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Journal of Food and Drug Analysis
1993, 1(2): 119 - 131

藥物食品分析
第一卷 第二期

Rapid Methods for The Detection of *Salmonella* in Foods

PETER FENG

Division of Microbiological Studies
Center for Food Safety and Applied Nutrition,
Food and Drug Administration, Washington, DC 20204 USA

ABSTRACT

Diagnostic methods for foodborne bacterial pathogens have changed dramatically in the past decade. Many systems previously used to analyze clinical specimens have been adapted for food analysis, and recent advances in biotechnology have introduced new technologies that can be applied to pathogen detections. Changes in *Salmonella* diagnostics are most evident because of the importance and worldwide prevalence of this foodborne pathogen. These new or modified methods for identifying *Salmonella* often use novel technologies and assay formats in combination with existing methodologies. In general, they may be classified into five types: new media formulations or modifications, miniaturized biochemical tests, nucleic acid-based assays, antibody-based assays, and automated or instrument systems. Most of these systems provide simpler and more rapid presumptive identification of *Salmonella* in foods. However, accuracy is often affected by the complexity and type of food analyzed. In this review, selected assays from each technology group are presented and some advantages and limitations of these novel systems are discussed.

Key words: Rapid methods, *Salmonella*, Foods.

INTRODUCTION

Traditional methods used to isolate and identify foodborne bacterial pathogens have relied extensively on selective enrichment, biochemical testing and, at times, serological confirmation. However, as a result of scientific advances in biotechnology, these traditional time-consuming, labor-intensive microbiological procedures have been altered dramatically. Novel concepts for identifying bacteria have been introduced and technologies already in existence have been modified and improved to make pathogen detection faster, more convenient, more sensitive, and more specific than conventional assays. The new diagnostic procedures used for food analysis include five areas of technology: A) new media formulations and B) miniaturized biochemical systems, which are modifications of traditional methods that simplify and improve the performance of isolation and identifcation procedures; C) DNA probes and D) antibody-based assays,

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which are fairly new concepts in the area of food analysis; and E) advancements in instrumentation technology, including automated systems for bacterial identification.

This paper examines the impact of these five areas of technological advancement on diagnostics in relation to *Salmonella* detection. As the most important foodborne pathogen worldwide, *Salmonella* is responsible for about 60% of all bacterial foodborne outbreaks in the United States [1]. With approximately 5 million analyses annually [2], *Salmonella* testing is one of the most lucrative in the diagnostics market and the one most affected by changes in technology. With few exceptions, the assays mentioned in this review are marketed commercially and commonly referred to as "rapid methods". However, most of these systems continue to rely on cultural methods to resuscitate injured cells and to select and amplify the number of target cells. Therefore, these "rapid methods" still require traditional pre-, selective- and post-enrichments or combinations for proper sensitivity and specificity.

MEDIA AND MEDIA MODIFICATIONS

New selective and differential media have been developed for distinguishing *Salmonella* spp. from other enteric bacteria. Rambach agar (Laboratories Technogram, Paris, France), which containspropylene glycol (PG), sodium desoxycholate, neutral red pH indicator, and the chromogenic substrate, 5-bromo-4 - chloro-3-indoyl β-D-galactopyranoside[3], is used to differentiate *Salmonella* from other bacteria by acid formation from PG and by the absence of β-galactosidase activity. Some species of *Pseudomonas* and *Acinetobacter* may give similar reactions to those of *Salmonella* on this medium [4]. Rambach agar has been evaluated using mostly clinical samples.

Another new selective and differential medium, xylose-lysine-tergitol 4 (XLT4), is essentially a modification of the xylose-lysine-desoxycholate (XLD) agar, with a surfactant, Tergitol 4, added to the xylose-lysine agar base as a selective inhibitor [5]. The XLT4 medium effectively inhibits *Proteus* spp. and other non-salmonellae; whereas isolates of *Salmonella* appear as black colonies on this medium. The XLT-4 medium has recently been evaluated in the isolation of *Salmonella* from drag-swab samples from commercial chicken flocks. Of 155 swab samples, the procedure using XLT4 isolated salmonellae from 73 samples. In comparison, conventional method, an ELISA kit from Kirkegaard and Perry Laboratories and the GENETRAK DNA probe assay each recovered salmonellae from 60, 72 and 67 samples, respectively[6]. Although XLT4 agar is not yet available commercially, a liguid medium is being marketed by Future Medical Technologies International, West Palm Beach, Florida.

