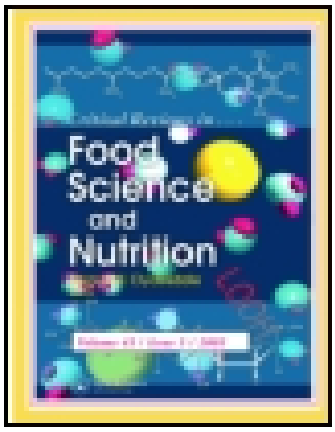

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Commercially Available Rapid Methods for Detection of Selected Foodborne Pathogens

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Abstract

Generally, the enumeration and isolation of foodborne pathogens is performed using culture-dependent methods. These methods are sensitive, inexpensive and provide both qualitative and quantitative assessment of the microorganisms present in a sample, but they are time consuming. For this reason, researchers are developing new techniques that allow detection of food pathogens in shorter times. This review identifies commercially available methods for rapid detection and quantification of *Listeria monocytogenes*, *Salmonella* spp., *Staphylococcus aureus*, and Shiga toxin-producing *Escherichia coli* in food samples. Three categories are discussed: immunologically-based methods, nucleic acid-based assays, and biosensors. This review describes the basic mechanism and capabilities of each method, discusses the difficulties of choosing the most convenient method, and provides an overview of the future challenges for the technology of rapid detection of microorganisms.

Keywords: food safety, *Staphylococcus aureus*, *Salmonella* spp., *Listeria monocytogenes, Shiga toxin-producing Escherichia coli* (STEC)

Introduction

The isolation and enumeration of pathogens in foods is commonly performed using conventional microbiological techniques that rely on standard culture methods. These methods are sensitive, inexpensive, and allow the qualitative and quantitative assessment of microorganisms (Feng, 2001a), but require several days to isolate and enumerate foodborne pathogens. Despite this limitation, standard culture methods are often regarded as the ñgold standardò by which all other methods are compared.

Advances in technology have permitted the development of rapid methods for the microbial analysis of foods (Feng, 2006). In general, any method that detects a foodborne pathogen in a shorter period of time, as compared to conventional methods, is considered a rapid method. The term ñrapid detection methodò also is used to describe an array of tests including biochemical kits, antibodies and nucleic acid-based methods, that are designed to speed up detection (Swaminathan and Feng, 1994). Despite the fact that rapid detection methods greatly reduce times, it is crucial to remember that a positive result for a foodborne pathogen is regarded only as ñpresumptive positiveò and must be confirmed by standard culture methods (Feng, 1996).

The utilization of commercial rapid detection methods demands that established sampling procedures are strictly followed. This approach is crucial because interpretations derived from a large batch of food may be based on a relatively small sample. If a sample is improperly collected, mishandled, contaminated, or is not representative, the results can be meaningless or worse, misleading. Along the same lines, it is also critical to follow the instructions given with the assay (Andrews and Hammack, 2003).

The present review attempts to identify methods that permit the rapid detection and quantification of selected pathogens: *Listeria monocytogenes, Salmonella* spp., *Staphylococcus aureus*, and Shiga toxin-producing *Escherichia coli* (STEC) developed for the food industry. It describes briefly the principles of these methods, as well as the relevant characteristics of the target pathogen. The scope of this review is restricted to commercially-available methods and references are provided for detailed protocols that are not discussed further. A summary of the commercially-available rapid methods also are presented for each foodborne pathogen.

Note: This review is not intended to endorse or recommend any commercial product and any omission of a commercial product is not intentional.

Rapid detection technologies

Rapid detection technologies are commonly grouped broadly into three categories: immunologically-based methods, nucleic acid-based assays, and biosensors (Goodridge et al., 2011; Mandal et al., 2011). Most rapid methods can analyze a sample in a few minutes to a few hours, but still lack sufficient sensitivity and specificity for direct testing of foods, so that the sample still needs to be culture-enriched before analysis (Feng, 1997). Although the enrichment step is often regarded as the main limitation in terms of speed, it may offer indirect benefits, such as the dilution of inhibitors often present in the food sample, the differentiation of viable from non-viable cells, and the revival of stressed or injured cells in the food sample. When choosing a rapid method, it is important to know that some rapid methods do not result in an isolated colony (Feng, 2001b).

Ideally, rapid detection methods should be validated by either one of the following organizations: Association of Analytical Communities (AOAC) in the United States, the

European Certification Organization for the Validation and Approval of Alternative Methods for the Microbiological Analysis of Food and Beverages (MicroVal) or the Association Française de Normalisation (AFNOR) in the European Union.

The validity of the method implies that the test has the ability to do what is intended to do (Forsythe, 2010). Validity is dependent upon the sensitivity and specificity of a method. Sensitivity is the probability of the test to detect a true positive, while specificity is the probability of the test to detect a true negative. These measures should be reported to facilitate comparison between rapid methods (Beumer and Hazeleger, 2009); however, such information is occasionally missing. While novel detection methods are released regularly, their acceptance by the industry depends not only on speed, but also on initial investment, cost, technical support, and ease of use, among other considerations.

Immunologically-based methods

Immunological methods rely on the specific binding of an antibody to an antigen. Immunoassay refers to the qualitative and quantitative determination of an antigen and antibody in a specimen by means of an immunological reaction. There is wide usage of this technology in the food industry due to high sensitivity, automation, and high specificity (Mandal et al., 2011). The specific binding of antibodies to their particular antigen, as well as the speed and simplicity of this interaction, has allowed the development of a wide variety of antibody assays based on immunochemistry (Feng, 1997).

