Review of *Salmonella* detection and identification methods: Aspects of rapid emergency response and food safety

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**A B S T R A C T**

*Salmonella* has been recognized as a major and important foodborne pathogen for humans and animals over more than a century, causing human foodborne illness as well as high medical and economical cost. Accordingly, the effort to develop efficient and reliable *Salmonella* detection methods continues. This paper reviews and describes the development and application of commercially available *Salmonella* detection methods. These are categorized into several groups based on the principle applied: conventional culture methods, immunology-based assays, nucleic acid-based assays, miniaturized biochemical assays, and biosensors. Conventional culture methods serve as the basis in food testing laboratories despite rather laborious and time-consuming protocols. Considerable progress in rapid methods using emerging technologies yield faster answers and higher throughput of samples. This paper also shows and analyzes *Salmonella* test results and summarizes the features and limitations of the studies involving *Salmonella* detection methods developed for emergency response, mainly by food emergency response laboratories participating during recent fiscal years. The emergency response laboratories utilize *Salmonella* detection methods possessing properties that include simplicity, versatility, high sensitivity, good specificity, and cost efficiency. Collaboration of the food emergency response laboratories in the development of these technologies is important essentially to compare for the purpose of continually improving *Salmonella* detection methods.

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1. Introduction

Salmonella bacteria represent the most common and primary cause of food poisoning in many countries for at least over 100 years (Alakomi & Saarela, 2009; Chalker & Blaser, 1988; Coburn, Grassl, & Finlay, 2007). Despite well-established instructions and measures for preventing salmonellosis (Salmonella food poisoning), the incidence and severity of human salmonellosis have significantly increased. Most salmonellosis cases are self-limiting (approximately 80%), but large outbreaks caused in schools, hospitals, and restaurants are not very common (Guiney et al., 1995). At present, over 2500 serotypes of Salmonella have been reported (Fierer & Guiney, 2001). Of these, the most common serotypes associated with human illness are Salmonella enterica serovar typhimurium (S. Typhimurium) and S. enterica serovar Enteritidis (S. Enteritidis) in the United States and European countries.

Approximately, 1.4 million human Salmonella infections occur annually in the United States with assumption of only 2% cases reported to Center for Disease Control and Prevention (CDC), resulting in about 16,000 hospitalizations with nearly 600 deaths (Cummings et al., 2010; Mead et al., 1999; Turner, 2010). The estimated total cost associated with Salmonella incidences may be up to several billion dollars annually (Frenzen et al., 1999; WHO, 2005). An estimated annual cost for a Salmonella control program has increased in some countries (WHO, 2005). Much higher incidences related to Salmonella may occur in some developing countries where relevant data is not readily available.

Salmonella is not considered to be fatal to healthy people or as a bioweapon agent. However, efforts have been made to develop and improve detection technologies for this organism because Salmonella may cause devastating foodborne illness. Salmonella detection methods are desirable to have sensitivity enough to detect one cell in a defined sample. The analysis time of conventional and rapid methods can vary with cell enrichment steps to reach minimal cell concentration enough for Salmonella detection. The cell enrichment process is typically lengthy in a conventional method whereas the rapid detection method generally requires at least $10^{12}$ cells $m^{-1}$ of Salmonella concentration for detection.

Salmonella surveillance and monitoring should be based on reliable and efficient detection methods, which should help improve the food safety (Rodríguez-Lázaro et al., 2007). It is essential that surveillance and monitoring should cover the entire food chain, preferably starting from investigation of feed and feed ingredients for Salmonella contamination (Alakomi & Saarela, 2009; Mead et al., 2010). Standardization, regulation, and international surveillance networks are also necessary to effectively prevent and control Salmonella pathogens. International Standards for the Salmonella tests such as ISO 6579:2002 (Amd 1:2007 Annex D) and the Nordic Committee on Food Analysis (NMKL) contain information about sample storage, sampling, and other critical steps in Salmonella analysis (Feldsine et al., 2003; Löfström, Hansen, & Hoofar, 2010; NordVal, 2009). Such standards are critical to ensure that the results are consistent and comparable among laboratories. Several countries have built regulations and guidelines, such as Regulation (EC) No 2160/2003, the EU Zoonoses Monitoring Directive (2003/99/EC), and the Food and Drug Administration (FDA) Food Code (EC, 2003a, 2003b; FDA, 2009). These regulations and guidelines provide or require information on Salmonella and control methods as well as help develop the food safety rules and regulatory policies, in order to reduce Salmonella pathogens at any stage of feed and food production (de Jong & Ekdahl, 2006; Tietjen & Pug, 1995).

Bioterror events, such as anthrax spores in contaminated letters, have greatly altered the view of the public and the scientific community about bacterial pathogens which could be used as weapons of biological terrorism, and about the needs and requirements of the biotechnology for detection of potential bioterrorism-employed pathogens (Cannons, Amuso, & Anderson, 2006). Salmonella spp. is one of such bacterial pathogen which has been purposely spread mainly using food as a carrier (Ashford et al., 2003; Török et al., 1997). For example, S. Typhimurium was used to infect residents by contaminating salad bars at the local restaurants in Oregon. The United States capability to respond to a bioterrorism event has been enhanced by integrated laboratory networks including the Food Emergency Response Network (FERN) and Laboratory Response Network (LRN) of Centers for Disease Control and Prevention (CDC) as well as to emerging infectious diseases.

The purpose of this paper is to review Salmonella detection methods including detection level, sensitivity, specificity, and sample matrices. Included in the discussion are a summary of Salmonella testing results and information from food emergency response laboratories available in a web-based database portal for recent fiscal years.

2. Conventional Salmonella detection methods

Traditional isolation of Salmonella spp. involves a nonselective pre-enrichment of a defined weight or volume of the sample, followed by a selective enrichment step, platting onto selective agars, and biochemical and serological confirmation of suspect colonies (Fig. 1). Different approaches of Salmonella enrichment using the unique biochemical physical properties of the organisms have been standardized by several regulatory agencies, such as International Organization for Standardization (ISO), Association of Official Analytical Chemists (AOAC), FDA, and Food Safety and Inspection Service (FSIS) of USDA. The current ISO horizontal method, ISO 6579:2002, consists of a pre-enrichment of samples in buffered peptone water (BPW) followed by a selective enrichment in Rappaport–Vassiliadis (soya base) (RVS) and Muller-Kaufmann Tetrathionate-Novobiocin (MKTTn) (ISO, 2002). Standard methods published for detection of Salmonella by other regulatory agencies are essentially similar to ISO 6579:2002. The conventional cultural
methods for *Salmonella* recovery basically include the following essential steps.