The new hydrophobic grid membrane filter (HGMF) (QA Life Sciences, San Diego, California) reduces colony overgrowth by trapping filtered bacteria in individual compartments. The original HGMF assay for *Salmonella*, which used selective lysine agar (SLA) as the isolation and presumptive identification medium, was adopted as Official action by the Association of official Analytical Chemists (AOAC) in 1986. The EF-18 selective agar (QA Life Sciences) currently used is a modification of SLA; it contains novobiocin to limit the growth of *Klebsiella* and sucrose for additional differential specificity. The HGMF/EF-18 method has been evaluated and approved as official first action by AOAC for analysis of *Salmonella* in all foods [7,8].

The Oxoid *Salmonella* Rapid Test (OSRT) (Oxoid Division, UNIPATH Co., Ogdensburg, NY) is a modification of existing *Salmonella* assays. OSRT combines traditional enrichment with a colorimetric system to deter-

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Table 1. Selected media and media modification used for the selection and differentiation of *Salmonella*

Medium	Assay format	Manufacturer	References
Rambach agar	selective & differential	Technogram (France)	3
EF-18/HGMF[a,b]	selective & differential	QA Life Sciences	8
OSRT[a]	selective & motility	Oxoid (UK)	9
XLT4[a]	selective & differential	Future Medical Technology International	5
MSRV[a]	selective & differential	not available	10
MUCAP[a]	biochemical	Biolife (Italy)	11

[a]HGMF, hydrophobic grid membrane filtration; OSRT, Oxoid *Salmonella* Rapid Test; XLT4, xylose-lysine-tergitol; MSRV, modified semisolid Rappaport-Vassiliadis; MUCAP, methyl-umbelliferyl caprilate reagent.
[b]Adopted by AOAC as official first action for identifying b*Salmonella* in all foods.

mine *Salmonella* motility through a semisolid selective medium at 41℃ [9]. OSRT will not detect nonmotile *Salmonella* isolates and provides only presumptive identification; it therefore requires serological confirmation.

The Modified Semi-solid Rappaport-Vassiliadis (MSRV) is another selective isolation medium for *Salmonella*. MSRV uses malachite green, novobiocin, magnesium chloride and incubation at 42℃ to selectively isolate salmonellae from foods [10]. Comparative analysis using 43 naturally contaminated and seeded food samples showed a procedure using MSRV to be comparable to standard methods and to selected rapid methods in the recovery of *Salmonella* from chocolate and biscuit ingredients[10].

The 4-methylumbelliferyl caprilate fluorescence test (MUCAP) manufactured by Biolife, M ilano, Italy, is not a medium but a reagent useful for the identification of *Salmonella*. The MUCAP reagent, consisting of an eight-carbon ester conjugated with 4-methylumbelliferone, interacts with Salmonella C_8 esterase to release the fluorogenic umbellifereone radical [11]. Evaluation of 3 rapid screening methods using 175 bacterial isolates showed the identification sensitivities of MUCAP, MicroScreen Latex (see antibody-based assays), and Rambach agar to be 100%, 96% and 91%, respectively, and the assay specificities were 80%, 96% and 100%, respectively [12]. These new selective media and reagents are listed in Table 1.