Latex agglutination test

The latex agglutination test is the most common immunological method that generally employs antibody-coated particles, latex beads, or colloidal gold particles Latex tests are performed manually and results are determined by simple observation. Briefly, the suspected pathogen, which is considered as the antigen (usually a single colony), is mixed with latex beads coated with antibodies. The visible clumping (or agglutination) of the latex beads will indicate the presence of the target antigen in a matter of few minutes. If the antigen is absent, agglutination will not occur and thus, no precipitant will be observed.

Reverse passive latex agglutination test

The reverse passive latex agglutination (RPLA), a test derived from the latex agglutination assay, allows the determination of toxin production by a bacterial culture and the presence or absence of toxins in a food extract (Feng, 1997). If the antigen is absent, the result is revealed as a compact dot formed in the bottom of the plate, as shown in Figure 1.

The test uses latex particles coated with rabbit antiserum, which reacts with the target antigen. If the antigen is present, the latex particles agglutinate, forming a ñlattice structure.ò This precipitate settles to the bottom of a V-shaped microtiter plate, exhibiting a diffuse appearance (Forsythe, 2010).

Enzyme-Linked Immunosorbent Assay

The enzyme-linked immunosorbent assay (ELISA) is the most popular antibody assay used for the detection of pathogens in foods (Candlish, 1991). ELISA utilizes an antibody bound to a solid matrix (such as a microplate well) that captures the antigen from enriched cultures. Once the antigen is immobilized, an enzyme-conjugated antibody is added, forming a complex with the antigen. This step is followed by the addition of a chromogenic, fluorescent, or

chemiluminescent enzyme substrate, which is used for visualization of the bound targets (Feng, 1997) as illustrated in Figure 2.

Lateral flow assay

The lateral flow assay (LFA) is a modification of the ELISA test. LFAs, are low cost, easy to use, disposable devices in the form of immunochromatographic line assays that generate detectable colored end products and give a presence/absence result (Goodridge et al., 2011). Briefly, the enriched sample is deposited into the reagent pad, and migrates through it by capillary action, encountering the conjugated antibodies (with colored particles) specific to the target on their pathway; they are then immobilized by anti-target analyte antibodies fixed in the pad that act as a capture zone (a control zone also will be present, containing antibodies specific for the conjugate antibodies). If the specific antigen is present, two visible lines (including control) of precipitation are formed, as shown in Figure 3.

One of the issues associated with LFAs is the potential for false positives, due to the interference from the sample matrix. As a result, these assays often need to be optimized for detection of bacteria from a specific food matrix (Goodridge et al., 2011).

Nucleic acid-based assays

Nucleic acids are biological molecules essential for life and include DNA (deoxyribonucleic acid) and RNA (ribonucleic acid). They are present in all living things and play a role in encoding, transmitting and expressing genetic information. Advances in molecular biology technology, particularly the Polymerase Chain Reaction (PCR), have allowed for more reliable microbial identification and surveillance. PCR has increased our ability to investigate foodborne outbreaks and identify the pathogen responsible for the disease. PCR techniques

provide increased sensitivity, allow for more rapid processing times, and enhance the likelihood of detecting bacterial pathogens (Lampel et al., 2000).

Polymerase chain reaction (PCR)

The PCR technique amplifies a single or a few copies of DNA by several orders of magnitude. PCR-based assays are widely used to evaluate foods for the presence or absence of specific pathogens. The main characteristic of this technology is that it allows the replication of a unique primer binding site sequence of the target organism(s), despite the presence of DNA from non-target microorganisms found in the food sample. For PCR, specific binding sites, sequence specific for the target species (i.e. virulence, or housekeeping genes), are selected. Following the PCR, the amplified product, (amplicon), can be detected using nucleic acid stains such as ethidium bromide, SYBR Green, PicoGreen, among others. Specialized literature on the use of molecular methods for pathogen detection is available from several sources (Levin, 2009b; Maurer, 2011; Sachse and Frey, 2003).

A basic PCR run comprises three phases: exponential, in which an exact doubling of product is accumulating at every cycle (assuming 100% reaction efficiency); linear, in which the reaction components are being consumed and the reaction is non-exponential; and plateau, in which the reaction has stopped and no more products are being made (Figure 3).

The reliability of PCR detection methods partially depends on the purity of the nucleic acid template and the presence of sufficient numbers of target molecules (Lampel et al., 2000). Foods are complex matrices, so certain steps are necessary to limit the effects of potential inhibitory compounds in food that may impair PCR amplification of the intended target. This requirement should be fulfilled, in addition to the enrichment steps that are frequently required,

to enhance PCR detection sensitivities and overcome problems of low pathogen numbers. Other means, including immunomagnetic separation or filtration, must be employed when selective enrichment methods are unsuitable (Lampel et al., 2000).

After PCR, the products are separated on agarose gels and are visualized after staining with a nucleic acid-binding dye, such as ethidium bromide. This procedure has several limitations: PCR is more time consuming due to the agarose gel step, when compared to sophisticated methods such as the quantitative PCR (qPCR, described below). PCR also is difficult to automate and thus, has throughput limitations. Finally, PCR results cannot be used to quantify the bacteria present in the starting material.

An additional amplification technique is the Loop-mediated Isothermal Amplification (LAMP). This method is regarded as a simple, rapid, specific and cost-effective nucleic acid amplification method when compared to PCR. Amplification and detection of genes can be completed in a single step, enabling visual discrimination of results without costly specialized equipment (Marlony et al., 2011; Mori et al., 2001; Ueda and Kuwabara, 2009).

Quantitative PCR (qPCR)

Quantitative, real time polymerase chain reaction (qPCR) is a technique that enables simultaneous detection and quantification of a DNA template. The principle is similar to PCR. However, qPCR incorporates fluorescent molecules that allow monitoring of amplification in real time. Consequently, this methodology is more advanced when compared to the traditional PCR (described above) where the product of the reaction is detected only after the PCR is complete.

