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Detection of *Salmonella* Species in Foodstuffs

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Abstract

Conventional methods to detect *Salmonella* spp. in foodstuffs may take up to 1 wk. Methods for pathogen detection are required. Real-time detection of *Salmonella* spp. will broaden our ability to screen large number of samples in a short time. This chapter describes a step-by-step procedure using an oligonucleotide probe that becomes fluorescent upon hybridization to the target DNA (Molecular Beacon; MB) in a real-time polymerase chain reaction (PCR) assay. The capability of the assay to detect *Salmonella* species from artificially inoculated fresh- and fresh-cut produce as well as ready-to-eat meats is demonstrated. The method uses internal positive and negative controls which enable researchers to detect false-negative PCR results. The procedure uses the buffered peptone water for the enrichment of *Salmonella* spp. and successfully detects the pathogen at low level of contamination (2–4 cells/25 g) in <24 h.

Key Words: Microbial food safety; fresh produce; ready-to-eat meat; enteric pathogens; salmonelosis; food surveillance.

1. Introduction

The detection of *Salmonella* species by regulatory agencies is still primarily based on traditional microbiological culture methods which may take up to 5 d to confirm the results ***(1)***. When a foodborne outbreak is suspected, faster the source of a pathogen can be identified, the sooner the public can regain confidence in the food supply ***(2)***. A rapid pathogen detection method helps identify source of pathogen during outbreaks investigation, reestablishing public confidence in the food supply. However, only 2 out of the 27 outbreak investigations on fresh produce clearly identified a point of contamination, which underscores the importance and need for rapid and accurate pathogen identification methods ***(3)***. Advances in biotechnology have permitted more rapid microbial identification

From: *Methods in Molecular Biology, Vol. 429: Molecular Beacons: Signalling Nucleic Acid Probes, Methods and Protocols* Edited by: A. Marx and O. Seitz © Humana Press Inc., Totowa, NJ

and surveillance ***(4,5)***, and polymerase chain reaction (PCR)-based detection methods have become valuable tools for investigating foodborne outbreaks and identifying the responsible etiological agents ***(6–8)***. The recently introduced real-time PCR-based format utilizes an internal fluorogenic probe that is specific to the target gene ***(9–12)***. During the PCR assay, the target gene is amplified and simultaneously recognized and monitored by the fluorescent probe moiety ***(13)***.

There are two types of fluorogenic PCR-based detection methods. One is based on a linear fluorogenic probe and requires the 5′–3′-nuclease activity of the DNA polymerase ***(9,14,15)*** (also known as TaqMan assays), while the other utilizes a fluorogenic probe which has flanking GC-rich arm sequences complementary to one another ***(12,13,16)*** (also known as Molecular Beacon, MB). In both types of real-time PCR probes, a fluorescent moiety is conjugated to one end of the sequence, and a quencher moiety is attached to the other end of the sequence. In the absence of target DNA sequences, the MB assumes a hairpin conformation with the two arms hybridizing to each other thus bringing the quencher into close proximity to the fluorophore (which results in no or low background fluorescence). When the target DNA is present the sequence in the loop region hybridizes, the hairpin of the MB opens, and the fluorophore and the quencher separate. In the open conformation, the fluorophore of the MB emits a detectable signal that is directly correlated with the quantity of the target template present in the PCR assay ***(13,17)***. The TaqMan assay differs from the MB method in that the generation of the fluorophore signal is dependent upon 5′–3′-nuclease activity to cleave the reporter dye from the linear probe ***(14,15,18)***.

Irrespective of the reporter technology employed in the PCR assay, its successful application to food samples, particularly to fresh produce, has been hindered by the lack of a convenient and relatively simple method for preparation of PCR-amplifiable DNA ***(6,19–22)***. We and others have reported the presence of inhibitory compounds of plant origin that interfered with PCR biochemistry resulting in false-negative data ***(5,19,22)***. Here we describe a simple, commercially available MB-based detection protocol for *Salmonella* spp. ***(10,23)***. The protocol enables detection of wide-range of *Salmonella* serovars in several food matrices such as fresh and fresh-cut produce, meat, poultry, and various ready-to-eat foods.

2. Materials

All buffers and double-distilled (or reverse osmosis)-purified water used in the protocol must be sterilized by autoclaving at 15 lb pressure for 15 min.