MINIATURIZED BIOCHEMICAL IDENTIFICATION SYSTEMS

Miniaturization of biochemical tests used to identify bacteria began in the late 1940s [13] and steadily gained in popularity, until presently a variety of these minisystems can be used to identify *Salmonella*. Typically, each system contains 15 to 20 biochemical tests designed to identify a specific bacterial group. Some systems, such as Enterobacteriaceae II, use inter-changeable biochemical discs and may be adapted for identifying

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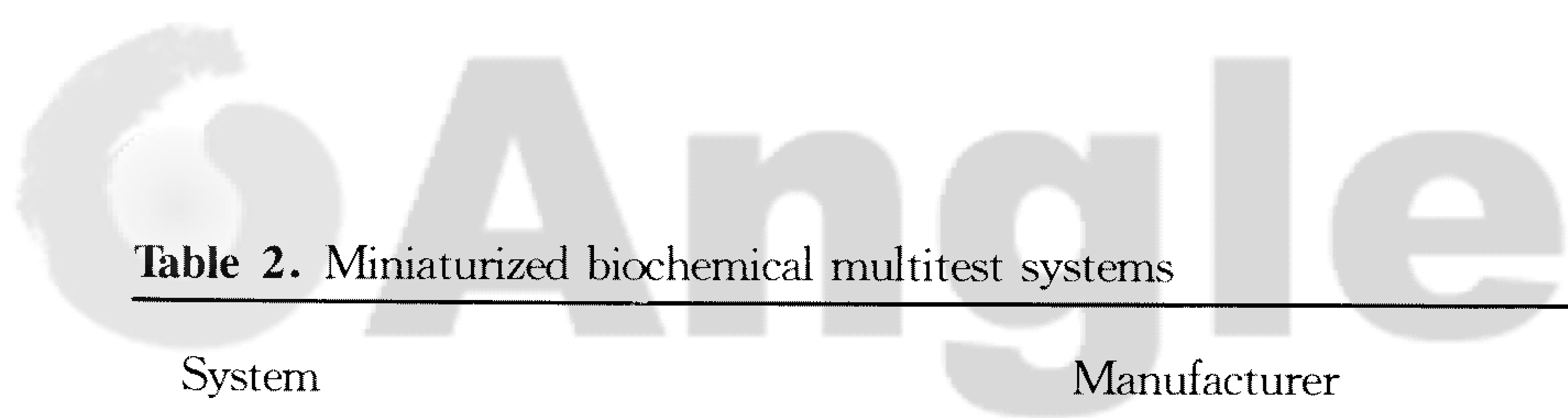

Table 2. Miniaturized biochemical multitest systems

System	Manufacturer	Reference
API-20E[a]	Analytab Products Inc. Plainview, NY	14-16
Enterotube II[a]	Hoffmann LaRoche Nettley, NJ	14, 16
Micro-ID[b]	Organon Teknika Durham, NC	14-16
Enterobacteriaceae II[a]	BBL Microbiology Systems Cockeysville, MD	14, 15

[a]Adopted by AOAC as official final action for *Salmonella* only.
[b]Adopted by AOAC as official final action for all enterics.

other groups of bacteria. For a few minisystems, the changes caused by biochemical reactions can be observed in 4 hours. Most systems, however, recommend 18 to 24 hours of incubation[13,14]. Studies have found these miniaturized biochemical systems to be economical, versatile, simpler, and generally showing 90 to 99% accuracy compared to conventional identification methods[13,14]. A partial list of commonly used miniaturized systems is given in Table 2. Although all of these systems can identify *Salmonella* isolates, only selected kits have been evaluated by collaborative studies and approved by the AOAC for *Salmonella* identification.

NUCLEIC ACID-BASED ASSAYS

Nucleic acid technolgy has had a dramatic impact on rapid diagnostic methods. Many DNA probe assays with different formats have been introduced to identify bacterial pathogens in foods. Recent developments in nucleic acid amplification technologies such as the polymerase chain reaction (PCR), Q-beta and others are also being actively explored as a means of increasing assay sensitvity. In the area of *Salmonella* diagnostics, many nucleic acid-based assays, targeting ribosomal RNA (rRNA), plasmids, and other virulence genes, have been or are being developed; however, only the DNA hybridization assay (DNAH) (GENETRAK, Framingham, Massachusetts) is marketed commercially (Table 3). The DNAH combines DNA probes with an enzyme immuno-

Table 3. DNA Prode and other nucleic acid-based assays for identifying *Salmonella*

Assay	Format	Manufacturer	Reference
DANH[a]	Colorimetric Probe	GENETRAK	17, 18
BIND[b]	Ice nucleation	DNA Plant Technologies	19, 20

[a]adopted by AOAC as official first action.
[b]being developed.