Two methods for detection of products during qPCR are commonly used. The first uses an intercalating dye such as SYBR Green. This detection method is suitable when a single target is being studied, since the dye will intercalate into any generated, double-stranded DNA (Sigma-Aldrich, 2011). The second detection method uses sequence-specific primers plus an oligonucleotide that is complementary to one strand of the amplicon. This oligonucleotide is commonly referred as a ñTaqman probe.ò The oligonucleotide is labeled on the 5ô end with a fluorescent dye and on the 3ô end with a quencher. The oligonucleotide by itself does not emit fluorescence, due to the proximity of the fluorophore and quencher. However, during the PCR reaction, the probe will form Watson-Crick base pairs with the template DNA between the PCR primer annealing sites. Upon primer extension, the probe will be degraded, due to the 5ô to 3ô exonuclease activity of *Taq* polymerase. This reaction uncouples the fluorophore and quencher and permits detection of the fluorophore (Sigma-Aldrich, 2011). With this detection method, multiplex PCR (mPCR) reactions that simultaneously detect two or more genes or organisms are made possible by incorporating primer and probes specific to each in a single reaction and covalently attaching fluorophores that emit at different wavelengths to each unique probe.

During amplification, the number of PCR cycles required for the fluorescent signal to reach a user-defined threshold is referred to as the cycle threshold (Ct). There is a direct correlation between the Ct and the number of target DNA copies present in the sample (Valasek and Repa, 2005). A standard curve can be generated from Ct values for a series of reactions using dilutions containing known quantities of target DNA. The amount of target in unknown samples can be derived by measuring the Ct and by using the standard curve to determine the starting copy number (Figure 4).

DNA Microarray Technologies

In general, microarrays are a set of arrangements of recognition ligands (i.e DNA, proteins, antibodies) that are immobilized in particular locations on a solid matrix (Rasooly and Herold, 2008). Specifically, DNA microarrays are used for the analysis of gene expression and variations of gene sequences (Sergeev et al., 2006). The most common DNA microarrays consist of immobilized single-stranded DNA probes spatially ordered on a glass matrix (Rasooly and Herold, 2008). The use of this technology for the genotyping and detection of foodborne pathogens is currently under development. Some examples of microarrays prototypes are those created for the detection of *Bacillus cereus* (Sergeev et al., 2006) and enterotoxigenic *E. coli* (Wang et al., 2010)

An advantage of DNA microarrays is their capability to obtain detailed genomic information of the pathogen, including relevant identiýcation, typing, virulence factors and antibiotic resistance (Rasooly and Herold, 2008). On the other hand, some disadvantages are their high cost, limited throughput and reproducibility (Goodridge et al., 2011).

Novel promising bead arrays are an alternative format of microarrays in which the capture probes are immobilized on silica beads that are in suspension, unlike the fixed matrix. Bead array technology has the potential to overcome microarrays limitations. However, neither of these products is yet commercially available for the detection of foodborne pathogens.

Biosensors

A biosensor is an analytical device that allows the detection of pathogens or toxins through an electrochemical, acoustic, thermal, or optical output (Luong et al., 1997). These biosensing systems include cell-based sensors (Banerjee and Bhunia, 2009; Ziegler, 2000), flow

cytometry combined with fluorescent antibodies (Kim et al., 2009), surface-enhanced Raman scattering (Kudelski, 2008), Fourier transform infrared spectroscopy (Berthomieu and Hienerwadel, 2009), and light-scattering technology (Banada et al., 2009; Bhunia, 2008; Bhunia, 2010). The advantages of biosensors, compared with other methods, are a higher sensitivity for microbial toxins and bacteria (Rasooly and Herold, 2006). Biosensors are being developed for several foodborne pathogens including *E. coli* O157:H7, *Staphylococcus aureus*, *Salmonella* spp. and *Listeria monocytogenes*. To date, the only biosensor method reported as commercially available is the Rapid B® from the Vivione-Biosciences Company (http://www.vivionebiosciences.com/technology.html), and is capable of detecting Salmonella and *E. coli* O157:H7.

Target pathogens

Staphylococcus aureus

Staphylococcus aureus is a cocoid-shaped, Gram-positive, facultative anaerobic bacterium. Some strains are capable of producing heat-stable proteins (enterotoxins) that cause staphylococcal food poisoning in humans, and in some cases, toxic shock syndrome. Symptoms of intoxication may include nausea, vomiting, and diarrhea. The presence of this bacterium or its enterotoxins in processed foods are indicators of poor sanitation (Bennet and Lancette, 2001) or inadequate handling. Immunoassay methods for detection of enterotoxins have been used for several years; however, improvements of this technology are still reported at the laboratory level. For example, the increased sensitivity for the detection of *S. aureus* enterotoxin A was recently achieved by using antibodies with higher antigen-binding affinity (Mizutani, et al. 2012).

Among nucleic-acid methods, real time PCR technique also is constantly being improved. For instance, detection of *S. aureus* is reported to be as short as ñnext day after receptionò and achieved through the development of primers with increased specificity, with the added advantage of being more sensitive, when compared to previous methods (Trnļíková et al., 2009).

Biosensor-based methods for *S. aureus* also are under research, but are not yet commercially available. Interesting efforts towards the development of this technology are reported, such as a newly developed method for detection of *S. aureus* and *E. coli* that combines electrochemical and immunological technology, with higher sensitivity for pathogen detection (10^2 CFU/mL) in as short as 1-2 hours (Tan, et al., 2011). The commercially available rapid assays for detection of *S. aureus* and/or its enterotoxins in a variety of food matrices, such as dairy, meat, poultry, and infant formula, are listed in Table 1.

