the enrichment medium (Posthuma-Trumpie et al. 2009). If the target organism is present, then it will bind with the colored particles. This conjugated cell/particle moves by capillary action until it finds the immobilized capture antibodies. Upon binding with these, it forms a colored line that is clearly visible in the device window, indicating a positive result (Betts and Blackburn 2009). As with other immunoassays, LFD also require previous enrichment. The technique is extremely simple to use and easy to interpret, requires no washing or manipulation, and can be completed within 10 min after culture enrichment (Aldus et al. 2003). There are various LFD on the market that have been validated for detecting different foodborne pathogens (Jasson et al. 2010; AOAC International 2011).

2.4.4.2 Enzyme-Linked Immunosorbent Assay and Enzyme-Linked Fluorescence Assay

The enzyme-linked immunosorbent assay (ELISA) is a biochemical technique that combines an immunoassay with an enzymatic assay. As LFD, it is a “sandwich” assay. An antibody bound to a solid matrix is used to capture the antigen from enrichment cultures and a second antibody conjugated to an enzyme is used for detection. The enzyme is capable of generating a product detectable by a change in color, or in the case of enzyme-linked fluorescence assay (ELFA) in fluorescence, which allows for indirect measurement using spectrophotometry (or fluorometry for ELFA) of the antigen present in the sample (microorganism or toxin) (Cohen and Kerdahi 1996; Jasson et al. 2010).

Detection using automated and robotic ELISAs is widely used since they can reduce detection times after enrichment to as low as 1–3 h (Thacker et al. 1996). Thus, the results can be obtained in 2–3 days instead of the 3–5 days needed by conventional methods (Leon-Velarde et al. 2009). There are many commercial enzyme immunoassays for detecting the main pathogens and toxins in foods (AOAC International 2011). Bacteriophage recombinant protein technology can also be integrated in detection methods as part of improved immunological qualitative tests (Jasson et al. 2010; Savoye et al. 2011) (Inset 2.1).

The success of an immunoassay depends on the specificity of the antibody. Using hybridoma technology, it has been possible to develop monoclonal antibodies that react only with one specific pathogen. The limit of detection for immunoassays is approximately 10^4 – 10^5 CFU/g (Table 2.1) depending on the type of antibody and its affinity for the corresponding epitope, which means that one or two previous enrichment stages are always required (Jasson et al. 2010).

2.4.5 Molecular Detection Methods

There has been an explosion in the past 15 years in the introduction of nucleic acid-based assays for the detection and identification of foodborne pathogens. There are many DNA-based assay formats, but only probes and nucleic acid amplification techniques have been developed commercially for detecting foodborne pathogens.

2.4.5.1 Fluorescent In Situ Hybridization

Fluorescent in situ hybridization (FISH) with oligonucleotide probes directed at rRNA is the most common method among molecular techniques not based on PCR. The probes used by FISH tend to be 15–25 nucleotides in length, and are covalently labeled at their 5′ end with fluorescent labels. After hybridization, the specifically stained cells are detected using epifluorescence microscopy (Wagner et al. 2003). The detection limit of this technique is around 10^4 CFU/g (Table 2.1). Following

pre-enrichment to reach these detection levels, the results can be obtained quickly (in about 3 h) (Bottari et al. 2006). FISH in combination with flow cytometry has been used for rapid culture-independent detection of *Salmonella* spp. on the surfaces of tomatoes and other fresh produce (Bisha and Brehm-Stecher 2010).

2.4.5.2 Polymerase Chain Reaction

Polymerase chain reaction (PCR) is a method used for the in vitro enzymatic synthesis of specific DNA sequences by *Taq* and other thermoresistant DNA polymerases. PCR uses oligonucleotide primers that are usually 20–30 nucleotides in length and whose sequence is homologous to the ends of the genomic DNA region to be amplified. The method is performed in repeated cycles, so that the products of one cycle serve as the DNA template for the next cycle, doubling the number of target DNA copies in each cycle (Hill 1996). The rapid increase in the number of copies of the target sequence that can be achieved with PCR-based methods makes them ideal candidates for the development of faster microbiological detection systems. Many PCR tests have been validated and commercialized to make PCR a standard tool used by food microbiology laboratories to detect pathogens in foods (Jasson et al. 2010; AOAC International 2011).

Conventional PCR relies on amplification of the target gene(s) in a thermocycler, separation of PCR products by gel electrophoresis, followed by visualization and analysis of the resulting electrophoretic patterns, a process that can take a number of hours. The specificity can be subsequently confirmed by sequencing the amplified fragment. PCR can be superior to culture for detecting the main pathogens in food samples (Abubakar et al. 2007).