2.1. Bacterial Strains and Culture Media

1. *Salmonella enterica* serovar Typhimurium ATCC 14028s and other serovar strains [Agona—SARB 1, Anatum—SARB 2, Dublin—SARB 12, Haifa—SARB 21,

Choleraesuis—SARB 4, Pullorum electrophoretic types Pu3 (SARB 51) and Pu4 (SARB 52) and Paratyphi A (SARB 42)] were obtained from the *Salmonella* Genetic Stock Center (Calgary, Alberta, Canada) and have been described previously ***(24)***.
Luria—Bertani (LB) broth and agar media (tryptone 10 g, yeast extract 5 g, NaCl 10 g, water 1 L (pH 7.2), add Difco granular agar 17 g L^{-1} for solid media), saline (0.9% NaCl in water (pH 7.0)), and buffered peptone water media (10 g bactopeptone, 5 g NaCl, 9 g $Na_2HPO_4 \cdot 12\ H_2O$, and 1.5 g KH_2PO_4 in 1 L of water, dissolve on magnetic stirrer, and heat if necessary. Final pH should be 7.2).
Orbital shaker incubator, 125-mL Erlenmeyer flasks, spectrophotometer to read optical density (A_{600}) of cell cultures.

2.2. DNA Extraction

1. Bench top microcentrifuge (10,000–12,000*g*, for 1.5 mL tubes, e.g., 5410, Eppendorf, Koln, Germany or similar), 1.5 mL screw-cap centrifuge tubes, micro-pippets and tips with incorporated cotton plugs, dry heat block or a boiling water bath (100°C), magnetic stir plate, vortex apparatus, and powder-free gloves (*see* **Note 1**).
   TE buffer: 1 mM EDTA, 10 mM Tris–HCl (pH 8.0); lysis reagent A (or Instagel matrix, *see* **Note 2**) (Bio-Rad Laboratories, Hercules, CA).

2.3. Processing of Food Samples

1. Stomacher Lab Blender 400 (Seaward Medical, London, UK).
2. Stomacher model 400 bags with incorporated filter (cat. no. 6041/STR, Seaward Medical).
3. Incubator.
4. Buffered peptone water (as described above).

2.4. Real-Time PCR

1. Thermal cycler with real-time data acquisition capability for FAM and Texas Red.
2. 96-well PCR plates with optically clear sealing tape or 8-strip 200 µL PCR tubes with optically clear flat caps.
3. iQ-check *Salmonella* MB-PCR detection kit (Bio-Rad Laboratories, cat. no. 357 8100) containing reagent B (dual-labeled oligonucleotide probe based on *iagA* gene with FAM at 5′-end and DABSYL at 3′-end); reagent C (pre-mixed Taq-DNA polymerase, $MgSO_4$, primer pairs for *iagA* and buffer); negative and positive controls (reagents D and E, respectively).

3. Methods

The real-time PCR system described below comprises the following steps: (i) artificial inoculation of foods with desired cell density and pre-enrichment of samples; (ii) DNA extraction from pure and mixed-cultures of various *Salmonella* serovar strains and from pre-enrichment broths; (iii) setting up the real-time PCR and data acquisition; and (iv) data analysis and confirmation of occurrence of *Salmonella* in food samples.

3.1. Artificial Inoculation of Foods and Pre-Enrichment

1. One day prior to the experiment, inoculate two to three colonies of *S. enterica* serovar Typhimurium (and other serovars, as needed) in 10 mL LB broth and grow in a shaker incubator at 37°C for 18–22 h at 220 rpm.
2. Begin with cleaning the work area with 5% bleach, and use powder-free gloves to keep PCR tube tops optically clean.
3. Dilute the overnight culture 1:10 and read A_{600} on a spectrophotometer. Take cells equivalent to 1.0 OD_{600} and make final volume to 1 mL with sterile saline, this represents approximately 10^9 cells mL^{-1}. Make appropriate 10-fold serial dilutions in saline to get 10^3 cells mL^{-1} or roughly 1 cell per 1 μL. Take a dry LB agar plate and spot several 5 and 20 μL spots, let the spots dry in a laminar flow. Incubate the plates overnight at 37°C and count colonies to determine actual inoculum level in food samples.
4. Simultaneously, take 25 g food materials and place in a stomacher bag 400 with incorporated filter. Add 5–10 μL diluted cell suspension to represent 5–10 *Salmonella* spp. cells. Perform several inoculations in triplicate to represent 10-fold inoculation on desired food matrices. Include multiple sample aliquots with and without addition of 50 μL saline per 25 g food sample to represent known negative-control samples.
5. Add 225 mL buffered peptone water and homogenize for 2 minutes at 120 strokes per min. Incubate the stomacher bags *without shaking* for 18–22 h at 37°C.