Pre-enrichment uses a nutritious nonselective medium to recover sublethally injured *Salmonella* cells while inhibiting the growth of competing flora (Tietjen & Fung, 1995). Most commonly used media in pre-enrichment step are BPW and lactose broth. The pre-enrichment process and formulas have been tested to increase the sensitivity of *Salmonella* detection. Antimicrobial resistant *Salmonella* spp. could be highly recovered by using appropriate antibiotics in pre-enrichment and selective media (Carrique-Mas & Davies, 2008). The incubated pre-enrichment media are inoculated into selective media containing two or more inhibitory agents such as bile salts, brilliant green, thiosulfate, deoxycholate, malachite green, novobiocin, tetrathionate, cycloheximide, nitrofurantoin, and sulphanilamide (Arroyo & Arroyo, 1995; Ha, Pillai, & Ricke, 1995; Ricke, Pillai, Norton, Maciorowski, & Jones, 1998). The use of inhibitors in a selective media allows continuous growth of *Salmonella* while suppressing the propagation of other bacteria (Tietjen & Fung, 1995). Rappaport–Vassiliadis (RV) medium and tetrathionate (TT) broth has been used as official *Salmonella* enrichment media in approved standard methods such as FDA Bacteriological Analytical Manual (BAM) and FERN *Salmonella* methods. Enrichment media has been modified to enhance *Salmonella* growth and thus to increase the sensitivity and selectivity.

The selective enrichment media containing *Salmonella* at the level of above 10^8 cells ml^-1 are streaked on solid selective media to isolate presumptive positive *Salmonella* colonies, simultaneously inhibiting the growth of other bacteria (Carrique-Mas & Davies, 2008; Ruiz et al., 1996; Tietjen & Fung, 1995). Commonly used plating media include Salmonella-Shigella agar (SS), brilliant green agar (BGA), bismuth-sulfite agar (BSA), Hektoen enteric (HE), and xylose-lysine-deoxycholate agar (XLD). The colonies of different *Salmonella* serotypes are differentiated by colors with the coliforms on these media (Mallinson et al., 2000; Ruiz et al., 1996). For example, *S. Typhi* on SS may appear as colorless colonies with black center. Lactose-fermenting *S. Arizona* serotype producing light pink-red halo around colonies can be also identified on XLD, but other lactose-fermenting serotypes such as *S. Montevideo* and *S. Virchow* may not be detected on the same agar medium. However, some serotypes are not distinctive and even missed on those media, yielding false negatives and increasing cost for additional tests (Carrique-Mas & Davies, 2008; Gruenewald, Henderson, & Yappow, 1991; Manafi, 2000; Reid, Porter, & Ball, 1993; Ruiz et al., 1996). The recovery and selectivity of *Salmonella* may be further enhanced by using two or more selective media or adding appropriate supplements to plating media such as novobiocin, malachite green, thiosulphate, and sulphanethazine (Arroyo & Arroyo, 1995; Carrique-Mas & Davies, 2008; Kang & Fung, 2000; Tietjen & Fung, 1995).

Presumptive *Salmonella* colonies isolated on plating media are typically incubated in triple sugar iron agar (TSI) and lysine iron agar (LIA) followed by urease test and additional tests for urease-negative cultures. TSI and LIA media use glucose-fermentation and lysine decarboxylase reactions for screening *Salmonella* spp., respectively. The cultures giving typical reactions for *Salmonella* are submitted for biochemical and serological identification tests. Serotyping identifies the serogroups of the cultures by antigenic analysis using agglutination reaction subject to antigenic structure. However, the same *Salmonella* serotype can vary with antigenicity due to change and loss of the surface antigens, reducing the sensitivity of serological methods (Hoorfar, Aherns, & Rådström, 1999; Sørensen, Alban, Nielsen, & Dahl, 2004). If *Salmonella* isolates cannot be serotyped under certain conditions, PFGE characterization might be useful in place of serological assays.

The conventional microbiological methods serve as the basis for analysis in many food safety and public health laboratories due to the ease of use, reliability of results, high sensitivity and specificity, and lower cost compared to emerging molecular-based technologies (Gracias & McKillip, 2004; Maciorowski, Herrera, Jones, Pillai, & Ricke, 2006). However, these procedures need to prepare multiple subcultures required for several identification steps, taking more than 5 days for complete isolation and confirmation. In addition, false positive results may occur due to competitive flora (e.g. *proteus*) (Naravneni & Jamil, 2005; Swaminathan & Feng, 1994). Under circumstances in which high throughput screening is required for a large number of samples, the laborious and time-consuming culture-based techniques may not properly address such a requirement.

The conventional methods have been improved to reduce cost and labor and to offer faster detection and identification of *Salmonella*. For example, chromogenic and fluorogenic growth media (e.g. SM-ID agar, Rambach agar, and BBL CHROMagar *Salmonella*) used for detection, enumeration, and identification directly on the isolation plate have shown to be convenient, reliable, and more specific and selective than conventional media (Alakomi & Saarela, 2009; Maciorowski et al., 2006; Manafi, 2000; Perry & Freydière, 2007). The test result using these selective media is typically available 1 day earlier than conventional methods but is not fast enough to respond to bioterrorism events, *Salmonella* outbreak, and product recall.

### 3. Rapid *Salmonella* detection methods

Several rapid methods using molecular cloning and recombinant DNA have been developed, validated, and are available on the market. These accelerated *Salmonella* detection procedures enable quick answers, reduce storage space of conventional supplies and samples, and increase the throughput of samples (Alakomi & Saarela, 2009; Blivet, Soumet, Ermel, & Colin, 1998; Tietjen &
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<th>Method and Format</th>
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media, modi can be divided into several categories including new selective media, dilution, centrifugation, 

Comi, results within a few hours to a day (Ferretti, Mannazzu, Cocolin, & Comi, 2001; Tietjen & Fung, 1995).