Listeria monocytogenes

L. monocytogenes is a facultative anaerobe, Gram-positive, motile, catalase-positive, rod-shaped, ubiquitous food-borne pathogen (Hitchins and Jinneman 2011; Posfay-Barbe and Wald, 2009). *L. monocytogenes* is often described as an opportunistic pathogen because it primarily affects susceptible vulnerable population groups (elderly, pregnant women, small children, etc). Listeria infection (listeriosis) symptoms might range from febrile gastroenteritis to meningitis, sepsis, infection of the fetus, or abortion (Schuppler and Loessner, 2010). Rapid detection kits are commonly used for the detection of *Listeria* spp. and *Listeria monocytogenes* in food samples. In the United States of America, further characterization of *L. monocytogenes* isolates is optional, with the exception of the isolates obtained by the Food and Drug Administration

(FDA). In this case, the isolates are required to be characterized by serological means, pulsed-field gel electrophoresis (PFGE), or by ribotyping (Hitchins and Jinnerman, 2011).

There are several commercially-available rapid assays that allow detection of *L. monocytogenes* in a variety of food matrices. These methods are listed and characterized to facilitate comparison in Table 2. Of note, there are considerably higher numbers of nucleic acid based methods, as compared with immunological-based methods.

Salmonella spp**.**

Salmonella spp. are rod-shaped, non-sporeforming, Gram-negative bacteria that can be motile. There are over 2500 Salmonella serotypes identified (CDC, 2009). Among them, *Salmonella* serotype Typhimurium and serotype Enteriditis are the most common outbreak-causing serovars in the U.S. In healthy populations, infection with *Salmonella* spp. causes diarrhea, fever and abdominal cramps. However, in elderly and infants, the bacteria can spread and even cause death if left untreated (CDC, 2012). Food products associated with human *Salmonella* spp. infections are diverse, ranging from eggs and poultry to pork and peanut butter.

The culture-based method for detection and isolation of *Salmonella* spp. in food is described in the ISO standard 6579:2002 (ISO, 2002), and requires up to five days for isolation and confirmation, with sensitivity as low as 1 CFU per 25 g of food analyzed. This level of sensitivity should also be achieved by rapid detection methods developed for *Salmonella* spp. (Marlony et al., 2011).

In general, immunologically-based methods target surface antigens, lipopolysaccharides, flagella, or fimbriae. Environmental conditions such as food matrix or enrichment methodology can affect antibody-specific antigen expression. Therefore, expression of these target antigens on

cells is critical to obtain false negatives. (Banada and Bhunia, 2008; Goodridge et al., 2011; Hahm and Bhunia, 2006; Marlony et al., 2011).

Currently, PCR analysis has been carried out with more than 30 *Salmonella*-specific genes (Levin, 2009a; Malorny et al., 2004). Several commercially-available rapid assays are available for the detection of *Salmonella* spp. in a variety of food matrices. Table 3 lists these methods to facilitate comparison.

***Shiga toxin-producing Escherichia coli* (STEC)**

Escherichia coli are a diverse group of Gram-negative and rod-shaped bacterium. Most strains of *E. coli* are harmless, but others are capable of producing the so-called Shiga toxins and are known as Shiga toxin-producing *E. coli* or STEC.

Domestic ruminants are recognized as the main reservoir for STEC. Therefore, human infections occurs primarily due to consumption of contaminated raw or undercooked beef, as well as raw milk and dairy products (De Boer and Heuvelink, 2000). This pathogen is responsible for severe gastrointestinal disorders such as hemorrhagic colitis (HC), and hemolytic uremic syndrome (HUS) (Karmali et al, 2010). Most cases of HC and HUS are caused by STEC strains of serotype O157:H7/H-. In recent years other STEC strains, known as non-O157 STEC (O26:H11/H-, O91:H21/H-, O103:H2, O111:H-, O113:H21, O121:H19, O128:H2/H, and O145:H28/H) have been also associated with HC and HUS (Karch et al., 2005).

Rapid immunological techniques are an alternative to traditional methods, reducing detection times of STEC in food samples. Some of these methods include latex agglutination test, ELISA, direct immunofluorescence and immunocapture techniques, among others.

Immunoassays rely on the detection of the O157 antigen allowing the screening of enrichment cultures for *E. coli* O157:H7 (De Boer and Heuvelink, 2000). A variety of commercial immunological-based methods such as ELISA and latex agglutination kits are available. The ELISA assay allows the simultaneous detection of different toxin sub-types, and uses specific antibodies that target the Shiga toxins or ganglioside Gb3 receptors in the enrichment broth. The formed antibody-target complex is then detected by secondary labeled antibodies (Stephan et al., 2011). Since immunological-based methods may exhibit some antibody cross-reactions with bacteria such as *Escherichia hermannii, Brucella abortus, Brucella melitensis, Yersinia enterocolitica* O:9*, Klebsiella* spp., and *Pseudomonas* spp. (Stephan et al., 2011), further characterization of suspected samples is necessary to avoid false positives.

Nucleic acid methods are often employed to confirm and further identify the presence of a suspected STEC. All commercial nucleic acid methods (ex. PCR) have at least one enrichment step of several hours to increase target microorganism numbers preceding the actual PCR procedure. Several commercially-available rapid assays have been developed for detection of STEC in a variety of food matrices. These methods are listed and characterized to facilitate comparison in Table 4.