3.2. DNA Extraction

3.2.1. DNA Extraction from Enriched Food Samples

1. After 18–22 h of incubation, carefully remove 1 mL liquid from the top 1–3 cm portion of the stomacher bag *without disturbing* the food debris (for oily foods, *see* **Note 3**) and place it in a screw cap Eppendorf centrifuge tube.
2. Centrifuge at 10,000 *g* for 2 min at room temperature and discard the supernatant.
3. Resuspend the pellet by vortexing briefly and add 200 μL lysis reagent A (*see* **Note 2**). Heat the tightly capped tube in a boiling/dry bath for 15 min at 100°C, and briefly chill on ice. Collect the supernatant in a new tube by centrifugation (10,000 *g* for 10 min at room temperature) and store at –20°C till further use.

3.2.2. DNA Extraction from Pure Salmonella spp. Cultures

1. Perform 10-fold serial dilution in saline from 1.0 OD (A_{600}) of cell suspension and perform a viable cell count using LB agar plates.
2. Take 5 μL cell suspensions from each dilution tube and add to a screw capped Eppendorf tube containing 195 μL of either lysis reagent A or Instagen matrix. Heat in a boiling water or dry bath for 15 min and proceed with DNA isolation as outlined in Section 3.2.1.
3. To determine detection limits using mixed serovar strains two different mixtures were prepared by pooling 1.0 OD_{600} cells from individual cultures. Mixture-1 contained serotypes Agona, Anatum, Dublin, Haifa, and Choleraesuis and Mixture-2 was made from serovars that do not appear to be responsive to attachment-mediated

acid tolerance (serotypes Pullorum electrophoretic types Pu3 and Pu4, and Paratyphi A). A 10-fold serial dilution was carried out before taking 5 μL aliquot for DNA isolation.

4. The following day, calculate genome equivalents per microliter of the lysis reagent (i.e., colony forming units per microliter) using viable cell counts data.

3.3. Performing Real-Time PCR

3.3.1. Thermal Cycle Parameters

1. Set the thermal cycle for the use of iQ check *Salmonella* kit as follows: cycle 1: 50°C for 2 min; cycle 2: 95°C for 10 min; cycle 3 (repeat 50 times) [95°C for 20 s, 55°C for 30 s (set fluorescence detection at this stage), 72°C for 30 s]; cycle 4: 72°C for 5 min; cycle 5: 4°C hold.
2. For fluorescence measurements select appropriate filters for FAM (excitation wavelength of 490 nm and an emission wavelength of 530 nm) and Texas Red (excitation wavelength of 575 nm and an emission wavelength of 620 nm) for each sample.
3. Turn on the optical system and thermal cycler at least 1 h before use.
4. Most real-time PCR instruments allow '*plate set up*' and have '*sample identifiers*' which allow assignment of microtiter plate position with individual samples, such as sample replicates, positive control, negative control, etc. It is necessary to define a plate setup that corresponds exactly to where the samples are loaded in PCR tubes.

3.3.2. PCR Mix Preparation

1. Prepare a master mixture (5 μL reagent B and 40 μL PCR amplification mix per tube) according to the number of reactions to be carried out.
2. To prepare a standard curve of log-genome equivalent versus threshold cycle (C_t), take 5 μL DNA from each of the 10-fold dilution series samples (from Section 3.2.2., step 1) and place into tubes (in duplicate). Add 45 μL of the master mix and incubate in ice till other samples are ready.
3. For analyzing food samples, prepare the ratio of 1:10 dilution of sample DNA by adding 18 μL water to 2 μL DNA which was originally isolated from samples incubated in the enrichment broth (from step 3.2.1.). Transfer 5 μL diluted and 5 μL undiluted DNA in individual tubes (in duplicate). To each tube, add 45 μL PCR master mix. Gently mix by tapping on the bench-top of flicking with fingers.
4. At least one positive and one negative control must be included in each PCR run.
5. Close the flat-top PCR caps on each tube and wipe clean the lid surface with a tissue paper.
6. Centrifuge all the PCR tubes at room temperature for 2 min at 4000*g* to get rid of bubbles and the PCR mix at the bottom of the tube (*see* **Note 4**).
7. Place all the tubes in the thermocycler block and orient tubes as per the 'design set up plate' file (Section 3.3.1., step 4).
8. Start the PCR program and the data collection should begin automatically.