Real-time PCR allows both the detection and quantification of a signal emitted by the amplified product by using the continuous measurement of a fluorescent label during the PCR reaction. The increase in fluorescence can be monitored in real time, which allows accurate quantification over several orders of magnitude of the DNA target sequence. Results can be obtained in an hour or less, which is considerably faster than conventional PCR. Real-time PCR has greatly increased the speed and sensitivity of PCR-based detection methods (Hanna et al. 2005). But the terms “rapid” and “sensitive,” when applied to PCR as a detection method for foodborne pathogens, must be used with caution (Jasson et al. 2010). PCR itself requires only about 30–90 min, but all methods for detecting foodborne pathogens using PCR require pre-enrichment times that may vary from 6–8 to 48 h. Aside from that, if a positive result for PCR is reached, this must be confirmed using cultures. Regarding sensitivity, the limit of quantification of real-time PCR with food samples is around 10^3 – 10^4 CFU/g (Table 2.1) (Rodriguez-Lazaro and Hernandez 2006; Navas et al. 2006; Jasson et al. 2010). This limit is still too high for quantitative detection, since most samples taken from throughout the food supply chain are usually contaminated with fewer pathogen cells (normally less than 100 CFU/g). Consequently, this requires some enrichment of the microorganisms to be done prior to analysis, which

turns all currently available commercial real-time PCR into qualitative detection methods (presence/absence) instead of quantitative.

In some cases, multiple different microorganisms may be detected in a single PCR reaction by amplifying the corresponding loci simultaneously. In this type of multiplex PCR reaction, all necessary primers are combined in a single tube for detecting the presence of the main pathogens associated with a given food (Kawasaki et al. 2009) or the main subtypes within a given species (Valadez et al. 2011).

2.5 Conclusions About Traditional and Rapid Methods

The process of selecting an appropriate method must consider the main criteria of the sensitivity of analysis, the time of detection, and the specificity of the test (Table 2.1). The cornerstone of any method is its accuracy. This consists of the sensitivity and the specificity. The intent in developing a rapid assay is to reduce the time required to obtain an accurate result.

Qualitative detection (presence/absence) tests are used if information concerning the presence of an organism in a specified quantity of food is required. The sensitivity of these tests is then defined by the quantity of food examined (Stannard 1997; Jasson et al. 2010). In many cases, the requirement of detection is less than one cell per 25 g of food, as small numbers of some pathogens may be sufficient to cause disease. Sensitive quantitative detection is usually achieved by traditional culture methods (Table 2.1). MPN determinations are suitable for low counts such as less than 100 per gram, and are widely used for estimates of levels below 10 per gram. Plate counts are generally used for counts of more than 10 CFU/g but are more accurate when levels exceed 100 CFU/g. With rapid methods, the lower limit of detection is almost always above 10^3 CFU/g of food (Table 2.1). Thus, rapid methods still lack sufficient sensitivity for direct testing (Feng 2007).

Traditional culture methods may require many days, resulting in very long assay durations. Most rapid methods for the detection of pathogens or toxins can be done in a few minutes to a few hours or at the utmost 1 day (Table 2.1). However, many detection systems need an enrichment, and positive results must be confirmed by the appropriate official method, which involve culturing, in many instances (Feng 2007). In spite of this, commercially available rapid detection methods, such as ELISA, LFD, and PCR, have substantially shortened the total time of the detection assay when compared to conventional methods (Table 2.1) (Leon-Velarde et al. 2009). They are, therefore, of great use in the rapid analysis of food with the goal of ensuring that only negative samples or lots are sent to market (Bohaychuk et al. 2005). A major disadvantage of alternative methods over culture methods is that most rapid methods involve damaging the cells. Therefore, viable cells for confirmation and further characterization can only be obtained by carrying out repeat analyses using standard culture procedures (Feng 2007).

Traditional culture methods use selective liquid or solid culture media to grow the target microorganism and simultaneously prevent the growth of other microorganisms present in the food. The selection of a specific DNA sequence that will serve as probe or primer, along with the conditions in which hybridization is carried out, will determine the specificity of the nucleic acid-based assays. Concerns with immunological techniques include problems with cross-reactivity and difficulties with obtaining species-specific assays selecting appropriate antibodies.

Although the method selected may be rapid and accurate, other factors, including the speed of sample processing and cost, must be considered. Sample preparation limits the speed of the assay and is one of the most challenging problems for the direct detection of bacteria in food. To be able to calculate the actual number of bacteria present in a food by rapid methods such as quantitative PCR (Q-PCR), no previous enrichment of the sample can be performed. If the target is present in low numbers and a small volume of sample is taken (as many PCR methods only require 0.1 mL or less), there is a chance that this subsample may not include the target organism. Thus, a labor-intensive preparation of the sample is needed in order to recover food cells and quantitatively extract the DNA and purify it. The major bottleneck of Q-PCR is therefore found in the preparation of the sample (Jasson et al. 2010). The potential application of biosensor technology to pathogen testing in foods offers many attractive features (Chap. 6). However, like most assays, the exquisite sensitivity achievable with cultures does not translate to food testing (Feng 2007). Microbial sensors are particularly applicable in fluid systems with little organic substances, but this technique can present problems in its efficacy in food systems containing fats and proteins that coat the sensor and render it inoperable. Therefore, adequate sample preparation techniques are another important consideration in developing biosensor assays for foods. The difficulties inherent in food testing apply to microarrays as well, since the sensitivity of these assays decreases when testing foods (Chap. 5). Hence, factors such as adequate sample preparation and the need for confirmation of results must be considered in designing microarrays for food testing (Feng 2007). More research is needed on techniques for separating microorganisms from the food matrix and for concentrating them before detection methods are carried out. The possibilities of combining different rapid methods, including improved technologies for separation and concentration of specific bacteria, and for DNA extraction and purification (Chap. 5), will facilitate the direct detection of pathogens in food (Mandal et al. 2011). The goal is to avoid the enrichment providing rapid alternatives to conventional quantitative culture methods.