Discussion

The implementation of Hazard Analysis and Critical Control Points (HACCP) systems requires food processors to test end product and environments as a way of verifying food safety during validation of the system. As such, microbiological testing remains a critical tool in process control monitoring, quality control, and surveillance. Although numerous rapid detection methods exist, conventional methods are still the first choice for the enumeration of pathogens,

indicator, and spoilage organisms (Beumer and Hazeleger, 2009). In the food industry, a robust sampling technique, as part of a continuously reviewed pathogen environmental monitoring program, is essential before the selection of any rapid detection kit.

A direct comparison between detection methods is difficult to achieve, mainly due to lack of performance measures provided by manufacturers of commercial rapid methods. Ideally, a direct comparison of time and performance measures, such as sensitivity and specificity, would provide a clear idea of false positives and false negatives expected from a specific rapid method. Additionally, some manufacturers report inclusivity and exclusivity as alternative performance measures, making a direct comparison of methodologies even more challenging.

A systematic comparison platform of rapid methods is expected from approval of third party organizations such as AOAC, but the validation process is oriented only to validate the claims the manufacturer makes about the product. This fact often obligates one to perform further on-site validation and optimization of rapid methods. This approach is especially true for immunological- and nucleic acid-based methods in which the food matrix has an important influence on the results.

The cost of the equipment is another important factor that often limits the use of rapid methods. An example of how manufacturers overcome this problem is provided by the GeneSeek Company (a Neogen Company, Lansing, MI USA). This company offers the service of sample analysis instead of selling the equipment, advertising a ñnext-dayò DNA-specific test result for seven pathogenic *E. coli* strains. Another example is from the DuPont Company which offers leasing options of their BAX® system to small and very small food processors, since the method is only suitable for large number of samples.

Although most of these rapid methods help to identify pathogens in a shorter timeframe, the enrichment procedure is still the most challenging bottleneck in the process of determining the presence or absence of foodborne pathogens.

As such, future research should focus on the development of better alternatives to speed the enrichment process. So far, magnetic immunoseparation remains a valuable tool to concentrate selected target microorganisms. Another innovative alternative is to use phages to eliminate undesirable microflora, increasing the probability of a positive detection (SDIX, 2011c, d). The finding of an alternative method or an optimal combination of methods that not only increases the probability of accurate detection, but also shortens the time of enrichment in a cost effective way, will likely improve the field of rapid methods and therefore, food safety in general.

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Table 1. List of commercially-available assays for the detection of *Staphylococcus aureus* and/or enterotoxins.

Method	Target	Company	Certification	Food matrices	Enrichment time (h)	Detection Time (h)	Total time (h)	Performance measure	Detection level	Reference
Immunologically-based methods										
TRANSIAÊ PLATE *Staphylococcal Enterotoxins Plus*	*Staphylo-coccal enterotox in A, B, C1, C2, C3, D and E*	BioControl	NR	Milk and dairy products	NR	2	NR	NR	0.25 ng enterotoxins/gram sample	(BioControl, 2011f)
3MÊ TecraÊ *Staph. aureus* Visual	*S. aureus*	3M	NR	NR	20 - 28	2	22-30	NR	1-5 CFU/3g	(3M, 2011b, c)

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Immunoassays

TRANSIAÊ PLATE *Staphylococcal Enterotoxins ID*	*Staphylo-coccal enterotox in A, B, C, D and E*	BioControl	NR	Milk and dairy products, meat, poultry, eggs, seafood	NR	2	NR	NR	20-60 pg/mL of each serological group (A-E)	(BioControl, 2011f, i)
Nucleic acid-based assays										
GENE-TRAK ®	*S. aureus*	Neogen®	AOAC	NR	NR	NR		NR	3 CFU/g	(Neogen, 2011a)
BAX® System *Staphylococcus aureus*	*S. aureus*	DuPontÊ	AOAC	Powdered infant formula (presence/ absence) ground beef	22-24 (Infant formula); 20-22 (Ground beef); 44-	1.5	23.5-25.5 (Infant formula) ; 21.5-23.5	SE=Equal or better than BAM* methods SP=Ó99%	1 CFU/g (Infant formula) 10 CFU/g (ground beef and	(DuPont, 2011f, h)

	and soy protein isolate (threshold testing)	48 (Soy protein Isolate)	(Ground beef); 45.5-49.5 (Soy protein isolate)	soy protein isolate)

SE=Sensitivity, SP=Specificity, NR=Not Reported. *Bacteriological Analytical Manual (BAM)

AOAC =Association of Analytical Communities AFNOR= Association Française de Normalisation.

Table 2. List of commercially-available assays for the detection of *Listeria monocytogenes.*

Method	Company	Certification	Food matrices	Enrichment time (h)	Detection Time (h)	Total time (h)	Performance measure	Detection level	Reference
Immunologically-based methods									
Reveal® for *L. monocytogenes*	Neogen®	AOAC	NR	30-48	2	32-50	NR	1-5 CFU/25 g	(Neogen, 2011d, g)
Nucleic acid-based methods									
BAX® System *L. monocytogenes* 24E	DuPontÊ	AFNOR AOAC	Dairy, meat, fish, vegetables, environment	24-28	3.5	27.5-31.5	NR	10^4 CFU/mL	(DuPont, 2011c)
GeneQuence	Neogen®	AOAC	Deli turkey, deli ham, deli roast beef, hot dogs, raw ground beef, raw ground pork,	42-46	1.9	43.9-47.9	NR	NR	(Neogen, 2011b)

			smoked salmon, cooked crab meat, raw shrimp, ice cream, pasteurized milk, brie cheese, parmesan cheese, lettuce, frozen peas, soy flour						
BAX® System *L. monocytogenes*	DuPontÊ	AOAC	Variety of foods	48	3.5	51.5	NR	10^5 CFU/mL	(DuPont, 2011b)
TRANSIAÊ PLATE *L. monocytogenes*	BioControl	AFNOR	Dairy, environment, meat, seafood, vegetable	NR	NR	46	NR	NR	(BioControl, 2011g)

Assurance GDSÊ for *L. monocytogenes*	BioControl	AOAC	NR	NR	NR	NR	NR	NR	(BioControl, 2011e)

SE=Sensitivity, SP=Specificity, NR=Not Reported

AOAC =Association of Analytical Communities AFNOR= Association Française de Normalisation.