Table 1.
Interpretation of Sample Results by MB PCR iQCheck *Salmonella* Spp. Assay

Sample	*Salmonella* spp. detection (FAM)	Internal control dNA detection (Texas Red)	Interpretation
Negative control	C_t = N/D	C_t > 20	Experimental set-up free of *Salmonella* spp.
Positive control	C_t > 10	Not significant	PCR kit components in good condition
Negative test sample	C_t = N/D	C_t > 20	Absence of *Salmonella* spp., PCR successful
Positive test sample	C_t > 10	Not significant	Positive identification for *Salmonella* spp.
False negative test sample	C_t = N/D	C_t = N/D	Inhibition of PCR N/D, none detected.

3.3.3. Data Analysis

1. The data can be analyzed directly at the end of the PCR run. It is necessary to analyze the positive and negative controls before sample analyses. Select the fluorophore to be analyzed, i.e., FAM for *Salmonella* and Texas Red for the internal control.
2. One can select to analyze a subset of the samples at one time, however, it is recommended to select a negative and positive control with each analysis.
3. Choose 'PCR baseline subtracted' data option (instead of background subtracted option) to analyze selected wells. Note the cycle number located just before the positive-control curve rises significantly (increase in the fluorescence reading) above the background noise. The calculation of threshold cycles can be done by the software or by entering user-defined cycle numbers (*see* **Note 5**). Select 'calculate threshold cycle' option and the threshold line will appear and it should cross all curves above all the background traces.
4. It is necessary to analyze the data with the second fluorophore, Texas Red. Repeat steps 3.3.3., -2 and -3 and analyze all the samples by selecting the second fluorophore (Texas Red).
5. Data for each fluorophore can be viewed and saved in a plate format or as a list of C_t values with the associated graphs. For the experiment to be valid, the controls must have results as summarized in **Table 1**. Otherwise the PCR needs to be repeated.
6. A positive *Salmonella* sample must have a C_t value ≥ 10 for FAM fluorophore. If no C_t value is obtained for FAM, then the interpretation of the result depends on the internal control: (a) the food aliquot is consider free of *Salmonella* spp. if there is no C_t value for FAM, and the internal control in Texas Red has a C_t ≥10; (b) if the internal control also has no C_t value, then it is not possible to interpret the data. In most instances, the data indicate an inhibition of the PCR. The 1:10 diluted DNA