2.6 Identification and Characterization of Microorganisms

After detection, miniature biochemical kits are often used to identify microorganisms quickly and easily. Advances in instrumentation have enabled automation of identification tests. These instruments can incubate the reactions and automatically monitor biochemical changes to generate a phenotypic profile, which is then

compared with a database stored in the computer to provide an identification (Stager and Davis 1992). Other automated systems identify bacteria based on compositional or metabolic properties, such as fatty acid profiles, carbon oxidation profiles, or other traits (Miller and Rhoden 1991).

It is also very common to use antibodies to detect specific antigens using simple agglutination reactions. There are several commercial methods based on agglutination, in which antibody-coated colored latex beads or colloidal gold particles are used for quick confirmation or serological identification of pure culture isolates of bacteria from foods (D'Aoust et al. 1991; van Griethuysen et al. 2001). A modification of latex agglutination, known as reverse passive latex agglutination (RPLA), tests for soluble antigens and is used mostly in testing for toxins in food extracts or for toxin production by pure cultures (Feng 2007).

Molecular methods based on the hybridization or amplification of nucleic acids may also be used to identify or confirm the identities of microorganisms, as well as a subspecific characterization. Molecular typing of a species can help not only to investigate the origin of the strains present in foods but also to establish an association of the various degrees of virulence (López et al. 2006) or antimicrobial resistance that may exist within a species to certain strains or subtypes. There are plenty of molecular genotyping and subtyping methods. Currently, the most widely used technique for microbial source tracking is pulsed-field gel electrophoresis (PFGE), which is based in macrorestriction analysis of bacterial DNA and usually is carried out in reference or public health laboratories (Foley et al. 2009). Ribotyping is the only molecular typing technique that has been marketed in a completely automated format and allows for the large-scale characterization and fingerprinting of strains for epidemiological investigation. Ribotyping is a variant of the restriction fragment length polymorphism (RFLP) technique, which employs probes based on rDNA. Results can be obtained within 16 h, and riboprint patterns can be stored to create a unique database (Pavlic and Griffiths 2009).

Inset 2.1 Detection of Enterohemorrhagic Strains of *Escherichia coli* in Food

Enterohemorrhagic *E. coli* (EHEC) is a subgroup of Shiga-toxin- (Stx) producing *E. coli* (STEC) that has caused major outbreaks and sporadic cases of hemorrhagic colitis (HC) and hemolytic uremic syndrome (HUS) (Inset 1.1). Among EHEC, *E. coli* O157:H7 is now recognized as an important human pathogen and together with *Salmonella*, *L. monocytogenes*, and *Campylobacter* is one of four major food safety parameters (Jasson et al. 2010).

There are other well-known EHEC serotypes that have caused illness worldwide, representing a growing public health concern. Some cases of non-O157 STEC illness appear to be as severe as cases associated with O157. One of the more recent examples of severe disease caused by non-O157 EHEC is the

(continued)

Inset 2.1 (continued)

2011 European outbreak of EHEC O104:H4 infection (Inset 1.1). Nevertheless, most cases of STEC attributed to non-O157 are less severe. There is much variation in virulence potential within STEC serotypes, and many may not be pathogenic. Of the more than 400 serotypes isolated, fewer than 10 (O26, O45, O91, O103, O111, O113, O121, O128, and O145) cause the majority of all STEC-related human illnesses (Mathusa et al. 2010).

Detection of *E. coli* O157:H7

Because of the low dose of *E. coli* O157:H7 needed to cause infection, sensitive and rapid detection methods for *E. coli* O157:H7 in food samples are necessary in order for the food industry to ensure a safe supply of foods. The sensitive detection of *E. coli* O157:H7 has been developed over recent years with the use of selective enrichment and through the development of methods such as IMS. Selective enrichment followed by IMS and subsequent spread plating of the concentrated target cells onto selective and differential agar appears to be the most sensitive and cost-effective method for the isolation of *E. coli* O157 from raw foods (Bolton et al. 1996).

There are different reference methods for detecting *E. coli* O157:H7 in foods such as the protocol ISO 16654 (International Organization for Standardization 2001), the USDA-FSIS method used for the analysis of both raw and ready-to-eat meat products and environmental samples (United States Department of Agriculture Food Safety and Inspection Service 2010a, b), and the FDA-BAM screening method for other foods (Feng and Weagant 2011).