Commercially available rapid methods for Salmonella detection can be divided into several categories including new selective media, modified or adapted conventional procedures, immunology-based assays, and nucleic acid–based assays (Alakomi & Saarela, 2009; Blivet et al., 1998; Eijkkelkamp, Aarts, & van der Fels-Klerx, 2009; Iqbal et al., 2000; Tietjen & Fung, 1995) (Table 1). Of these methods, ELISA and PCR procedures show comparable specificity and sensitivity to conventional methods. ELISA assays are able to detect Salmonella concentration at the level of 10^5–10^7 ml^-1 while PCR-based assays provide the level of sensitivity of 10^4 ml^-1 after enrichment. The sensitivity and specificity of these methods largely depend on the background microflora, sample matrix, presence of non-culturable cells, and inhibitory substances (e.g., fats, proteins, polysaccharides, heavy metals, antibiotics, and organic compounds) (Alakomi & Saarela, 2009; Mozola, 2006; Naravani & Jamil, 2005). It has been reported that the sensitivity and detection limits of the methods were improved by additional sample preparation and purification techniques such as modification of pre-enrichment and enrichment media, dilution, centrifugation, filtration, flow injection, chromatography, organic solvent extract, and fluorescence hybridization (Kutter, Hartmann, & Schmid, 2006; Mozola, 2006; Polaczyk et al., 2008; Wolfs, Glencross, Thibaudeau, & Griffiths, 2006).

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<td>Vitek 2</td>
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* ELISA, enzyme-linked immunoabsorbent assay; LA, latex agglutination; IMS, immunomagnetic separation; ECL, electrochemiluminescence, PCR, Polymerase chain reaction.

Fung, 1995). The rapid method may be defined as one which allows the detection of Salmonella spp. in samples and delivers reliable results within a few hours to a day (Ferretti, Mannazzu, Cocolin, Comi, & Clementi, 2001; Tietjen & Fung, 1995).

Of immunology-based assays, ELISA is the most commonly used assay for the detection of antigens or products of Salmonella spp. The different ELISA systems have been developed and commercially available in kit form. In the ELISA assay, an antigen specific to Salmonella spp. is bound to the appropriate antibody linked to a solid matrix. After forming the antigen–antibody complex, the concentration of the antigen and the presence of Salmonella can be measured through the change in color caused by the enzymatic cleavage of a chromogenic substrate (Blivet et al., 1998; Tietjen & Fung, 1995). Alternatively, the presence of antibodies in samples infected with Salmonella spp. can be detected using antibodies coupled to the solid phase of ELISA (Wiuuff et al., 2000). ELISAs have also been used to detect antibodies for development of vaccines against Salmonella infections (Meneaksi et al., 1999). Several commercial validated ELISA systems designed for rapid detection of Salmonella in raw or processed products are available: Assurance GDS™ for Salmonella (BioControl Systems, Inc., Bellevue, WA), TECRA Salmonella (Tecra International Pty Ltd, French Forest, New South Wales, Australia), Salmonella ELISA Test SELECTA/OPTIMA (Bioline APS, Denmark), and Vitek Immuno Diagnostic Assay System (VIDAS) (bioMérieux, Hazelwood, MO) (Table 1). The agglutination technique employs latex particles coated with antibodies which react with antigens on the surface of Salmonella cells to form visible aggregates for identification of Salmonella positive samples (Thorns, McLaren, & Sojka, 1994; Tietjen & Fung, 1995). The assays are specific, uncomplicated, and reliable so that generally, they have been used as a confirmatory analysis technique, rather screening test for Salmonella organisms (Eijkkelkamp et al., 2009; Love & Sobsey, 2007). There are several commercial kits available on the market (Table 1).

3.1.1. Enzyme-linked immunoabsorbent assay (ELISA)

Table 1 (continued)

3.1.2. Latex agglutination assay

The agglutination technique employs latex particles coated with antibodies which react with antigens on the surface of Salmonella cells to form visible aggregates for identification of Salmonella positive samples (Thorns et al., 1994; Tietjen & Fung, 1995). The assays are specific, uncomplicated, and reliable so that generally, they have been used as a confirmatory analysis technique, rather screening test for Salmonella organisms (Eijkkelkamp et al., 2009; Love & Sobsey, 2007). There are several commercial kits available on the market, including Spectra (May & Baker Diagnostics Ltd., Glasgow, Scotland, UK), Wellcolox color Salmonella (Wellcolox, Merseyside, UK), Salmonella Latex test (Oxoid, Basingstoke, UK), Bactigen (Wampole Laboratories, Cranbury, NJ), and SlideX (Bio-Mérieux, Marcy L’Etoile, France).

3.1.3. Immunodiffusion assays

The Salmonella 1-2 Test system (BioControl Systems, Inc., Bothell, WA) for detection of motile Salmonella is a commercial kit that operates on the basis of immunodiffusion reaction (D’Aoust & Sewell, 1988; Flowers & Kllat, 1989; Nath, Neidert, & Randall, 1989; Tietjen & Fung, 1995). Before inoculation into the system unit which consists of two connected chambers, the sample is pre-enriched for 24 h. The enriched sample is inoculated to a tetrahionate brilliant green broth in the inoculation chamber. Salmonella then moves out of the inoculation chamber into the mobility chamber in which antibody has been added onto a distal surface of a semisolid medium. Salmonella in the mobility chamber is immobilized by forming an antigen–antibody complex. After incubation for 14 h, the readable three-dimensional immunodiffusion band is produced. Modifications in an enrichment step before inoculation and an increase of incubation time improved the effectiveness of detection of Salmonella spp. (Nath et al., 1989). The
modified and original assays have shown to be equivalent with the reference culture method.

3.1.4. Immunochromatography (dipstick) assays

The commercial *Salmonella* test kits, based on the dipstick format, include the Tecra® *Salmonella* Unique® test (Tecra International Pty Ltd, French Forest, New South Wales, Australia) and the PATH-STIK (Celsis, Inc., Edison, NJ). The Tecra *Salmonella* Unique™ test which consists of the immunoenrichment and detection step is a screening procedure for *Salmonella* detection. The PATH-STIK *Salmonella* test is an immunological dipstick assay for one-step detection of *Salmonella*. The dipstick contains an assay control and all reagents used for *Salmonella* detection. Prior to the dipstick assay, a sample is pre-enriched and selectively enriched in media. The enriched sample is applied to the test unit and *Salmonella* in the sample is captured onto the dipstick. Unlike the Tecra® *Salmonella* Unique™ test, the PATH-STIK doesn’t include a washing step, requiring only 30 min for analysis (van Beurden, 1992; Brinkman, van Beurden, Mackintosh, & Beumer, 1995).