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assay, with the key components being a polydeoxyadenosine-tailed capture probe and a fluorescein-labeled detector probe, both of which are targeted at regions of rRNA unique to *Salmonella*. In the presence of salmonellae, both probes bind to the same target, which in turn is immobilized to a polydeoxythymidine coated dipstick. The dipstick-bound complex is detected by use of a horseradish peroxidase conjugated antibody to fluorescein and a colorimetric enzyme substrate[17]. The DNAH assay was recently modified to include an additional probe specific for subgenus V *Salmonella* and the assay time was also shortened to 2. 5 hours. This modified DNAH assay has been been approved as official first action by AOAC for screening *Salmonella* in all foods [18].

The Bacterial Ice Nucleation Diagnostics (BIND) assay for *Salmonella* from DNA Plant Technology Corporation, Oakland, California, is a unique nucleic-acid based test still being developed. BIND assay is based on the principles of ice crystal formation, where small volumes of pure water may be supercooled to -40℃ and remain liquid. However, in the presence of ice nucleation protein, such as that encoded by the *ina* gene, ice crystals form at -2℃ [19,20]. To develop a test specific for *Salmonella*, the *ina* gene was cloned into P22, a transducing bacteriophage of *Salmonella*. In the presence of salmonellae, *ina* is transduced into the bacteria by P22 and the gene is activated by the host cell to produce ice nucleation proteins . This specific host-phage interaction is then observed ty ice crystal formation, using a freezing-indicator dye that is green and fluorescent in the liquid state, but becomes orange and nonfluorescent when the medium freezes[19,20]. Preliminary analysis of food seeded with *Salmonella* showed that the BIND assay was not susceptible to interferences by food particles or by high levels of normal flora. It was also able to identify injured salmonellae without the need for cultural resuscitation or enrichment [20].

ANTIBODY-BASED ASSAYS

Many of the assays developed for detecting foodborne *Salmonella* are antibody-based systems (Table 4). These commercially available test kits use monoclonal , polyclonal, or combinations of antibodies, and are designed in various formats, including enzyme-linked immunosorbent assay (ELISA), latex agglutination, immunodiffusion, dipstick, or combinations of these technologies.

At least eight commercial ELISA systems are available for detecting *Salmonella* in foods. However, two of these kits, Report and Biopro, are both manufactured by Bioenterprises PTY, Australia, hence, are identical to the TECRA system. All ELISA assays for *Salmonella* use a " sandwich " or capture format, in which antibody-coated polystyrene wells are used to capture salmonellae antigen from the postenrichment medium. A second *Salmonella* antibody conjugated with either the horseradish peroxidase or alkaline phosphatase enzyme is added to form a "sandwich" complex, which is detected by a colorimetric enzyme substrate. Aside from this basic format, the various ELISA kits have minor design variations or patented modifications. In many kits, the reaction wells are removable, and only the number of wells (tests) needed for an analysis are used. Others, such as the BacTrace kit, provide the capture antibody as a reagent; therefore, the wells must be coated with the capture antibody before use [21]. The Assurance EIA system increases binding efficiency by including an additional antibody, trade-marked Assur-Link, which supposedly reduces steric and size hindrances between antigen and antibody-conjugate. ELISAs provide only presumptive identification; hence, positive results must be confirmed by conventional culture methods.