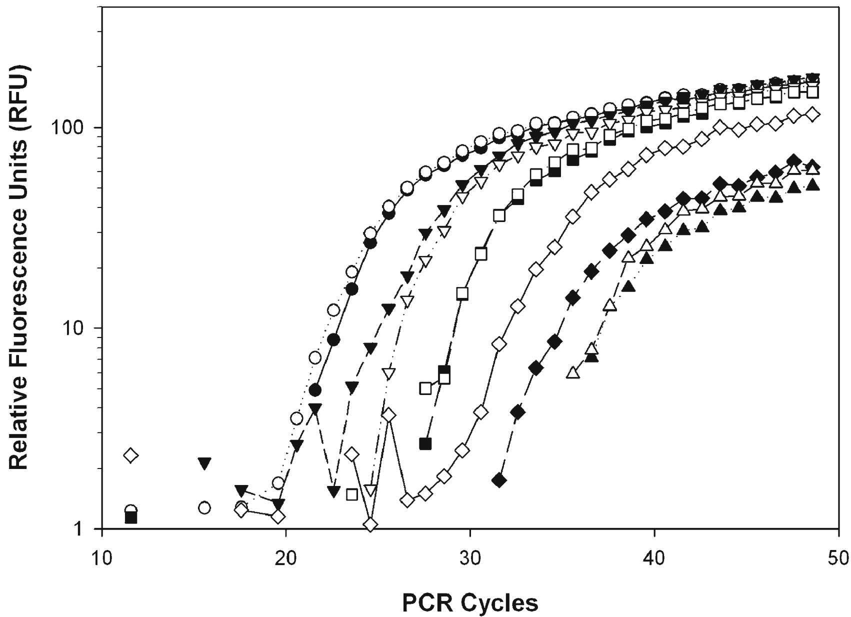

Fig. 1. Amplification plot of a 10-fold serial dilution series of *Salmonella enterica* serovar Typhimurium. Sample replicates (open and closed symbols) with cells per assay 10^0 (Δ, ▲); 10^1 (◇, ♦); 10^2 (□, ■); 10^3 (∇, ▼); and 10^4 (◦, •) using a molecular beacon probe and real-time PCR assay. Real-time detection was done by measuring fluorescence of FAM during the annealing step of each PCR cycle (*X*-axis). Relative fluorescence units are plotted on the *Y*-axis. (Data obtained from Ref. ***(10)***, With permission.)

samples are useful in this respect, and occasionally one may have to dilute the DNA sample 1:25 to get successful amplification of the internal control.

7. We observed that the relative fluorescence from the MB probe increased with number of PCR cycles when cells per assay of serovar Typhimurium increased from 10^0 to 10^4 (**Fig. 1**). MB probe was also able to detect both mixtures of different serovars as effectively as serovar Typhimurium, and the C_t values decreased linearly with increasing target quantity per PCR assay (**Fig. 2**).
8. The variability for detection and quantitation between various serovar mixtures was minimal and correlation coefficient values were 0.98 and 0.94 were observed for mixtures 1 and 2, respectively. The correlation coefficient for serovar Typhimurium was 0.93 (**Fig. 2**).
9. The MB probe used in this study was able to detect *Salmonella* spp. from variety of fresh and fresh-cut produce at a very low level of contamination (i.e., at 1–3 CFU per 25 g of produce) (**Table 2**) ***(10)***, as well as from poultry ***(23)*** and ready-to-eat meats (Patel and Bhagwat, unpublished work).

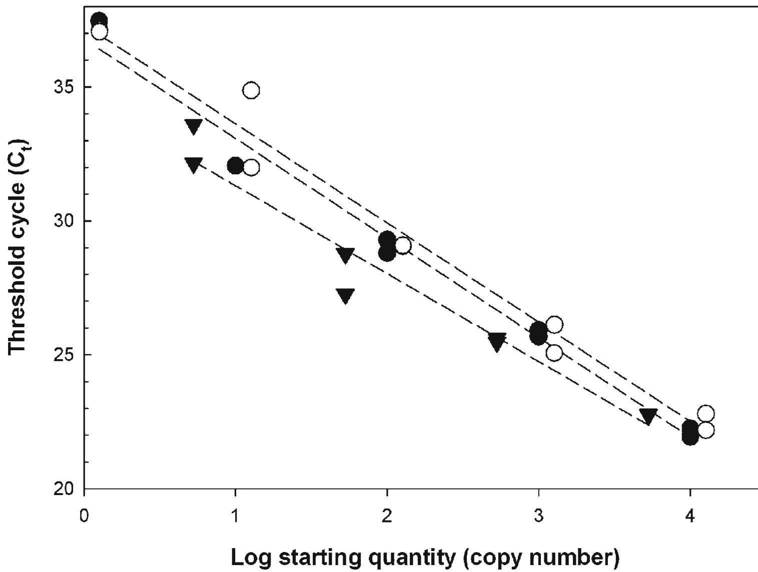

Fig. 2. Standard curve for a 10-fold serial dilution series of *Salmonella* strains (10^6 to 10^0 cells per assay, in duplicate) plotted as the threshold cycle (C_t) on the *Y*-axis using *S. enterica* serovar Typhimurium. Symbols: ∘, serovar Typhimurium (data from **Fig. 1**); •, mixture of serovars Agona, Anatum, Dublin, Haifa, and Choleraesuis; or ▼, serovars Pullorum electrophoretic types Pu3, and Pu4, and Paratyphi A. The target copy number per assay is on the *X*-axis. (Data obtained from Ref. ***(10)***, With permission.)

10. We also observed variation in enrichment of *Salmonella* spp. inoculated on different food matrices ***(10)***. For example, the C_t values for the detection of *Salmonella* in alfalfa and cilantro were much higher (25.37 ± 4.6 and 32.66 ± 2.86, respectively) than the C_t values observed for cantaloupe and mixed-salad (17.74 ± 0.28 and 19.66 ± 2.02, respectively), even though all produce was inoculated at 3–4 CFU per 25 g (**Table 2**).