3.2. Nucleic acid-based assays

The nucleic acid-based assays are *Salmonella* detection tests that utilize a specific nucleic acid target sequence within the organism. The assays have been most intensively explored and developed for the past decade among *Salmonella* detection methods because they offer some advantages of sensitivity, specificity, and inclusivity over other methods, rapidly identifying *Salmonella* without obtaining pure cultures (Glynn et al., 2006; Mozola, 2006). Two major techniques of the assays are direct hybridization (DNA probe) and amplification (PCR) methods. The great progress of the assays allows the detection of very low numbers of organisms in the sample and high throughput of a large number of samples for routine analysis (Cohen et al., 1993; Mozola, 2006; Rasmussen, Rasmussen, Larsen, Hoff-Jorgensen, & Cano, 1994).

3.2.1. Polymerase chain reaction (PCR)

The PCR assay is the preferred non-cultural technique for *in vitro* primer-mediated enzymatic amplification of specific segments of DNA for the detection of *Salmonella* pathogens (Cocolin, Manzano, Cantoni, & Comi, 1998; McKillip & Drake, 2004; Wolcott, 1991). The PCR assay can exponentially amplify a single specific sequence to one million-fold of DNA segment within 2 or 3 h using a thermostable DNA polymerase. The amplified products can be detected by several means (e.g. gel-based systems and real-time PCR) (Eijkelkamp et al., 2009; Mozola, 2006). The development and advancement of the technique for amplifying specific segment of DNA improve the specificity and sensitivity enough for detecting even one molecule of target DNA in a defined sample. Due to the capability to detect such a low concentration of *Salmonella*, enrichment times are considerably shorter to reach sufficient concentration needed for reliable detection by the PCR compared to other assays. The equivalency was reported between the PCR assay and the standard culture methods in detecting *Salmonella* in food products (Aabo, Andersen, & Olsen, 1995; Cano, Rasmussen, Sanchez Fraga, & Palomares, 1993; Eyigor, Carli, & Unal, 2002; Stone, Oberst, Hays, McVey, & Chengappa, 1994). The PCR assays have been developed, validated, and standardized according to the general guidelines and requirements by ISO (IOS 20838:2006, ISO/DIS 22119, ISO 16140:2003, ISO 6579:2002, ISO 22174).

Commercial kits based on the PCR technology have been developed and successfully used for routine *Salmonella* screening in the industry and emergency response laboratories. The PCR-based commercial kits and systems with different specificity and sensitivity include ABI Prism 7500 (Applied Biosystems, Warrington, UK), Probelia (Sanofi –Diagnostics Pasteur, Marnes-la-Coquette, France), BAX system (DuPont Qualicon, Wilmington, DE), TagMan (PE-Applied Biosystems, Foster City, CA), Gene-Trak (Neogen Corporation, Lansing, MI), iQ-Check™ PCR (BioRad Laboratories, Hercules, CA), LightCycler (Roche Diagnostics, Manheim, Germany), and SmartCycler (Cepheid Inc., Sunnyvale, CA). The BAX system is the first commercially available PCR method using a single tablet combining all reagents (primers, enzyme, and deoxyribonucleotides) needed for PCR to rapidly amplify and detect *Salmonella* spp. (Bennett., Greenwood, Tennant, Banks, & Betts, 1998; Hoorfar et al., 1999; Maciorowski et al., 2006).

The real-time PCR (RT-PCR) system detects accumulated PCR product by monitoring the increase fluorescence signal using the integrated real-time thermocycler and fluorescence detector within a system. This helped overcome the problem of false positive results posed by amplicon contamination (Maurer, 2011). The false positive results can be further excluded by an internal standard used for ensuring the absence of inhibitors. The RT-PCR system employing automated DNA extraction, amplification, and detection has improved the ability of industry and emergency response laboratories in identifying *Salmonella* from a variety of sample matrices. The RT-PCR methods available on the market can be classified into several categories based on the principle and approach of the methods. Several fluorescent techniques have been developed and incorporated into the RT-PCR systems for simplicity and cost saving in detecting the target *Salmonella* sequences.

3.2.2. DNA probe hybridization assay

A DNA probe hybridization assay uses a DNA probe with a sequence complementary to the target sequence of a DNA or RNA molecule in the target organism (de Boer & Beumer, 1999; Fung, 2002; Mozola, 2006). In this assay, the target cells are first lysed and the produced nucleic acids are purified prior to hybridization with a DNA probe. The unbound probe to a target sequence is eliminated by washing. The stable hybrid with the labeled DNA can be detected using different detection techniques, such as radioisotopes and enzymatic reactions. The presumptive *Salmonella* results are typically confirmed by the culture methods.

The DNA probe hybridization assays have some advantages in specificity and sensitivity of identifying *Salmonella* in samples compared to other methods. The assays are effective to rapidly screen the large numbers of samples and highly specific to detect the target cells without cross-reactions with the other organism (Agron et al., 2001; de Boer & Beumer, 1999; Dunbar & Jacobson, 2007; Mozola, 2006). This may allow more rapid distribution of *Salmonella*-free products and more frequent monitoring at important investigation sites. However, the required sensitivity may be achieved only in the presence of sufficient concentration of target organisms after pre-enrichment (Jones, Law, & Bej, 1993). The amplification of target nucleic acid sequence and DNA probe might be an alternative way to increase the sensitivity of the assays (Tietjen & Fung, 1995).

The Gene-Trak® *Salmonella* Assay (Neogen Corporation, Lansing, MI) is the first introduced commercial assay. The second generation of this assay is based on a hybridization format of two probes, a polyadenylic acid (poly dA) tail on the capture probe and a polyadenylic acid (poly dA) tail on the target probe. This assay is equivalent to the original dipstick format assay and greatly simplifies the procedure for eliminating the enzyme incubation and washing steps and by lowering a hybridization temperature (Eijkelkamp et al., 2009; Mozola, 2006). The microwell format assay was also introduced and available on the market under the name GeneQuence™ *Salmonella*. 
The different colorimetric DNA hybridization assays available in the market have less than 48 h of a test time frame. Since the assays require higher detection limits of enrichment culture for positive signal, they need a longer enrichment time to increase the concentration of Salmonella in the final culture. However, the total test time with certain samples can be shortened to a significant extent. GeneQuence® Salmonella showed a sensitivity of 97.1% using a new 2-stage procedures including 24–27 h pre-enrichment and selective enrichment followed by a 2 h testing time in selected foods (Alles, Peng, Wendorf, & Mozola, 2007). The assays based on such rRNA hybridization approach have certain benefits in designing a probe because rRNA sequences are highly conserved, stable, and abundant in a bacteria cell, providing various targets and increasing the assay sensitivity (Mozola, Peng, & Wendorf, 2007).