Table 3. List of commercially-available assays for the detection of *Salmonella* spp.

Method	Company	Certification	Food matrices	Enrichment time (h)	Detection time (h)	Total time (h)	Performance measure	Detection level	Reference
Immunologically-based methods									
3MÊ TecraÊ Unique Salmonella	3M	AFNOR	Ground black pepper, soy flour, dried whole eggs, chocolate milk, non-fat dry milk, raw deboned turkey	16-20	5-6.5	21-26.5	NR	1-5 CFU/3g	(3M, 2011f, g)
3MÊ TecraÊ Salmonella Visual Immunoassay	3M	AOAC	NR	NR	NR	NR	NR	NR	(3M, 2011a, e)
RapidChek® Salmonella	SDIX	AOAC	Raw meat, poultry, deli meats, orange	24	0.17 hours	24.17	NR	NR	(SDIX, 2011b, c)

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Method	Company	Certification	Food matrices	Enrichment time (h)	Detection time (h)	Total time (h)	Performance measure	Detection level	Reference
			juice, cheese, liquid eggs, milk						
TRANSIAÊ PLATE Salmonella Gold	BioControl	AFNOR AOAC	Animal feed, dairy products, egg products, environmental samples, meat, pastries, RTE meals, seafood, vegetables	NR	NR	24 with TAG 24 supplement	NR	NR	(BioControl, 2011h, j)
RapidChek® SELECT Ê Salmonella	NR	AFNOR AOAC	Deli meats, liquid eggs, raw chicken, carcass rinsates, ground beef,	Enrichment 1 (16-22); enrichment 2 (16-22)	0.17	32.17 - 44.17	NR	NR	(SDIX, 2011d, e)

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Method	Company	Certification	Food matrices	Enrichment time (h)	Detection time (h)	Total time (h)	Performance measure	Detection level	Reference
			environmental samples						
Reveal® 2.0	Neogen®	AOAC	Chicken carcass rinse, raw ground turkey, raw ground beef, hot dogs, raw shrimp, RTE meals, dry pet food, ice cream, fresh spinach, cantaloupe, peanut butter, environmental swabs	NR	0.25		NR	1 CFU/analytical Unit; 10^6 CFU/mL post enrichment	(Neogen, 2011e)

Method	Company	Certification	Food matrices	Enrichment time (h)	Detection time (h)	Total time (h)	Performance measure	Detection level	Reference
			from stainless steel surfaces, sprout irrigation water						
Nucleic acid-based methods									
GeneQuence®	Neogen®	NR	Raw whole egg, dried whole egg, nonfat dry milk, chocolate, refrigerated dough, soy flour, egg noodles, cheese powder, cake mix, walnuts, food dye,	24-48	2	26-50	NR	1-5 CFU/25 g	(Neogen, 2011c)

Method	Company	Certification	Food matrices	Enrichment time (h)	Detection time (h)	Total time (h)	Performance measure	Detection level	Reference
			black pepper, dried fruit, frozen fruit, mushrooms, surimi, raw ground beef, raw pork, raw fish, raw turkey, raw chicken, beef franks, dry pet food, peanut butter						
BAX® System Salmonella	DuPont Ê	AOAC AFNOR	Variety of foods	8*-24 * MP media	3.5	11.5-27.5	Ó98% Ó98%	10^5 CFU/mL	(DuPont, 2011d, g)
Assurance GDSÊ	BioContr	AOAC	NR	NR	NR		NR	NR	(BioContro

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Method	Company	Certification	Food matrices	Enrichment time (h)	Detection time (h)	Total time (h)	Performance measure	Detection level	Reference
for *Salmonella*	ol	AFNOR							l, 2011b)
Biosensors									
RAPID-BÊ	Vivione Biosciences	NR	NR	0	6.5	6.5	NR	1 CFU/ g	(Vivione-Bioscience s, 2012)

NR=Not Reported

AOAC =Association of Analytical Communities AFNOR= Association Française de Normalisation.

Table 4. List of commercially-available assays for the detection of STEC, including *Escherichia coli* O157:H7.

Method	Manufacturer	Certification	Food matrices	Enrichment time (h)	Detection Time (h)	Total time (h)	Performance measure	Detection level	Reference
Immunologically-based methods									
Reveal® for *E. coli* O157:H7	Neogen®	AOAC	NR	8-20	0.25	8.25-20.25	NR	1 CFU/25 g 1 CFU/375 g (raw beef products)	(Neogen, 2011f)
Assurance® EIA EHEC	BioControl	AOAC	Apple cider, beef trim, cheese, cooked ground beef, cooked	NR	NR	10 for raw beef samples, 20 other foods	NR	NR	(BioControl, 2011a, d)

Method	Manufacturer	Certification	Food matrices	Enrichment time (h)	Detection Time (h)	Total time (h)	Performance measure	Detection level	Reference
			ground poultry, cooked ham, ice cream, liquid egg, liquid infant formula, liquid milk, nuts, pasta, raw ground beef, raw ground lamb, raw						