Currently, there are at least 22 commercial assays available for the detection and/or identification of *E. coli* O157:H7 that have been officially validated for use in food testing (AOAC International 2011). These rapid methods are mainly based on the use of antibodies or PCR.

Detection of Non-O157 EHEC

Except for O157:H7, there are no clear regulations to address the presence of other EHEC strains in foods. This is partly due to the difficulties in discerning EHEC from STEC strains that have not been implicated in illness and may not be pathogenic (Mathusa et al. 2010). These strains have few reliable biochemical or morphological characteristics (besides Stx production) that allow them to be distinguished from commensal *E. coli*. Thus, to detect EHEC other than O157 and phenotypic variants of *E. coli* O157 in food, methods for the detection of virulence factors and/or genes must be used.

While there is no standardized protocol for non-O157 EHEC, IMS has been shown to be useful in the recovery of specific serogroups from food and

(continued)

Inset 2.1 (continued)

fecal samples (O'Sullivan et al. 2007). Together with O157, paramagnetic beads coated with antibodies against serogroups O26, O103, O111, and O145 are also commercially available. There are also commercial selective agars that differentiate among O157 and those four EHEC serogroups on the basis of color, which is determined by a chromogenic compound and a mixture of selected carbohydrates (O'Sullivan et al. 2007).

Real-time PCR assays have been developed for the detection of EHEC that carry the major associated virulence genes *eae*, *stx1*, and *stx2* (Inset 1.1) and/or serogroup-specific gene targets. Serogroup target genes include *wzx* (O-antigen flippase in O26), *wzy* (O-antigen polymerase in O103), *galE* (galactose operon in O103), *Wbdl* (transferase gene in O111), and *Sil* (silver resistance in O145) (O'Sullivan et al. 2007). Some of these targets have been combined into multiplex assays (O'Sullivan et al. 2007; Valadez et al. 2011). Unfortunately, when confirmation of the isolates inoculated in food samples is carried out, the recovery of some serogroups was lower than that of others (Fratamico et al. 2011), and for serogroup O111, false negatives were often found (Verstraete et al. 2012). In the case of the 2011 European outbreak of EHEC O104:H4 infection, simple diagnostic screening tools to detect the outbreak strain in clinical specimens and foods were rapidly obtained. Three days after having the first isolate, a specific multiplex PCR was developed and made public, allowing for the specific identification of the strain, together with a novel real-time PCR assay for its detection in foods (Scheutz et al. 2011).

The USDA-FSIS procedure to detect and isolate non-O157 STEC serogroups utilizes a multiplex real-time PCR detection assay followed by culture isolation (United States Department of Agriculture Food Safety and Inspection Service 2010c). The culture isolation of non-O157 EHEC involves IMS followed by plating onto commercial chromogenic agars. Typical colonies from these plates are confirmed using multiplex PCR assays and biochemical identification. In the FDA method, the enrichment procedure and real-time PCR screening assay for O157 STEC have also been validated for the detection and recovery of other non-O157 EHEC as well (Feng and Weagant 2011). Commercial real-time PCR assays have also been designed for each of the non-O157 STEC O serogroups most commonly associated with human illness (Lin et al. 2011).

Identification and Characterization of EHEC

Serotyping is based on the use of specific antisera and the detection of O- and H-antigens expressed by these bacteria. Currently, a total number of 181 O-antigens and 53 H-antigens are available. Kits for O serotyping and H serotyping for STEC are commercially available. However, a minority of strains

(continued)

Inset 2.1 (continued)

do not serotype satisfactorily (O'Sullivan et al. 2007). Serotyping can also be done by molecular methods including analysis of gene/DNA sequences within and just outside the O-antigen cluster and *fliC* (H-antigens) sequence analysis. This molecular serotyping is based on DNA sequencing, DNA hybridization (microarray), PCR, or PCR-RFLP (O'Sullivan et al. 2007). The importance of serotyping was highlighted when during the 2011 European outbreak of EHEC O104:H4, other HUS-associated EHEC serogroups (O157, O91, and O103) were also identified, giving misleading results. This shows the need for the development of rapid serotyping and pathotyping methods for all HUS-associated *E. coli* strains (Friedrich 2011).

Genes encoding variants of Stx, intimin, enterohemolysins, and other potential pathogenicity factors (Inset 1.1) can be used as targets for characterization and typing. Single-nucleotide polymorphism (SNP) assays detect nucleotide substitutions (SNPs) by using sequencing, PCR, or microarrays. While *stx1* has a relatively conserved nucleotide sequence, several variants of *stx2* have been described (O'Sullivan et al. 2007). Neupane et al. (2011) have shown that overexpression of *stx2* is common in strains associated with HUS and that SNPs that may affect *stx2* expression could be useful in differentiating between highly virulent strains.