3.3. Miniaturized biochemical assay

Miniaturized biochemical assay employs the reduced volumes of reagents, media, and vessels for rapid characterization of Salmonella to replace conventional biochemical tests and to provide more results in the same span of time (Fung, 2002; Ge & Meng, 2009; Tietjen & Fung, 1995). Many microbiologists have developed and used diverse miniaturized biochemical systems to rapidly confirm isolated organisms from a larger number of samples. Most miniaturized biochemical systems consist of disposable sterilized microtiter plates, a multiple inoculation device, and 10–20 media or substrates specially designed to target organisms. Salmonella spp. are identified based on the assimilation and utilization of special substrate and automated colony and cell density measurement. The test procedures include placing pure cultures into wells of a microtiter plate each of which represents independent inoculation in the conventional method, holding up to 96 different cultures. After 16–24 h incubation at a desirable temperature, the presence or absence of Salmonella on solid media or liquid media can be determined by reading color changes caused by the reaction of specific compositions and metabolites with reagents with the aid of a manual identification code or an automatic reader interfaced with a computer.

Diverse miniaturized kits for rapid biochemical characterization of Salmonella are commercially available, including API 20E (bioMérieux sa, Marcy-l’Etoile, France), Enterotube II (BBL Diagnostics, Sparks, MD), Enterobacteriaceae Set II (previously known as Mitek II, BBL Microbiology Systems, Cockeysville, MD), MICRO-ID (Remel, Lenexa, KS), EPS (Enteric Pathogens Screen Card) (Vitek Systems, Hazelwood, MO), GNI (Gram Negative Identification Card) (Vitek Systems, Hazelwood, MO), Microscan (Baxter Diagnostics, West Sacramento, CA), Quad Enteric Panel (Micro-Media Systems, San Jose, CA), Quantum II (Abbott Laboratories, Diagnostic Division, Abbott Park, IL), Sensititre (Sensititre, Salem, NH), Tri Panel (DifcoPasco Laboratories, Wheat Ridge, CA), Vitek 2 (bioMérieux sa, Marcy-l’Etoile, France), and Walk Away (Dade MicroScan, West Sacramento, CA). The majority of these commercial kits were first used for comparative analyses of clinical samples and later applied for food microbiological samples (Fung, 2002; Stager & Davis, 1992). These systems use similar approaches and procedures ideal for analyzing large number of samples and isolates. The sensitivity and specificity of these systems, particularly the latest ones, easily exceed 95% with the higher sample throughput, but the analysis cost may significantly increase if a sophisticated automated identification system is used. Most miniaturized biochemical assay kits were initially designed for identification of gram-negative bacteria including Salmonella, Shigella, and Enterobacteriaceae, and later expanded to gram positive bacteria, anaerobes, and even yeasts and molds (Gracias & McKillip, 2004).

The previous studies reported that miniaturized biochemical systems are accurate, efficient, convenient, labor-saving, space-saving, and more economical compared to the conventional culture methods (Cox, Fung, Bailey, Hartman, & Vasavada, 1987; Fung, 1997; Holmes, 1989; Kalamaki, Price, & Fung, 1997; Stager & Davis, 1992). Except for the MICRO-ID and APE 20E system requiring 4 h of incubation, the results are typically obtained after 24 h of incubation at an appropriate temperature in other systems. These miniaturized biochemical systems and kits have significantly contributed to improve accuracy, efficiency, and capacity in detecting Salmonella spp. It is anticipated that in the future they will continue to play an important role in the food, industrial, or environmental microbiology areas.

3.4. Biosensors

Biosensor technology encompasses an applied microbiology approach to bacterial detection (Alonso-Lomillo, Domínguez-Renedo, & Arcos-Martínez, 2010; Baeumner, 2003; Fung, 2002; Lazcka, Campo, & Munoz, 2007) that defines a molecule or a group of molecules by incorporating materials immobilized on the surface of a physicochemical transducer or transducing microsystem. A recognition signal is generated when a specific analyte binds to the biological recognition element. The signal can be changes in mass, oxygen consumption, potential difference, refractive index, pH, current, and other parameters (Baeumner, 2003; Eijkkelkamp et al., 2009). It is anticipated that a biosensor technique may replace existing immunology- and nucleic acid-based assays (Alocilja & Radke, 2003; Lazcka et al., 2007; Van Dorst et al., 2010).

Biological recognition elements used in biosensor application include enzymes, antibodies, nucleic acids, whole cells, tissue/whole organisms, and biomimetic material. The signal recognition and transduction in the biosensor is achieved by different types of transducers: electrochemical, optical, thermometric, micro-mechanical, and other miscellaneous transducers according to which the biosensors have been often classified by several authors (Goepel & Heiduschka, 1995; Ivanitski, Abdel-Hamid, Atanasov, Wilkins, & Stricker, 2000; Leonard et al., 2003).

3.4.1. Enzyme biosensor

An enzyme biosensor is an analytical device that integrates an enzyme with a transducer to produce a signal resulting from different physicochemical changes caused by enzyme-catalyzed reactions. The signal is converted into measurable responses such as current, temperature change, and light absorption to determine target analyte concentration (Baeumner, 2003). Enzyme biosensors are highly selective, rapid, and easy to use while they are expensive and sometimes lose their activities when immobilized on a transducer. Enzymes used in this type of biosensor include glucose oxidase, galactosidase, glucoamlyase, lactate oxidase, and other enzymes (Fung, 2002).

3.4.2. Nucleic acid biosensor

A nucleic acid biosensor which consists of a nucleic acid as a biological recognition element and a signal transducer is based on the hybridization of complementary strands of DNA or RNA molecules for detection of organisms (Baeumner, 2003; Eijkkelkamp et al., 2009; Lazcka et al., 2007). In addition to detection of bacteria and other pathogens, nucleic acid biosensors have been applied to food and environmental samples for detection of toxic compounds and biotechnology products using different signal transduction systems such as quartz crystal microbalances, surface plasmon resonance, surface acoustic wave sensor, and evanescent wave biosensors (Deisingh & Thompson, 2004; Feriotto, Borgatti,
Salmonella pathogens, and believed as one of the future directions to detect many current problems including real time detection of a variety of attention from microbiologist due to their great potential to solve (Biliteswski, 2000; Rogers, 2000). However, actual application to pathogen detection because of their incompatibility with some biological samples. Despite some obstacles and challenges in the research and application of the micro- and nano-biosensors, rapid development and integration of associated core technologies seems to provide no doubt that in the future they would be one of important technologies in microbiology and have a great value in the market.