Among the ELISAs listed in Table 4, TECRA has official final action status from the AOAC for detection of *Salmonella* in all foods

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Table 4. Antibody-based assays for detecting *Salmonella*

Assay	Format	Manufacturer	Reference
TECRA[a]	ELISA[b]	Bioenterprises (Australia)	29
Report[c]	ELISA	3M	
Biopro	ELISA	International Bioproducts	
Salmonella-Tek[d]	ELISA	Organon-Teknika	30
Assurance EIA[d]	ELISA	Biocontrol	
MICROELISA[c,d]	ELISA	Dynatech	31
BacTrace	ELISA	Kirkegaard & Perry	21
EQUATE	ELISA	Binax	32
SEROBACT	Agglutination	Remel	
Oxoid	Agglutination	Oxoid (UK)	
Bactigen	Agglutination	Wampole	33
Microscreen	Agglutination	Mercia (UK)	24
Spectate	Agglutination	May & Baker (UK)	24
1-2[d]	Immunodiffusion	Biocontrol	28
Immunocapture	Dipstick/ELISA	Bioenterprises (Australia)	
PATH-STIK	Dipstick-EIA	LUMAC (The Netherlands)	

[a]Adopted by AOAC as official final action.
[b]ELISA, enzyme-linked immunosorbent assay.
[c]No longer available commercially.
[d]Adopted by AOAC as official first action.

[7], and the Assurance EIA and the Salmonella-Tek assay are currently on official first action status. In a recent analysis, two ELISA methods were evaluated and shown to be comparable to the AOAC/Bacteriological Analytical Manual method in the recovery of *Salmonella* from low-moisture foods [22]. Of the 300 samples analyzed, conventional method identified 206 *Salmonella*-positive samples. Salmonella- Tek and TECRA showed equivalent results, but, also had 7 and 13 false negative reactions, respectively. The performance of the Salmonella-Tek assay was recently improved by the addition of novobiocin to the postenrichment media and by elevating the incubation temperature of enrichment to 42°C[23].

Many antibody-based assays for *Salmonella* use the agglutination format in which latex particles coupled with polyvalent antisera are used to presumptively identify salmonellae from a selective enrichment medium. However, information on the use of these assays to detect *Salmonell a* in food is limited. The few comparative studies

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showed that the efficiency of selected latex systems on identifying pure culture isolates ranged from 66 to 89%[24]. Furthermore, significant false positive identifications were due mostly to antibody cross reactivity with *Citrobacter freundii* and *Esherichia coli*[24]. Antibody cross reactivity with *Citrobacter* has also been reported with other antibody-based systems [25]. Latex systems were slightly more efficient in the analysis of naturally contaminated food, but only after extensive selective enrichment of the food samples [24]. The use of latex assays to detect *Salmonella* in foods should be evaluated further. Suspended particulate matter in foods have affected the interpretation of agglutination reactions [24], and the antibody specificity of some systems need to be improved.

The 1-2 Test (Biocontrol, Bothell, Washington) is the only antibody-based assay that uses the immunodiffusion format to identify *Salmonella* in foods. This test consists of a L-shaped apparatus with two connecting chambers, one of which is filled with semisolid agar. A *Salmonella*-specific antibody is inoculated into the agar while an aliquot from selective enrichment is dispensed into the inoculation chamber. Motile salmonellae, if present, travel from the inoculation chamber into the agar-filled motility chamber, where they react with the diffusing antibody to form a line of immunoprecipitation[26]. Nonmotile salmonellae are not identified by the 1-2 Test, and the manufacturer specifies two different enrichment protocols, depending on the types of food being analyzed[27,28]. Recently, 1-2 Test, specific for *S. enteritidis* was introduced. Two antibody-based commercial *Salmonella* identification systems use dipsticks in their assay format (Table 4). The PATH-STIK system, made by LUMAC (The Netherlands) and markted by Integrated BioSolutions, Monmouth, New Jersey is essentially a "sandwich" ELISA. However, the assay uses a dipstick rather than polystyrene wells. An enzyme-conjugated antibody and a dipstick coated with a second antibody to *Salmonella* are used to simultaneously capture and bind salmonellae from a 20 to 30-hr selective enrichment culture. The "sandwich" complex bound to the dipstick is then identified with a colorimetric enzyme substrate. Because the PATH-STIK assay requires no washing steps and all reagents are provided in dropper bottles, pipeting is minimal. The test can be completed in 30 minutes after culture enrichment, and an assay control is included on every dipstick to ensure that the test was conducted properly. Although no data has been published on the performance of this system for detecting *Salmonella* in foods, results of the preliminary studies done by the manufacturer seems promising. The LUMAC test showed a detection sensitivity of 91.4% and a specificity of 98.6% in the analysis for *Salmonella* in ice cream samples and were comparable to conventional methods and other rapid assay systems.