4. Notes

1. We prefer round-bottom 1.8 mL centrifuge tubes over conical tubes since round-bottom tubes tend to decant solutions cleaner, do not trap air bubbles and retain cell pellets reproducibly.
2. The lysis reagent A provided in the PCR-kit is not enough to perform DNA extractions from several reference strains to generate reference quantitative DNA templates. Among other lysis procedures, Instagel matrix (cat. no. 730-6030,

Table 2.
Comparative Analyses of Detection Frequencies of *Salmonella enterica* serovar Typhimurium from Artificially Inoculated Fresh Produce and Ready-to-Eat Meats by the MB Probe Real-Time PCR and Using Conventional Selective Media

Food matrices used for artificial inoculation	Contamination level (CFU/25 g)	Detection of *S. enterica* serovar typhimurium after one step enrichment (18 h)		
		Detection frequency (conventional) selective media[1]	Molecular beacon PCR	
			Detection frequency	Quantitation (C_t value[2])
Fresh produce				
Alfalfa sprouts	3.7 ± 1.2	9/9	9/9	25.37 ± 4.60
Cilantro	4.1 ± 2.0	8/8	8/8	32.66 ± 2.86
Parsley	8.5 ± 3.5	4/6	6/6	25.9 ± 2.20
Celery	4.9 ± 1.2	4/6	6/6	26.3 ± 3.50
Cauliflower	4.9 ± 1.2	2/4	4/4	25.4 ± 2.40
Fresh-cut produce				
Green onion	8.5 ± 3.5	6/6	6/6	29.5 ± 2.40
Mixed salad	3.3 ± 1.2	6/6	6/6	19.16 ± 2.02
Bell peppers	4.9 ± 1.2	6/6	6/6	32.6 ± 4.9
Cantaloupe	3.4 ± 1.5	6/6	6/6	17.74 ± 0.28
Ready-to-eat meat				
Turkey	3.4 ± 1.5	11/11	11/11	20.32 ± 2.55
Chicken	2.22 ± 0.11	8/8	8/8	18.9 ± 1.66
Ham	3.4 ± 1.5	12/12	12/12	18.16 ± 2.49
Bologna	3.4 ± 1.5	12/12	12/12	17.36 ± 1.57

[1]Standard microbiological procedure with selection on *Salmonella-Shigella* agar media was followed ***(25)***.

[2]C_t value is defined as the cycle at which a significant increase in fluorescence is first recorded.

Bio-Rad Laboratories) closely matches lysis reagent A for DNA extraction and performance during the real-time PCR assay ***(7)***.

3. In case of fatty foods, there may be a layer of oil/fat floating at the top. Immerse the pipette tip underneath the oil/fat layer that may have accumulated depending on food material being analyzed.
4. It is important to avoid bubbles at the bottom of each PCR tube (or PCR plate) by performing careful pipetting. To eliminate any bubbles from a PCR plate, hold the sealed plate in hand and force the solution to the bottom of the wells with a sharp single action movement.

5. In order to visualize correctly an amplification curve and set the threshold, it is recommended to change the graph properties from default linear scale to a semi-log scale (fluorescence units on the *Y*-axis on log-scale; PCR cycles on a linear scale on the *X*-axis).