3.4.3. Antibody-based biosensors
Antibody-based biosensors are analytical devices which use an antibody as a biological recognition element attached to a signal transducer to quantify the target analyte concentration (Biliteswski, 2000; Rogers, 2000). Antibodies are the most commonly used biological recognition element due to their high specificity and affinity to antigen, diversity, and availability on the market. Antibody-based biosensors can be divided into three main assay formats including an immobilized antibody, immobilized antigen, and non-immobilized antibody or antigen whose characteristics are largely determined by the type of signal transduction mechanisms (Rogers, 2000).

3.4.4. Micro- and nano-biosensors
The field of micro- and nano-biosensors has been drawing much attention from microbiologist due to their great potential to solve many current problems including real time detection of a variety of pathogens, and believed as one of the future directions to detect Salmonella spp. (Chen et al., 2005; Mozola, 2006; Scaria et al., 2008). However, actual application to Salmonella detection is rather limited so far because of the instrumental complexity and immature stage of the technology development (Alakomi & Saarela, 2009). However, the techniques are rather restricted in application to pathogen detection because of their incompatibility with some biological samples. Despite some obstacles and challenges in the research and application of the micro- and nano-biosensors, rapid development and integration of associated core technologies seems to provide no doubt that in the future they would be one of important technologies in microbiology and have a great value in the market.

4. Salmonella detection methods for food emergency response laboratories
Salmonella detection is critical for emergency responders who require robust and preferably portable laboratory-based detection systems. The methods for Salmonella detection typically recommended by the FDA and the FSIS of the USDA are based on cultural, serological, and biochemical properties using selective media. However, rapid methods for Salmonella detection have become increasingly important for effectively monitoring food products and are considered desirable as a future approach and national strategy. Rapid test kits recommended by the US Food Emergency Response Network for Salmonella include the VIDAS Salmonella (SLM) and immuno-concentration Salmonella (ICS) methods, the Tecra Unique Salmonella test, and the polymerase chain reaction (PCR)-based BAX™ system, which were chosen with the expectation of providing results within 24 h. These test kits are approved for screening purposes by the AOAC and have been widely used by food testing laboratories.

During the past four fiscal years, food emergency response laboratories have performed single lab and/or multi-lab validations involving rapid Salmonella detection platforms. Several studies have focused on the recovery of Salmonella spp. from a variety of sample matrices spiked with Salmonella strains. According to the results of these studies, detection levels varied with sample matrix and spiking concentration. For example, when spiked at the same low concentration Salmonella was easily identified in cat food and vegetable burgers, while its detection in other matrices such as baby oatmeal, peanut butter, liquid eggs, and cantaloupe was more difficult. Several food matrices, including milk, strawberries, and creamed corn, displayed an inverse relationship between detectability and concentration. Furthermore, wild-type Salmonella enteritidis showed much lower recoveries from liquid egg products relative to the American Type Culture Collection (ATCC) strains. In comparison studies of recovery of Salmonella from culture media, Yersinia pestis enrichment (YPE) broth and buffered peptone water (BPW) were as effective as lactose broth, a standard enrichment medium, in some sample matrices. Overall, xylose lysine deoxycholate (XLD) agar exhibited a higher recovery of Salmonella spp. than Hektoen enteric (HE) and bismuth sulfite (BS) agars, which may be explained by the selectivity of the media and the physiological properties of the strains used in the studies. Sample preparation techniques also appeared to affect the recovery of the spiked samples. For example, results obtained with the VIDAS® SLM and BAX® Q7 Salmonella PCR assays indicated that washing with soaking and soaking alone were more effective for extraction of Salmonella than surface washing only.

Comparative and collaborative studies of the rapid methods for screening of Salmonella have been performed by many food emergency response laboratories by using a variety of spiked sample matrices. However, as seen in Table 2, only limited numbers of methods and platforms were extensively evaluated and compared within integrated laboratory networks during this specific period. In the validation studies, a suspension of Salmonella cells at the appropriate turbidity was serially diluted for spiking into samples prior to assessing the test kits. Enrichment using BPW or YPE broth for the PCR-based methods offered a rapid alternative to the standard culture method for identifying Salmonella spp. Of the PCR methods tested, the BAX™ system showed comparable sensitivity and specificity to conventional culture methods in most sample matrices spiked at low and high concentrations of Salmonella cells, with the exception of some false results due to cross-contamination of PCR products. No differences in identifying Salmonella spp. were found between the standard BAX™ system and the BAX™ Q7 system.

Overall, there was little or no difference between the rapid methods, which displayed equally high accuracies in identification of the target organisms regardless of the matrix. Comparable results were found between BAX and SmartCycler PCR platforms in single laboratory testing. Some laboratories reported that the performance and accuracy of the SmartCycler PCR system was what dependent on the characteristics of the sample matrix. It was also reported that the sensitivity of the systems was further improved by increasing enrichment time. Taken together, these observations seem to imply that the difference between these methods may be mainly due to the matrix properties and the possible presence of PCR inhibitors, rather than the inherent effectiveness of the assay systems.

Several laboratories have compared the BAX™ Q7 PCR and VIDAS Salmonella (SLM) assays. The results showed no significant difference between the two assays in detecting Salmonella spp. in most food matrices. Indeed, most failures of the two assays in Salmonella detection appeared to be related to the matrix effect. Several laboratories have indicated that the VIDAS assay might be more reliable than the BAX system based on the study results and on the discussions moderated through a web-based database portal. However, the VIDAS assay was insufficiently sensitive for certain matrices (e.g., bean sprouts), and requires a secondary enrichment procedure to reduce the effects of background flora. Furthermore, there is only limited information available on how the BAX™ and VIDAS systems interact with matrices.