In the Immunocapture/ELISA system an antibody-coated dipstick selectively captures salmonellae from the preenrichment medium. The salmonellae-bound dipstick is then introduced directly into postenrichment medium for amplification, and detected by TECRA ELISA. The Immunocapture/ELISA system is supposedly more sensitive with fewer false positive reactions, because competing bacteria are not captured by the antibody on the dipstick. In addition, because specific capture of *Salmonella* eliminates the need for cultural selective enrichment, the testing procedure is shortened and simplified.

Recently, a variation of the Immunocapture system has been introduced in the form of immunomagnetic particles. These assays use magnetic particles coated with *Salmonella*-specific antibody to selectively capture this organism from the enrichment medium. Captured salmonellae is

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Table 5. Automated identification and detection systems for *Salmonella*

Trade Name	Assay Format	Manufacturer	References
Quantum II	Biochemical	Abbott Diagnostics	39
GNI[a, b]	Biochemical	Vitek	7, 40
MIS[b]	Fatty acids	Microbial ID	14, 15
Biolog	Carbon utilization	Biolog	37
M1000S[a]	Conductance	Malthus	38

[a]Adopted by AOAC as official first action.
[b]GNI, Gram-negative identification; MIS, microbial identification system.

easily separated using magnetic force and can be assayed using various methods, including PCR [(34)] and conductance measurements [(35)]. A magnetic immuno PCR assay was used to directly detect *Salmonella* in fecal samples [(36)].

INSTRUMENTATION AND AUTOMATED ASSAYS

Several instrumentation systems that use different technologies are available for identifying *Salmonella* (Table 5). The Quantum II (Abbott Diagnostics, Irving, Texas) and the Automicrobic System with the Gram-Negative Identification (GNI) card from Vitek/bioMerieux (Hazelwood, Missouri) are based on biochemical test profiles of pure culture isolates. The GNI system has beem collaborative studied and approved as official first action by the AOAC for the identification of *Salmonella* spp. and other members of the family Enterobacteriaceae [(7)]. Other automated identification systems include the Microbial Identification System (MIS) (Microbial ID, Newark, Delaware), which is based on fatty acid profiles [(15)]; and the Biolog Identification System (Biolog, Hayward, California), which obtains bacterial identification profiles by measuring oxidation of carbon sources [(37)].

The M1000S (Malthus Division, Radiometer America, Westlake, Ohio) is not an identification system; rather it is an automated system for detecting *Salmonella* in foods. The instrument is essentially a microbiological analyzer which measures changes in electrical conductivity of the specially formulated medium [(38)]. *Salmonella* positive reactions can be completed in less than 24 hours, including the preenrichment step; negative reactions require 40 to 46 hours to complete. The M10000S system has been validated in comparison with standard methods by using *Salmonella* seeded food samples. The method was approved official first action by the AOAC for determining the presence of *Salmonella* spp. in all foods[(7)].

CONCLUSIONS

The National Academy of Sciensces, Institute of Medicine, has suggested placing greater emphasis on the use of improved or rapid methods for identifying pathogens in foods. However, as evidenced in this review, microbiologists are faced

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with a deluge of "rapid" methods available for identifying *Salmonella* in foods, not to mention tests being developed in other countries that were not discussed in this paper. Although most of these" rapid" methods are well designed, many of them have not been evaluated extensively to ensure that their accuracy is comparable to that of standard methods. However, of those systems that have been examined, most generally showed equivalent sensitivities to conventional microbiological procedures in detecing *Salmonella* in foods [25,32,41,42]. One important observation from these comparative studies, however, was that sensitivity of " rapid" methods and the incidences of false-positive reactions varied greatly among the assay systems, depending on the type of food analyzed. Assay performance has routinely been affected by the complexity of food matrices, which also interferes with the accurate or reproducible comparison of test systems. Therefore, it is critical for individual users to validate each new assay, as some methods may be more suited to testing certain food types or situations than others.