There are numerous PCR-based methods for the detection of EHEC virulence factors, but the time and cost involved with large-scale screening efforts and population level analyses have limited the size and scope of studies. Combining the high-throughput performance of microarrays with the specificity and sensitivity of real-time Q-PCR, Gonzales et al. (2011) identified and evaluated a panel of 28 genetic markers that can be used with high-throughput PCR to virulotype, serotype, and preliminarily subtype large numbers of isolates. The panel includes known virulence and regulatory genes, O-antigen genes, and select prophage regions of O157 and non-O157 EHEC. This has the potential to become an integral tool in outbreak, environmental, and genetic investigations of EHEC.

A different microarray has been developed for the identification of STEC strains with a high potential for human virulence. It is based on the genetic identification of 12 O-types and 7 H-types of STEC including the most clinically relevant EHEC serotypes (Bugarel et al. 2010). The genes selected for determination of the O-antigens showed a high specificity and concordance with serology. The microarray also had a high specificity for EHEC-associated virulence factors, and it was used during the 2011 European outbreak of EHEC O104:H4 infection (Chap. 5).

References

- Abubakar I, Irvine L, Aldus CF, Wyatt GM, Fordham R, Schelenz S, Shepstone L, Howe A, Peck M, Hunter PR (2007) A systematic review of the clinical, public health and cost-effectiveness of rapid diagnostic tests for the detection and identification of bacterial intestinal pathogens in faeces and food. *Health Technol Assess* 11:1–216.
- Aldus CF, van Amerongen A, Ariens RMC, Peck MW, Wichers JH, Wyatt GM (2003) Principles of some novel rapid dipstick methods for detection and characterization of verotoxigenic *Escherichia coli*. *J Appl Microbiol* 95:380–389.
- Aminul Islam M, Heuvelink AE, Talukder KA, de Boer E (2006) Immunoconcentration of Shiga toxin-producing *Escherichia coli* O157 from animal faeces and raw meats by using Dynabeads anti-*E. coli* O157 and the VIDAS system. *Int J Food Microbiol* 109:151–156.
- AOAC INTERNATIONAL (2011) Performance Tested Methodssm Validated Methods. <http://www.aoac.org/testkits/testedmethods.html>. Last updated: October 2011. Accessed 18 November 2011.
- Betts R, Blackburn CW (2009) Detecting pathogens in food. In: Foodborne pathogens: hazards, risk analysis and control, 2nd edn. Edited by: Blackburn CW, McClure PJ. Woodhead Publishing, Oxford, UK. pp. 17–65.
- Bhunia AK (2008) Biosensors and bio-based methods for the separation and detection of food-borne pathogens. *Adv Food Nutr Res* 54:1–44.
- Bisha B, Brehm-Stecher BF (2010) Combination of adhesive-tape-based sampling and fluorescence *in situ* hybridization for rapid detection of *Salmonella* on fresh produce. *J Vis Exp* 44 pii:2308.
- Blodgett R (2010) Most probable number from serial dilutions. Bacteriological Analytical Manual Appendix2. <http://www.fda.gov/Food/ScienceResearch/LaboratoryMethods/BacteriologicalAnalyticalManualBAM/ucm109656.htm>. Accessed 10 November 2011.
- Bohaychuk VM, Gensler GE, King RK, Wu JT, McMullen LM (2005) Evaluation of detection methods for screening meat and poultry products for the presence of foodborne pathogens. *J Food Prot* 68:2637–2647.
- Bolton FJ, Crozier L, Williamson JK (1996) Isolation of *Escherichia coli* O157 from raw meat products. *Lett Appl Microbiol* 23:317–321.
- Bosilevac JM, Shackelford SD, Brichta DM, Koohmaraie M (2005) Efficacy of ozonated and electrolyzed oxidative waters to decontaminate hides of cattle before slaughter. *J Food Prot* 68:1393–1398.
- Bottari B, Ercolini D, Gatti M, Neviani E (2006) Application of FISH technology for microbiological analysis: current state and prospects. *Appl Microbiol Biotechnol* 73:485–494.
- Bugarel M, Beutin L, Martin A, Gill A, Fach P (2010) Micro-array for the identification of Shiga toxin-producing *Escherichia coli* (STEC) seropathotypes associated with Hemorrhagic Colitis and Hemolytic Uremic Syndrome in humans. *Int J Food Microbiol* 142:318–329.
- Chain VS, Fung DY (1991) Comparison of Redigel, Petrifilm, spiral plate system, Isogrid, and aerobic plate count for determining the numbers of aerobic bacteria in selected foods. *J Food Prot* 54:208–211.
- Chen WT, Hendrickson RL, Huang CP, Sherman D, Geng T, Bhunia AK, Ladisch MR (2005) Mechanistic study of membrane concentration and recovery of *Listeria monocytogenes*. *Biotechnol Bioeng* 89:263–273.
- Cohen AE, Kerdahi KF (1996) Evaluation of a rapid and automated enzyme-linked fluorescent immunoassay say for detecting *Escherichia coli* serogroup O157 in cheese. *J AOAC Int* 79:858–860.
- Comas-Riu J, Rius N (2009) Flow cytometry applications in the food industry. *J Ind Microbiol Biotechnol* 36:999–1011.
- Cools I, D’Haese E, Uyttendaele M, Storms E, Nelis HJ, Debevere J (2005) Solid phase cytometry as a tool to detect viable but non-culturable cells of *Campylobacter jejuni*. *J Microbiol Methods* 63:107–114.