Several immunomagnetic separation (IMS) systems such as the Dynal BeadRetriever, Pathatrix, and BioVeris M1M Platform were validated and assessed for their efficiency in recovering and isolating different strains of Salmonella from sample matrices (Table 2). Typically, after an overnight incubation, a sample aliquot
was subjected to immunomagnetic separation and the two bacterial fractions (immuno-bound and unbound) were plated onto agarified medium and/or processed using the Roche LightCycler 2.0 RT-PCR, BAX System Q7 PCR, or Applied Biosystems 7500. Irrespective of manufacturer and concentration of Salmonella, the IMS systems were generally effective for improving the recovery from sample matrices and allowing for shorter turnaround times. However, the recovery from some food matrices (e.g., bean sprouts, cooked ham, ground beef, and peanut butter) was identical with or without the use of an IMS system at all spiking levels, indicating that the system efficiency is highly dependent on sample matrix. In addition, the IMS systems appeared to be not fully functional when the complexity of the sample matrices increased. Compared to manual methods, the automated IMS systems further increased the speed and productivity, and minimized cross-contamination by significantly reducing sample transfer time and pipetting steps.

Apart from the rapid methods, CHROMagar™ Salmonella appeared to be useful for colony isolation, in particular, in highly contaminated matrices. This medium could conceivably replace MacConkey agar as the growth agar of choice because it was easier to read and more quickly differentiated Salmonella from other organisms. Commercial biochemical assays used by food emergency response laboratories for confirmation of typical Salmonella colonies isolated on selective plating agars such as CHROMagar™ include API 20E, Enterotubes, and Vitek II Compact. One potential advantage of the automated Vitek II Compact system is that it could provide higher sample throughput compared to the API 20E, which was limited to testing smaller sample volumes.

As stated above, diverse and rapid commercial assays for identifying Salmonella in foods have been evaluated and validated by food emergency response laboratories. However, most studies have used artificially spiked samples, which may not truly reflect the physiological state and acclimation of naturally contaminated samples. Previous studies have indicated that the currently available rapid methods might produce a relatively high rate of false negative results for naturally contaminated samples. Although the rapid methods require much shorter detection times compared to conventional methods, some of them still appear to suffer from lack of sensitivity and specificity for certain sample matrices. Additionally, the PCR-based detection platforms require extensive sample preparation steps for some complex matrices and interpretation of the data can be relatively complicated. Finally, as implied by the outcomes of the studies, participation of the testing laboratories and their collaborative efforts is crucial to improve the methods and technologies needed to achieve the goals of integrated laboratory networks and to benefit the laboratories involved in Salmonella investigations in future.

5. Selection of Salmonella detection methods for rapid emergency response

Salmonella detection methods to quickly identify Salmonella contamination sources are vital for immediate actions of emergency responders. Considerable progress has been made to shorten analysis time of the methods while maintaining or increasing sensitivity and specificity for detection of Salmonella. However, despite significant progress in development of a variety of assays to detect and control Salmonella, the routine use of the assays has been limited due to some reasons. First of all, the assays have not been properly selected for sample matrices to be tested and the sampling and sample preparation have not been optimized for the methods and samples. Besides, the detection limits of the methods have not been accurately considered in evaluating the test results. Another reason may be a long and inappropriate enrichment time.

Table 2

<table>
<thead>
<tr>
<th>Method</th>
<th>Sample matrix</th>
</tr>
</thead>
<tbody>
<tr>
<td>PCR assays</td>
<td></td>
</tr>
<tr>
<td>ABI 7500 Fast</td>
<td>Cheese, cookie, cookie, ground beef, ice cream, liquid egg, peanut butter, potato salad, crackers, snack bar</td>
</tr>
<tr>
<td>BAX® PCR system</td>
<td>Baby carrot, beef stick, black pepper, cilantro, cilantro flakes-dried, cooked ham, cooked ham, deli ham, ground beef, beef, ground beef, burger, ham, hamburger, hot pepper, hot dog, infant formula, jalapeno pepper, liquid egg, milk, orange juice, peanut butter, potato salad, powdered formula, pretect ready-to-eat (RTE) lettuce, serrano pepper, spinach, sponge sicle, tomato, vegetable juice, whole head romaine lettuce</td>
</tr>
<tr>
<td>LightCycler</td>
<td>Alfalfa sprout, baby oatmeal, broccoli, cantaloupe, cat food, chicken, creamed corn, frozen spinach, ground beef, hot dog, jalapeno pepper, lettuce, liquid egg, milk, orange juice, peanut butter, strawberry, tomato, vegetable burger, watermelon</td>
</tr>
<tr>
<td>SmartCycler</td>
<td>Baby carrot, cheese, chicken breast deli meat, cookie, deli ham, ground beef, ice cream, liquid egg, milk, orange juice, peanut butter, strawberry, tomato, vegetable burger, watermelon</td>
</tr>
<tr>
<td>Luminex</td>
<td>Alfalfa sprout, chocolate, bean sprout</td>
</tr>
<tr>
<td>ELISA assay</td>
<td>Cilantro, cilantro flakes-dried, jalapeno pepper, pretect ready-to-eat (RTE) lettuce, serrano pepper, spinach, whole head romaine lettuce</td>
</tr>
<tr>
<td>VIDAS</td>
<td></td>
</tr>
<tr>
<td>IMS assays</td>
<td></td>
</tr>
<tr>
<td>Dynal BeadRetriever</td>
<td>Chicken breast deli meat, cooked ham, ground beef, hamburger, liquid egg, milk, orange juice, peanut butter, potato salad, raisin, raw pork sausage, spinach</td>
</tr>
<tr>
<td>M1M analyzer</td>
<td>Alfalfa sprout, baby oatmeal, broccoli, cantaloupe, cat food, chicken, creamed corn, frozen spinach, ground beef, hot dog, jalapeno pepper, lettuce, liquid egg, milk, orange juice, peanut butter, strawberry, tomato, vegetable burger, watermelon</td>
</tr>
<tr>
<td>PATHATRIX</td>
<td></td>
</tr>
<tr>
<td>Miniaturized biochemical assays</td>
<td></td>
</tr>
<tr>
<td>API 20E</td>
<td>Chicken breast deli meat, raisin, raw pork sausage</td>
</tr>
<tr>
<td>Enterotubes</td>
<td>Liquid egg, milk, orange juice, peanut butter, spinach</td>
</tr>
<tr>
<td>Vitek®</td>
<td>Black pepper, ground beef, peanut butter, potato salad, powdered formula</td>
</tr>
<tr>
<td>Media</td>
<td>CHROMagar Ground beef, liquid egg, potato salad</td>
</tr>
</tbody>
</table>

* PCR, Polymerase chain reaction; ELISA, enzyme-linked immunosorbent assay; IMS, immunomagnetic separation.
et al., 2000; Mozola, 2006; Tietjen & Fung, 1995). Multiplexing to simultaneously detect more than one target organism and the ability to differentiate between naturally contaminated and artificially spiked samples are also desirable parameters to be included into consideration in selection of the method. Currently, any single method may not fulfill all these requirements (Cannons et al., 2006; Mahon, Murphy, Jones, & Barrow, 1994; Settanni & Corsetti, 2007). The following parameters are particularly important to be carefully considered in analytical methods for identification of Salmonella.

