Salmonella Detection in Foods: Present Status and Research Needs for the Future

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ABSTRACT

Standard cultural procedures generally require 4 to 5 d for presumptive evidence of Salmonella in foods. Attempts at greater method brevity have resulted in the use of selective enrichment cultures as test material for short immunological tests including fluorescent antibody (FA), enrichment serology (ES), enzyme-linked immunosorbent assay (ELISA), direct immunoenzyme (DI) and membrane filter-disc-immunoimmobilization (MFDI) assays. Nonimmunological tests such as the limulus amebocyte lysate (LAL) test to detect Salmonella cell wall antigens in preenrichment cultures or application of the ELISA, the hydrophobic-grid-membrane (HGMF) techniques or other rapid diagnostic tests to preenrichment cultures are indicated.

Standard cultural methods for the isolation of Salmonella in foods are labor-intensive and generally require 4 to 5 d for presumptive identification of salmonellae. Typically, a food sample is preenriched overnight in a nonselective broth medium, enriched in one or more selective liquid media and plated on differential agar media. Presumptive Salmonella colonies are screened biochemically by conventional tube media and confirmed serologically with polyvalent and single grouping somatic and flagellar antisera (14, 18, 33, 34).

Nonselective enrichment (preenrichment) of foods increases method sensitivity through facilitated recovery of the few stressed or uninjured salmonellae usually present in foods (3, 21). Recent work suggests that the choice of preenrichment medium is not critical for recovery and that media of low and high nutritive capacity are equally effective for detection of Salmonella in raw and processed foods (4, 24). Attempts at method brevity through reduction of the preenrichment incubation period have led to conflicting reports. Although short (≤6 h) incubation of preenrichment cultures is desirable because it allows presumptive identification of salmonellae one day earlier than with standard cultural methods, short preenrichment engenders unacceptably high numbers of false-negative results (3, 4). Pure culture work also showed that 6 h is not sufficient for the resuscitation of injured cells in lactose or tryptone soy broths (35). In contrast, short preenrichment incubation periods performed satisfactorily in the isolation of salmonellae from soy and egg products (20, 27).

Method brevity can also be achieved by omitting the preenrichment step and suspending food samples directly in selective enrichment broth media (direct enrichment). This approach is recommended for the isolation of Salmonella in products known or suspected to contain high numbers of competing microorganisms (33, 34). However, several reports indicate that direct enrichment may adversely affect method sensitivity (5, 13). Combination of the preenrichment and enrichment steps into a single procedure has also been suggested to reduce analytical time (28). In this approach, selective agents were added to nonselective basal medium previously incubated for 4 h, and incubation of the mixture resumed for a total of 24 h at 35°C. Results with heat- and freeze-injured salmonellae and with artificially contaminated foods compared favorably with standard cultural methods. The potential of short selective enrichment periods has generated little research interest, presumably because of reports on increased detection of salmonellae with prolongation (16 to 96 h) incubation of enrichment media. For example, rate of isolation increased substantially when meat and environmental samples preenriched in buffered peptone water were selectively enriched in Muller-Kauffman tetrathionate brilliant green broth for more than 24 h (5, 13, 36). Two recent studies, however, support the use of abbreviated (6 h) selective enrichment for detection of Salmonella in raw and finished products. Analysis of naturally and artificially contaminated foods by the hydrophobic-grid-membrane filter technique using overnight preenrichment in nutrient broth and a 6-h selective enrichment in tetrathionate brilliant green (TBG) and selenite cystine (SC) enrichment broths identified 97.8% of samples positive by a standard cultural procedure (6). Short (6 h) incubation of TBG and SC enrichment broths following overnight preenrichment of feeds and feed ingredients in nutrient broth yielded results identi-
cal to those obtained by a standard procedure (D'Aoust, in press); results from a continuing study with other raw and processed foods are most encouraging.