- Cunningham AE, Rajagopal R, Lauer J, Allwood P (2011) Assessment of hygienic quality of surfaces in retail food service establishments based on microbial counts and real-time detection of ATP. *J Food Prot* 74:686–690.
- D'Aoust J-Y, Sewell AM, Greco P (1991) Commercial latex agglutination kits for the detection of foodborne *Salmonella*. *J Food Prot* 54:725–730.
- D'Haese E, Nelis HJ (2002) Rapid detection of single cell bacteria as a novel approach in food microbiology. *J AOAC Int* 85:979–983.
- Favrin SJ, Jassim SA, Griffiths MW (2003) Application of a novel immunomagnetic separation-bacteriophage assay for the detection of *Salmonella* Enteritidis and *Escherichia coli* O157: H7 in food. *Int J Food Microbiol* 85:63–71.
- Fedio WM, Jinneman KC, Yoshitomi KJ, Zapata R, Wendakoon CN, Browning P, Weagant SD (2011) Detection of *E. coli* O157:H7 in raw ground beef by Pathatrix™ immunomagnetic-separation, real-time PCR and cultural methods. *Int J Food Microbiol* 148:87–92.
- Feng P (2007) Rapid methods for the detection of foodborne pathogens: current and next-generation technologies. In: Food microbiology, fundamentals and frontiers, 3rd edn. Edited by: Doyle MP, Beuchat LR. ASM Press, Washington, D.C. pp 911–934.
- Feng P, Weagant SD (2011) Diarrheagenic *Escherichia coli*. Bacteriological Analytical Manual, Chapter 4A. <http://www.fda.gov/food/scienceresearch/laboratorymethods/bacteriologicalanalyticalmanualbam/ucm070080.htm>. Accessed 10 November 2011.
- Foley SL, Lynne AM, Nayak R (2009) Molecular typing methodologies for microbial source tracking and epidemiological investigations of Gram-negative bacterial foodborne pathogens. *Infect Genet Evol* 9:430–440.
- Fratamico PM, Bagi LK, Cray WC Jr, Narang N, Yan X, Medina M, Liu Y (2011) Detection by multiplex real-time polymerase chain reaction assays and isolation of Shiga toxin-producing *Escherichia coli* serogroups O26, O45, O103, O111, O121, and O145 in ground beef. *Foodborne Pathog Dis* 8:601–607.
- Friedrich AW (2011) Enterohaemorrhagic *Escherichia coli* O104:H4: are we prepared now? *Euro Surveill* 16 pii:19938.
- Fu Z, Rogelj S, Kieft TL (2005) Rapid detection of *Escherichia coli* O157: H7 by immunomagnetic separation and real-time PCR. *Int J Food Microbiol* 99:47–57.
- Fung DY, Cox NA, Bailey JS (1988) Rapid methods and automation in the microbiological examination of food. *Dairy Food Sanit* 8:292–296.
- Gonzales TK, Kulow M, Park DJ, Kaspar CW, Anklam KS, Pertzborn KM, Kerrish KD, Ivanek R, Döpfer D (2011) A high-throughput open-array qPCR gene panel to identify, virulotype, and subtype O157 and non-O157 enterohemorrhagic *Escherichia coli*. *Mol Cell Probes* 25:222–230.
- Hanna SE, Connor CJ, Wang HH (2005) Real-time polymerase chain reaction for the food microbiologist: technologies, applications, and limitations. *J Food Sci* 70:R49–R53.
- Hermida M, Taboada M, Menéndez S, Rodríguez-Otero JL (2000) Semi-automated direct epifluorescent filter technique for total bacterial count in raw milk. *J AOAC Int* 83:1345–1348.
- Hill WE (1996) The polymerase chain reaction: application for the detection of foodborne pathogens. *CRC Crit Rev Food Sci Nutr* 36:123–173.
- Hunter DM, Leskinen SD, Magaña S, Schlemmer SM, Lim DV (2011) Dead-end ultrafiltration concentration and IMS/ATP-bioluminescence detection of *Escherichia coli* O157:H7 in recreational water and produce wash. *J Microbiol Methods* 87:338–342.
- International Standards Organization (2001) Microbiology of food and animal feeding stuffs – horizontal method for the detection of *Escherichia coli* O157. ISO 16654.
- Jantzen MM, Navas J, Corujo A, Moreno R, Lopez V, Martínez-Suarez JV (2006a) Review. Specific detection of *Listeria monocytogenes* in foods using commercial methods: from chromogenic media to real-time PCR. *Span J Agric Res* 4:235–247.
- Jantzen MM, Navas J, de Paz M, Rodríguez B, da Silva WP, Nuñez M, Martínez-Suárez JV (2006b) Evaluation of ALOA plating medium for its suitability to recover high pressure-injured *Listeria monocytogenes* from ground chicken meat. *Lett Appl Microbiol* 43:313–317.