5.1. Matrix effect and sample preparation

The efficacy of a rapid method is significantly influenced by sample matrix (Mozola, 2006; Ricke et al., 1998; Ricci, Volpe, Micheli, & Palleschi, 2007; Stevens & Jaykus, 2004). Therefore, it is critical to verify if the method is fitting for the sample matrix to be tested. Regardless of the detection methods, the sample preparation is a critical and challenging step that should be optimized to better separate the target organisms from inhibitory substances in a sample, which may improve signal to noise ratios and lower false-positive results (Koyuncu, Andersson, & Haggblom, 2010; Ricke et al., 1998; Saroj, Shashidhar, Karani, & Bandekar, 2008). Immunomagnetic separation and molecular imprints systems are excellent tools for sample concentration and purification. The rapid methods that can be used for crude samples and require minimal sample preparation will be desirable, particularly for field and on-site usage.

5.2. Selectivity and specificity

The sensitivity and specificity are extensively examined during the method development and independent studies. However, care should be given to compare sensitivity and specificity rates of the methods presented in the published literature and on websites because the study design and sample matrices used are different between the studies. It is desirable that both sensitivity and specificity are as high as possible and can be used to determine whether a confirmation test will be conducted or not. According to the previous studies and the manufacturer’s claim, the sensitivity and specificity rates of some commercial assays are more than 99% (Eijkelkamp et al., 2009). However, such a high level of these properties may not be achievable by most of commercially available rapid assays. Even the validation bodies such as AOAC, AFNOR, and MicroVal do not specify acceptance criteria and values for such properties. In the meantime, it should point out that the detection capability of the rapid methods needs to be determined by considering the bioavailability of toxins and assessment of food safety, rather than by simply measuring concentrations of pathogens by the methods because all Salmonella isolates are not equally pathogenic for humans.

5.3. Analysis time

In emergency response situations, the rapid methods should be able to give the results preferably within a few hours to a day and perform on-site (Skrotstrup, Nicolaelsen, & Justesen, 2008). However, the commercially available rapid methods which produce the test results in the next day are rather limited. The presumptive Salmonella results need to be confirmed by culture and biochemical identification methods, requiring at least additional 24 h and more for completion. The use of microplates with the rapid methods may offer the advantage of reducing analysis time and increasing the capacity of the methods when processing a large number of samples.

5.4. Cost efficiency

The laboratorians seek the rapid methods with lower operational and maintenance costs with comparable accuracy to avoid expense and time consuming methods. However, the public health risk and cost associated with false test results might have much greater negative impacts than the benefits from the use of a low-cost method (Eijkelkamp et al., 2009; Tietjen & Fung, 1995).

6. Future aspects of Salmonella detection methods

In order to replace conventional procedures and to meet the food emergency and safety needs, the rapid methods should be simple, fast, precise, sensitive, specific, stable, versatile, and cost-effective. These requirements are in part associated with improvement of sample preparation and reduced time and labor for analysis in the methods. According to the literature and previous studies by food emergency response laboratories on testing and validating the methods, none of commercially available methods for detection of Salmonella seem to meet all the requirements for adequate emergency response to Salmonella incidence.

More reliable and efficient new assays are needed to replace existing conventional methods and to meet the food emergency and safety needs although considerable works should be done before routine use. In addition, the sensitivity and specificity of the new assays should be more uniformly assessed under a well-defined study design.

The development and improvement of separation and concentration methods that efficiently isolate, concentrate, and purify target Salmonella spp. and simultaneously eliminate interfering components directly from food samples is needed to further increase sensitivity and selectivity of the rapid detection methods and provide real-time screening of the samples for Salmonella in significantly less detection time than that required for cultural enrichments. The removal of the need for cultural enrichment would allow food emergency response laboratories to detect low levels of Salmonella contamination in a sample matrix. Further research needs to focus on the development of separation and concentration methods to be simple, rapid, low-cost, and applicable to a variety of sample matrices and background microflora.

The development of accurate field and on-site systems may also help improve the efficiency of food emergency response networks, reducing analysis time and the work load for reference laboratories. The progress of microarray and automation technologies will further increase the capacity of the food emergency response laboratories for screening Salmonella. Since a single Salmonella bacterium can be rapidly multiplied into more than a million in a few hours, the methods should be capable of cultivating extremely low concentration of the target organisms in samples. The assay which enables to simultaneously detect multiple pathogens will provide additional benefits and advantages to the laboratories. Perhaps more importantly, it is needed to develop the schemes and procedures to validate the efficiency of new assays in food emergency response laboratories for coordinating of the results using dependable and representative control samples.

7. Conclusions

Presently, many commercial detection methods are available and vary in detection limit, sensitivity, specificity, and cost efficiency with sample matrices to be tested. Despite of great advances in technologies, current Salmonella detection methods are not fast and reliable enough for emergency responders to determine the desired directions within the appropriate period of time, being required for further improvements and higher efficiency of the
methods. Food emergency response laboratories should choose the most acceptable method or a combination of methods having the desired analytical properties to meet food emergency and safety needs, based on their own needs and requirements. The present study indicates that validation and comparative studies for the test kits should be set up under the identical test conditions to better compare and evaluate the test results among different food emergency response laboratories. A study on the effect of sampling and sample size on the recovery of the target organism might also help obtain more accurate test results.

Due to increasing population mobility and the wide distribution of feed/food products, Salmonella outbreaks and salmonellosis cases are occurring across state and national boundaries. Such widely dispersed outbreaks and salmonellosis may limit the public health resources for detection and investigation of Salmonella outbreaks. During the past years, some integrated laboratory networks have effectively met demands from public and emergency responders, organized training and the inter-laboratory comparative studies, and coordinated the test results of the participating laboratories through online reporting and data sharing system. With years of experience, they should efficiently integrate the emergency networks for food safety and harmonization of Salmonella detection methods to provide timely investigation of a widely dispersed multistate Salmonella outbreak and respond to potential future events of bioterrorism.

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