Recent years have seen research efforts directed at development of rapid immunological methods based on enrichment cultures as test material (Fig. 1). In the immunofluorescent antibody (FA) technique, a portion of an enrichment culture is fixed and stained on a glass slide and the reaction between somatic and/or flagellar antigens and conjugated fluorescent antibodies is visualized by fluorescence microscopy. The sensitivity of the technique is overshadowed by a high incidence of false-positive reactions and need for well-trained analysts (31). Recognition of FA as a laborious analytical procedure led to the development of a semiautomated system for the direct immunofluorescent detection of Salmonella (23,32). The two-component system includes a slide processor which sequentially filters, stains and rinses samples of enrichment cultures, and a fluorometer which reads total fluorescence intensity of stained samples. A positive sample is recognized when its total fluorescence intensity exceeds an arbitrary threshold value. Deficiencies in the design and operating characteristics of this system, failure to detect Salmonella in certain food types, and high numbers of false-positive results were noted in both studies. Enrichment serology (ES) is another immunological method which provides for the presumptive identification of salmonellae one day earlier than with standard cultural methods (15,25). In this procedure, portions of selective enrichment cultures are post-enriched for 6 h in a non-selective M broth; flocculation from a mixture of Spicer-Edwards flagellar antiserum and M broth culture is indicative of Salmonella. Prolonged incubation (24 h) of the M broth reportedly increases method sensitivity (1). The ES procedure compares favorably with conventional cultural techniques, but fails to detect nonmotile strains. The enzyme-linked immunosorbent assay (ELISA) method was recently applied to the detection of Salmonella in foods (22). In this method, a sample of enrichment culture is placed in the well of a microfiltration plate and reacted with rabbit polyvalent flagellar (H) antiserum; unbound antiserum is removed by repeated washings. A goat anti-rabbit globulin coupled to alkaline phosphatase is then added to the well and incubated for 1 h at room temperature; unbound globulin is removed by washing. In the presence of a Salmonella-contaminated sample, addition of the alkaline phosphatase substrate produces a visible color reaction that can be quantitated spectrophotometrically. ELISA performed well in the identification of naturally contaminated food samples where no false-negative and few false-positive results were reported (22). A direct immunoenzymatic (DI) method utilizing polyvalent flagellar antiserum conjugated to horseradish peroxidase was compared to fluorescent antibody (FA) and conventional cultural techniques (29). In this procedure, fixed smears of enrichment cultures are reacted with conjugated antiserum. Upon addition of the peroxidase substrate, positive samples exhibit cells with brown cell envelopes and flagellae under light microscopy. This immunoenzymatic technique detected 90% of contaminated raw meat samples and produced an appreciable number of false-positive reactions. A membrane filter-disc-immunomobilization (MFDI) technique has been used successfully for the rapid detection of salmonellae in mechanically deboned poultry meat and in raw and pasteurized egg products (9,20). Following preenrichment in a nonselective medium, samples are selectively enriched for 4 to 6 h at 35°C. A portion of the enrichment culture is then filtered through a 0.45-μm membrane filter which is inverted and placed on the surface of a semi-solid selective medium. A paper disc impregnated with Salmonella polyvalent flagellar antiserum is placed in close proximity to the inverted filter. Motile salmonellae migrate into the semi-solid medium to form a distinct black line of immobilization upon contact with the diffusing flagellar antiserum. Sensitivity of this novel technique reportedly exceeded that obtained with conventional cultural procedures (9,20).

Several nonimmunological diagnostic techniques based on enrichment cultures as test material have also been proposed for the rapid isolation or identification of Salmonella. The lysine-iron-cystine-neutral red (LICNR) broth is a nonselective medium that provides for a 24-h presumptive identification of salmonellae in dairy products through development of typical color reactions (11). Salmonella decolorizes the red broth medium to yellow with a concomitant blackening of the medium. Lactose and sucrose biotypes behave as typical strains and H₂S-negative

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**Figure 1. Methods for detection of Salmonella in foods**

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biotypes produce an alkaline reaction after 48 h of incubation. Application of the LICNR broth to foods other than dairy products met with limited success because of interaction between components of food samples and LICNR and inhibition of typical color development (16). High numbers of LICNR false-negative results from selenite cystine enrichment cultures undermine the value of this broth medium as an alternate to plating media (2). A radiometric method based on the liberation of $^{14}$CO$_2$ from $^{14}$C-dulcitol in the presence of polyvalent H antisera has been proposed for the rapid detection of Salmonella in meat, eggs and coconut (26). Although the reaction mechanism remains unclear, components of the polyvalent antiserum presumably block Salmonella-mediated breakdown of dulcitol. Presumptive identification of salmonellae is based on the ratio of $^{14}$CO$_2$ liberated from test and control reaction mixtures.