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Method	Manufacturer	Certification	Food matrices	Enrichment time (h)	Detection Time (h)	Total time (h)	Performance measure	Detection level	Reference
			ground pork, raw ground poultry, raw milk, surimi						
RapidChek *E. coli* O157 (including H7)	SDIX	AOAC	Boneless beef trim, ground beef	8 - 18 (depending on sample size)	0.17	8.17- 18.17	NR	NR	(SDIX, 2011a)
3MÊ TecraÊ *E. coli* O157 Visual Immunoassays	3M	NR	NR	18-24	1.25	19.25-25.25	NR	NR	(3M, 2011d)
FoodChek *E.*	FoodChek	AOAC	Raw ground	6 for trim ï	0.5	< 8	NR	NR	(FoodChek,

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Method	Manufacturer	Certification	Food matrices	Enrichment time (h)	Detection Time (h)	Total time (h)	Performance measure	Detection level	Reference
coli O157 test kit	Systems Inc.		beef, N60 trim and carcass swab	7 for ground beef					2013)
Nucleic acid-based methods									
BAX® System *E. coli* O157:H7 real-time	DuPontÊ	AOAC AFNOR	Raw ground beef, beef trim, spinach, lettuce	9 (65 g ground beef); 10 (275 g beef trim); 8 (25 g spinach and lettuce)	0.92	9.92 (ground beef);10.92; (beef trim); 8.92 (spinach and lettuce)	NR	10^4 CFU/mL	(DuPont, 2011e)
BAX® System *E. coli*	DuPontÊ	AOAC AFNOR	Meat, fruits vegetable,	8*-24 * express	3.5	11.5-27.5	NR Ó99%	10^4 CFU/mL	(DuPont, 2011e)

Method	Manufacturer	Certification	Food matrices	Enrichment time (h)	Detection Time (h)	Total time (h)	Performance measure	Detection level	Reference
O157:H7 MP			animal feed, dairy and environment	media					
BAX® System *E. coli* O157:H7	DuPontÊ	AOAC	Raw ground beef, beef trim	8*-24 *immuno-magnetic separation + MP media	3.5	11.5-27.5	NR	10^5 CFU/mL	(DuPont, 2011a)
Assurance GDSÊ for *E. coli* 0157:H7	BioControl	AOAC	NR	NR	N R	NR	NR	NR	(BioControl, 2011c)
Biosensors									
RAPID-BÊ	Vivione Biosciences	NR	NR	0	6.5	6.5	NR	1 CFU/ g	(Vivione-Biosciences,

Method	Manufacturer	Certification	Food matrices	Enrichment time (h)	Detection Time (h)	Total time (h)	Performance measure	Detection level	Reference
									2012)

NR=Not Reported

AOAC =Association of Analytical Communities; AFNOR= Association Française de Normalisation

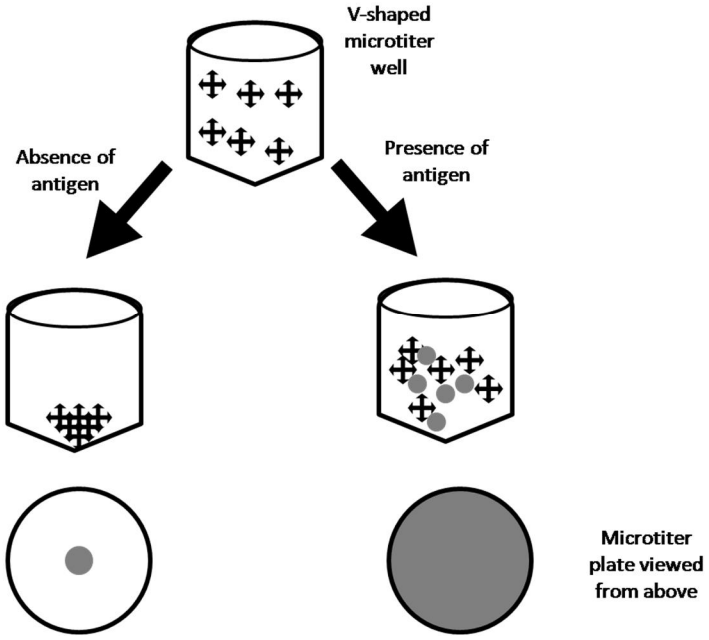

Figure 1. Diagram of reverse passive latex agglutination.

Adapted from (Forsythe, 2010).

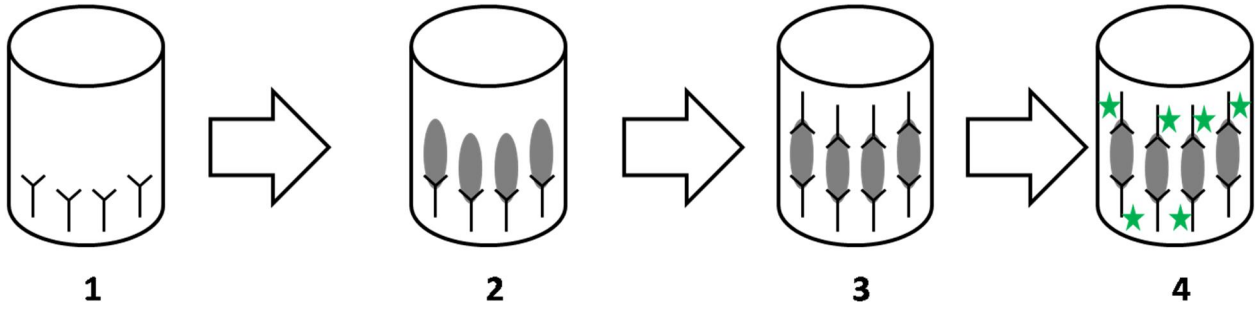

Figure 2. Simplified diagram of an immunologically-based method, ELISA technology (TECRA®).