References

1. Bhagwat, A. A. (2006) In *Microbiology of Fresh Produce* (Matthews, K. R., ed.), pp. 121–165, American Society for Microbiology, Washington, DC.
2. Bhagwat, A. A. and Lauer, W. (2004) Food borne outbreaks in raw produce can be prevented. *Food Quality* **11,** 62–63.
3. NACMCF (1999) National Advisory Committee on microbiological criteria for foods. Microbiological safety evaluations and recommendations on fresh produce. *Food Control* **10,** 117–143.
4. Feng, P. (1997) Impact of molecular biology on the detection of foodborne pathogens. *Molec. Biotechnol.* **7,** 267–278.
5. Bhagwat, A. A. (2003) Simultaneous detection of *Escherichia coli* O157:H7, *Listeria monocytogenes* and *Salmonella* strains by real-time PCR. *Int. J. Food Microbiol.* **84,** 217–224.
6. Hill, W. E. (1996) The polymerase chain reaction: applications for the detection of foodborne pathogens. *Crit. Rev. Food Sci. Nutr.* **36,** 123–173.
7. Bhagwat, A. A. (2004) Rapid detection of *Salmonella* from vegetable rinse-water using real-time PCR. *Food Microbiol.* **21,** 73–78.
8. Hines, E. (2000) PCR-based testing: unraveling the mystery. *Food Quality* **7,** 22–28.
9. Hoorfar, J. and Radstorm, P. (2000) Automated 5′ nuclease PCR assay for identification of *Salmonella enterica. J. Clin. Microbiol.* **38,** 3429–3435.
10. Liming, S. H. and Bhagwat, A. A. (2004) Application of a molecular beacon—real-time PCR technology to detect *Salmonella* species contaminating fruits and vegetables. *Int. J. Food Microbiol.* **95,** 177–187.
11. Liming, S. H., Zhang, Y., Meng, J., and Bhagwat, A. A. (2004) Detection of *Listeria monocytogenes* in fresh produce using molecular beacon- real-time PCR technology. *J. Food Sci.* **69,** 240–245.
12. Chen, W., Martinez, G., and Mulchandani, A. (2000) Molecular beacons: a real-time polymerase chain reaction assay for detecting *Salmonella. Anal. Biochem.* **280,** 166–172.
13. Tyagi, S. and Kramer, F. R. (1996) Molecular beacon: probes that fluoresce upon hybridization. *Nat. Biotechnol.* **14,** 303–308.
14. Nogva, H. K. and Lillehaug, D. (1999) Detection and quantification of *Salmonella* in pure cultures using 5′-nuclease polymerase chain reaction. *Int. J. Food Microbiol.* **51,** 191–196.
15. Kimura, B., Kawasaki, S., Fujii, T., Kusunoki, J., Itoh, T., and Flood, S. J. A. (1999) Evaluation of TaqMan PCR assay for detecting *Salmonella* in raw meat and shrimp. *J. Food Protect.* **62,** 329–35.
16. McKllip, J. L. and Drake, M. A. (2000) Molecular beacon polymerase chain reaction detection of *Escherichia coli* O157:H7 in milk. *J. Food Protect.* **63,** 855–859.

17. Higuchi, R., Fockler, C., Dollinger, G., and Watson, R. (1993) Kinetic PCR analysis: real-time monitoring of DNA amplification reactions. *Biotechnology* **11,** 1026–1030.
18. Chen, S., Yee, A., Griffiths, M., et al. (1997) The evaluation of a fluorogenic polymerase chain reaction assay for the detection of *Salmonella* species in food commodities. *Int. J. Food Microbiol.* **35,** 239–250.
19. Liao, C.-H. and Shollenberger, L. M. (2003) Detection of *Salmonella* by indicator agar media and PCR as affected by alfalfa seed homogenates and native bacteria. *Lett. Appl. Microbiol.* **36,** 152–156.
20. Miller, D. N. (2001) Evaluation of gel filtration resins for the removal of PCR-inhibitory substances from soils and sediments. *J. Microbiol. Methods* **44,** 49–58.
21. Heller, L. C., Davis, C. R., Peak, K. K., et al. (2003) Comparison of methods for DNA isolation from food samples for detection of Shiga toxin-producing *Escherichia coli* by real-time PCR. *Appl. Environ. Microbiol.* **69,** 1844–1846.
22. Shearer, A. E. H., Strapp, C. M., and Joerfer, R. D. (2001) Evaluationof a polymerase chain reaction- based system for detection of *Salmonella* Enteritidis, *Escherichia coli* O157:H7, *Listeria* spp., and *Listeria monocytogenes* on fresh fruits and vegetables. *J. Food Protect.* **64,** 788–795.
23. Patel, J. R., Bhagwat, A. A., Sanglay, G. C., and Solomon, M. B. (2006) Rapid detection of Salmonella from hydrodynamic pressure-treated poultry using molecular beacon real-time PCR. *Food Microbiol.* **23,** 39–46.
24. Gawande, P. V. and Bhagwat, A. A. (2002) Inoculation onto solid surfaces protect *Salmonella* spp. during acid challenge: a model study using polyethersulfone membranes. *Appl. Envron. Microbiol.* **68,** 86–92.
25. Andrews, W. H., June, G. A., Sherrod, P. S., Hammack, T. S., Amaguana, R. M., and Andrews, W. H. (1998) In *Bacteriological Analytical Manual* (Jackson, G. A., ed.), pp. 5.01–5.20, AOAC International, Gaithersburg, MD.