Other novel techniques are of equal interest. The Felix 0-1 phage at concentrations of $10^5$ to $10^{12}$ plaque-forming units per ml lyses most Salmonella strains (>90%) with minimal cross-reactions with nonsalmonellae (30,37). The technique can be used as an alternate or adjunct to biochemical screening of presumptive isolates by conventional tube media or rapid diagnostic kits (7,12). The reduced bacteriolytic activity of the 0-1 phage against salmonellae belonging to somatic groups E$_1$-E$_4$ recently led to development of a phage mixture (0-1/G$_{47}$) exhibiting a >94% specificity for somatic group E organisms (10). Recent work showed that refrigeration of preenrichment and enrichment broth cultures for 72 h offers greater operational flexibility without adversely affecting method sensitivity (3). In a comparative study involving six laboratories, samples found to be contaminated by a standard cultural procedure were also identified from refrigerated preenrichment (93%) and enrichment (92%) cultures. A recently completed study sponsored by the International Commission on Microbiological Specification for Foods (ICMSF) further underlined the reliability of the refrigeration approach (D’Aoust, in press).

It is evident that recent efforts in the development of rapid methods have focused primarily on selective enrichment broth cultures as test material and failed to explore the feasibility of preenrichment-dependent analytical schemes (Fig. 1). This apparent lack of research interest presumably arises from the few salmonellae usually present in naturally contaminated foods and the contention that competitive microflora readily overgrow Salmonella in nonselective media. Recent studies have shown that populations of $10^5$ to $10^7$ salmonellae/ml develop in overnight preenrichment cultures and that growth characteristics are independent of bacterial strain and incident level of contamination (4). Preenrichment cultures therefore provide the minimal cell concentrations for reliable test results by the enrichment serology ($10^7$/ml), fluorescent antibody ($10^5$/ml), and ELISA ($10^6$/ml) techniques and underline the possible application of these three immunological methods to preenrichment cultures. Preliminary results on detection of Salmonella in preenrichment cultures by the FA (8) and ELISA (22) techniques are encouraging. Furthermore, monoclonal antibodies adsorbed to latex particles or as a high titer antiserum preparation would likely increase the sensitivity of the ES, FA and ELISA procedures through enhanced specificity and affinity of immunologic reactants to Salmonella antigens. Background fluorescence from food debris in FA screening of preenrichment cultures could be minimized through prefiltration of the culture or short enrichment in a selective or nonselective medium.

The Limulus amoebocyte lysate (LAL) test is widely used for detection of bacterial endotoxins (19,38). The test depends on activation of a Ca$^{+2}$-dependent clotting enzyme by bacterial endotoxin (lipopolysaccharide), cleavage of a coagulogen into peptide chains by the activated enzyme, and formation of a stable gel protein matrix through disulfide bridges between peptide chains. The technique is extremely sensitive and can detect picograms of endotoxin. Recent work on gel formation with bacterial polymers other than lipopolysaccharides suggests that the LAL test could be modified to respond specifically to Salmonella cell envelope or exocellular constituents. The intrinsic sensitivity of the LAL test conceivably could provide for the detection of Salmonella cell envelope antigens or trace amounts of enterotoxin in preenrichment cultures. The radioimmunoassay technique (17) should also be considered for the rapid detection of enterotoxin in preenrichment and enrichment cultures.

A recent report on the reliability of short (6 h) selective enrichment (D’Aoust, in press) suggests evaluation of the ES and FA techniques with 6-h enrichment cultures for an accelerated presumptive identification of salmonellae. The hydrophobic-grid-membrane filtration (HGMF) technique has been used successfully for the detection of Salmonella in enrichment cultures (16). In this method, a hydrophobic substance applied to the surface of a membrane filter divides the filter into independent square compartments (grid cells). Filtration of a bacterial suspension randomly distributes microorganisms into various grid cells. The inoculated HGMF filter is then transferred to the surface of an agar medium and incubated until colonies develop in grid cells. Colony counts are converted into numbers of organisms in the test material using a statistically derived standard curve. A study on the ability of the HGMF technique to effect separation and distinguish salmonellae from competitive flora in preenrichment cultures using selective and/or differential agar media would be indicated.

The last decade has seen major advances in developmental methodology, notably the ELISA technique because of its specificity, sensitivity and amenability to automation. Further testing of the ELISA technique and adaptation of the LAL and radioimmunoassays for the identification of Salmonella enterotoxin or cell envelope antigens in preenrichment cultures would provide for more rapid identification of salmonellae in foods. Innovative research efforts must be encouraged to meet the growing needs of regulatory and industrial sectors for cost-efficient and sensitive methods.

REFERENCES