1) Antibodies specific for the target pathogen are fixed on the surface of the plastic wells; 2) Antibodies capture the target pathogen antigens if present, and all un-bound material in the sample is removed; 3) Added enzyme-conjugated antibodies specific for the target pathogen completes the ñsandwichò; 4) A colorimetric or fluorescent substrate specific for the enzyme is added, and the amount of product formed over time can be used to quantify the amount of target bacteria present in the initial sample.

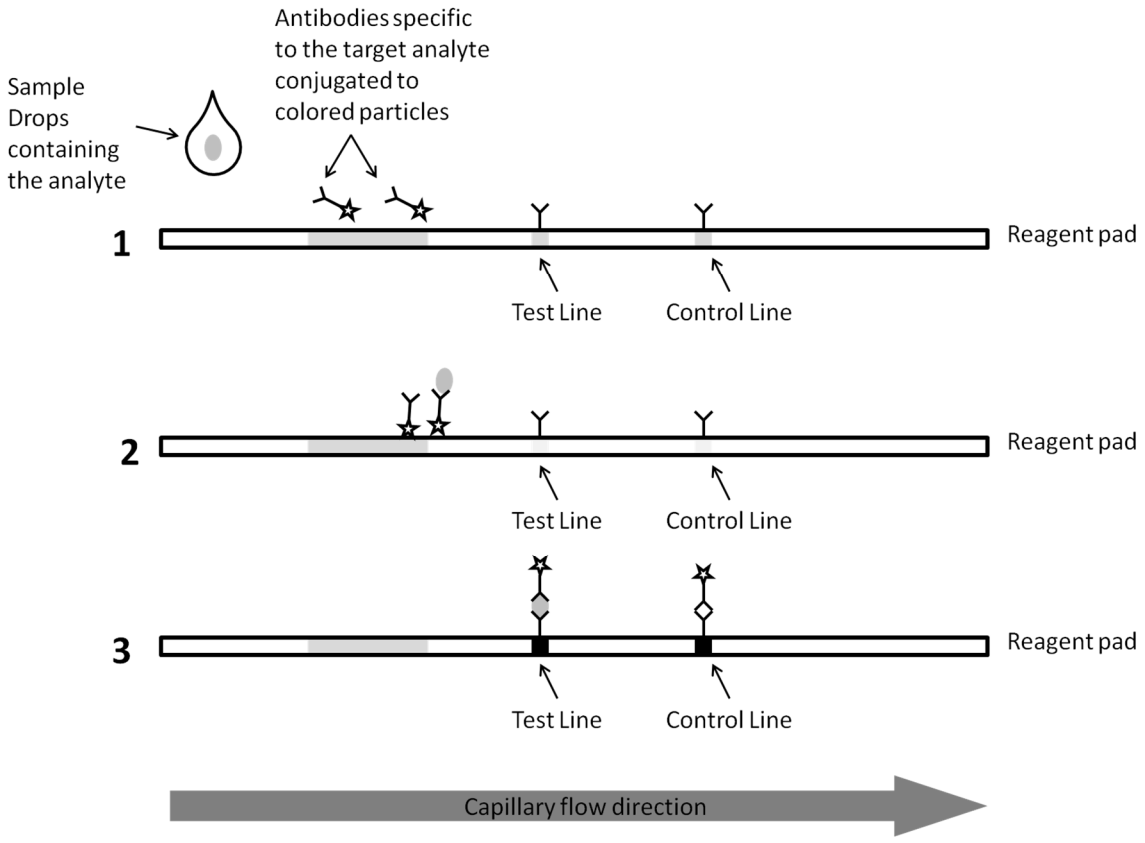

Figure 3. Simplified diagram of the lateral flow assay.

(1) Enriched sample is deposited in the reagent pad; (2) Migration thorough the reagent pad and attachment to conjugated antibodies; (3) two visible (analyte and control) lines represent a positive result.

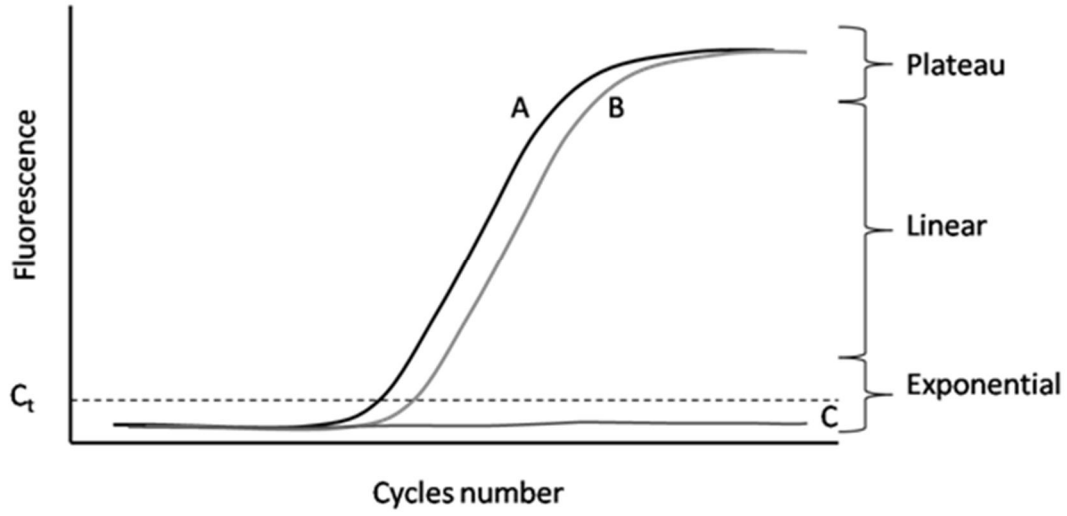

Figure 4. Simplified scheme of a PCR curve.

Curve A has a higher amount of template, as compared to curve B. Curve C is a negative control (DNA absent).