- Jasson V, Jacxsens L, Luning P, Rajkovic A, Uyttendaele M (2010) Review. Alternative microbial methods: An overview and selection criteria. *Food Microbiol* 27:710–730.
- Kannan P, Yong HY, Reiman L, Cleaver C, Patel P, Bhagwat AA (2010) Bacteriophage-based rapid and sensitive detection of *Escherichia coli* O157:H7 isolates from ground beef. *Foodborne Pathog Dis* 7:1551–1558.
- Kawasaki S, Frataimico PM, Horikoshi N, Okada Y, Takeshita K, Sameshima T, Kawamoto S (2009) Evaluation of a multiplex PCR system for simultaneous detection of *Salmonella* spp., *Listeria monocytogenes*, and *Escherichia coli* O157:H7 in foods and in food subjected to freezing. *Foodborne Pathog Dis* 6:81–89.
- Leon-Velarde CG, Zosherafatein L, Odumeru JA (2009) Application of an automated immunomagnetic separation-enzyme immunoassay for the detection of *Salmonella enterica* subspecies *enterica* from poultry environmental swabs. *J Microbiol Methods* 79:13–17.
- Lin A, Sultan O, Lau HK, Wong E, Hartman G, Lauzon CR (2011) O serogroup specific real time PCR assays for the detection and identification of nine clinically relevant non-O157 STECs. *Food Microbiol* 28:478–483.
- López V, Suárez M, Chico-Calero I, Navas J, Martínez-Suárez JV (2006) Foodborne *Listeria monocytogenes*: are all the isolates equally virulent? *Rev Argent Microbiol* 38:224–234.
- Manafi M (2000) New developments in chromogenic and fluorogenic culture media. *Int J Food Microbiol* 60:205–218.
- Mandal PK, Biswas AK, Choi K, Pal UK (2011) Methods for rapid detection of foodborne pathogens: an overview. *Am J Food Technol* 6:87–102.
- Mathusa EC, Chen Y, Enache E, Hontz L (2010) Non-O157 Shiga toxin-producing *Escherichia coli* in foods. *J Food Prot* 73:1721–1736.
- Miller JM, Rhoden DL (1991) Preliminary evaluation of Biolog, a carbon source utilization method for bacterial identification. *J Clin Microbiol* 29:1143–1147.
- Navas J, Ortiz S, Lopez P, Jantzen MM, Lopez V, Martinez-Suarez JV (2006) Evaluation of effects of primary and secondary enrichment for the detection of *Listeria monocytogenes* by real-time PCR in retail ground chicken meat. *Foodborne Pathog Dis* 3:347–354.
- Neupane M, Abu-Ali GS, Mitra A, Lacher DW, Manning SD, Riordan JT (2011) Shiga toxin 2 overexpression in *Escherichia coli* O157:H7 strains associated with severe human disease. *Microb Pathog* 51:466–470.
- O'Sullivan J, Bolton DJ, Duffy G, Baylis C, Tozzoli R, Wasteson Y, Lofdahl S (2007) Methods for detection and molecular characterisation of pathogenic *Escherichia coli*. Co-ordination action FOOD-CT-2006-036256. Pathogenic *Escherichia coli* network. Editors O'Sullivan J, Bolton DJ, Duffy G, Baylis C, Tozzoli R, Wasteson Y, Lofdahl S. <http://www.antimicrobialresistance.dk/data/images/protocols/e%20coli%20methods.pdf>. Accessed 15 November 2011.
- Olsvik O, Popovic T, Skjerve E, Cudjoe KS, Hornes E, Ugelstad J, Uhlen M (1994) Magnetic separation techniques in diagnostic microbiology. *Clin Microbiol Rev* 7:43–54.
- Pavlic M, Griffiths MW (2009) Principles, applications, and limitations of automated ribotyping as a rapid method in food safety. *Foodborne Pathog Dis* 6:1047–1055.
- Pettipher GL, Watts YB, Langford SA, Kroll RG (1992) Preliminary evaluation of COBRA, an automated DEFT instrument, for the rapid enumeration of microorganisms in cultures, raw-milk, meat and fish. *Lett Appl. Microbiol* 14:206–209.
- Posthuma-Trumpie GA, Korf J, van Amerongen A (2009) Lateral flow (immuno) assay: its strengths, weaknesses, opportunities and threats; a literature survey. *Anal Bioanal Chem* 393:569–582.
- Pyle BH, Broadaway SC, McFeters GA (1999) Sensitive detection of *Escherichia coli* O157:H7 in food and water by immunomagnetic separation and solid-phase laser cytometry. *Appl Environ Microbiol* 65:1966–1972.
- Rodriguez-Lazaro D, Hernandez M (2006) Isolation of *Listeria monocytogenes* DNA from meat products for quantitative detection by real-time PCR. *J Rapid Method Automat Microbiol* 14:395–404.
- Samkuty PJ, Gough RH, Adkinson RW, McGrew P (2001) Rapid assessment of the bacteriological quality of raw milk using ATP bioluminescence. *J Food Prot* 64:208–212.