Most" rapid" tests for identifying *Salmonella* in foods are easy to perform and the entire procedure can be completed in about half the time required for standard procedures. Hence, speed and simplicity are advantages of"rapid" systems. The price for these benefits, however, may be reflected in the cost which range from US $ 2.40 to $ 10.00 per test. Furthermore, as some systems can only be purchased directly from the manufacturers, availability of reagents may be another limiting factor.

By far, the biggest disadvantage that most "rapid" systems have in common may be their continued reliance on enrichment schemes. Most "rapid" systems can be completed in a few hours; but, the requirement for time-consuming enrichments generally lengthen the procedure to a few days. In addition, " rapid" methods are recommended for use only in presumptive detection; therefore, all positive results must still be confirmed by appropriate official (often cultural) methods and serology.

This discussion of the "rapid" systems is intended for general reference and does not indicate officoal endorsement or approval of their use in food analysis. The AOAC status is mentioned for some methods that have been validated or evaluated by collaborative studies; however, this information cannot be kept current because the assays are continually being modified or improved.

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Correspondence to: Peter Feng *Accepted for Publication: March 12. 1993*

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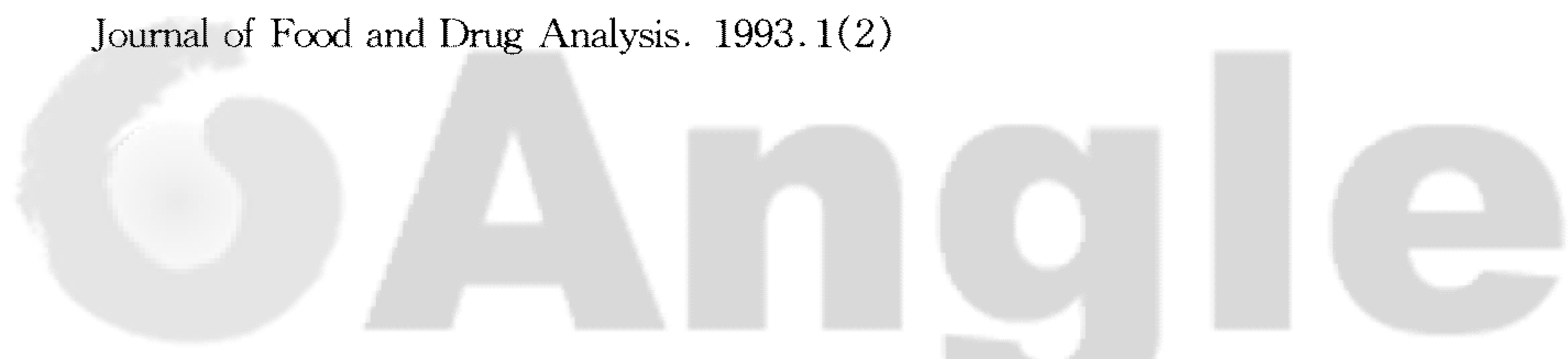
Journal of Food and Drug Analysis. 1993. 1(2)



食品中沙門氏桿菌之快速檢驗法

馮 寄 新

摘 要

在過去十年裡，有關食品中毒病原菌之鑑定方法已有顯著的改變。許多過去用於檢驗臨床檢體的方法已陸續應用於食品當中，特別是近來在生物技術方面的進步，已有許多新的技術被應用於病原菌之檢測。由於沙門氏桿菌是一種常見且重要的食品中毒病原菌，因此與其有關的檢驗方法之改變尤其顯著。這些新開發或改良的沙門氏桿菌鑑定方法大多是將新技術及分析原理與現存的方法合併使用。一般而言可分為五大類：新的或改良的培養基組成；簡化的生化試驗；核酸技術；免疫抗體法以及自動或儀器鑑定系統。大部分的方法可更快速及簡易地篩檢食品中的沙門氏桿菌。然而，由於食品種類的不同及其組成分的複雜性，常會影響檢測之準確性。此篇論文在於介紹以上各類技術方，並探討其應用上的限制及利弊。