- Savoye F, Feng P, Rozand C, Bouvier M, Gleizal A, Thevenot D (2011) Comparative evaluation of a phage protein ligand assay with real-time PCR and a reference method for the detection of *Escherichia coli* O157:H7 in raw ground beef and trimmings. *J Food Prot* 74:6–12.
- Scheut F, Nielsen EM, Frimodt-Møller J, Boisen N, Morabito S, Tozzoli R, Nataro JP, Caprioli A (2011) Characteristics of the enteroaggregative Shiga toxin/verotoxin-producing *Escherichia coli* O104:H4 strain causing the outbreak of haemolytic uraemic syndrome in Germany, May to June 2011. *Euro Surveill* 16 pii:19889.
- Seo KH, Brackett RE, Frank JF, Hilliard S (1998) Immunomagnetic separation and flow cytometry for rapid detection of *Escherichia coli* O157:H7. *J Food Prot* 61:812–816.
- Stager CE, Davis JR (1992) Automated systems for identification of microorganisms. *Clin Microbiol Rev* 5:302–327.
- Stannard C (1997) Development and use of microbiological criteria for foods. *Food Sci Technol Today* 11:137–177.
- Stevens KA, Jaykus LA (2004) Bacterial separation and concentration from complex sample matrices: A review. *Crit Rev Microbiol* 30:7–24.
- Thacker JD, Casale ES, Tucker CM (1996) Immunoassays (ELISA) for rapid, quantitative analysis in the food-processing industry. *J Agric Food Chem* 44:2680–2685.
- Torlak E, Akan IM, Gokmen M (2008) Comparison of TEMPO EC and TBX medium for the enumeration of *Escherichia coli* in cheese. *Lett Appl Microbiol* 47:566–570.
- United States Department of Agriculture Food Safety And Inspection Service (2010a) Detection, isolation and identification of *Escherichia coli* O157:H7 from meat products, MLG 5.05, 10/01/10. http://www.fsis.usda.gov/PDF/MLG_5_05.pdf. Accessed 22 November 2011.
- United States Department of Agriculture Food Safety And Inspection Service (2010b) FSIS procedure for the use of *Escherichia coli* O157:H7 screening tests, MLG 5A.02, 10/01/10. http://www.fsis.usda.gov/PDF/MLG_5A_02.pdf. Accessed 22 November 2011.
- United States Department of Agriculture Food Safety And Inspection Service (2010c) Detection and isolation of non-O157 Shiga-toxin producing *Escherichia coli* strains (STEC) from meat products, MLG 5B.00, 10/01/10. http://www.fsis.usda.gov/PDF/MLG_5B_00.pdf. Accessed 7 November 2011.
- Valadez AM, Debroy C, Dudley E, Cutter CN (2011) Multiplex PCR detection of Shiga toxin-producing *Escherichia coli* strains belonging to serogroups O157, O103, O91, O113, O145, O111, and O26 experimentally inoculated in beef carcass swabs, beef trim, and ground beef. *J Food Prot* 74:228–239.
- van Griethuysen A, Bes M, Etienne J, Zbinden R, Kluytmans J (2001) International multicenter evaluation of latex agglutination tests for identification of *Staphylococcus aureus*. *J Clin Microbiol* 39:86–89.
- Veal DA, Deere D, Ferrari B, Piper J, Attfield PV (2000) Fluorescence staining and flow cytometry for monitoring microbial cells. *J Immunol Method* 243:191–210.
- Verstraete K, Robyn J, Del-Favero J, De Rijk P, Joris MA, Heyndrickx M, De Zutter L, De Reu K (2012) Evaluation of a multiplex-PCR detection in combination with an isolation method for STEC O26, O103, O111, O145 and sorbitol fermenting O157 in food. *Food Microbiol* 29:49–55.
- Wagner M, Horn M, Daims H (2003) Fluorescence in situ hybridisation for the identification and characterisation of prokaryotes. *Curr Opin Microbiol* 6:302–309.
- Wawerla M, Stolle A, Schalh B, Eisgruber H (1999) Impedance microbiology: applications in food hygiene. *J Food Prot* 62:1488–1496.
- Wu VCH (2008) A review of microbial injury and recovery methods in food. *Food Microbiol* 25:735–744.
- Yang L, Bashir R (2008) Electrical/electrochemical impedance for rapid detection of foodborne pathogenic bacteria. *Biotechnol Adv* 26:135–150.

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