Letters in Applied Microbiology

Letters in Applied Microbiology ISSN 0266-8254

ORIGINAL ARTICLE

Detection of *Salmonella* spp. from large volumes of water by modified Moore swabs and tangential flow filtration

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Significance and Impact of the Study: Large-volume water samples may be screened for the presence of *Salmonella* both preseason and preharvest. This will provide better data from which to make risk management decisions to improve fresh produce safety. The time required to complete screening (2 days) will make it more practical to screen surface waters for *Salmonella* prior to use during produce production, to facilitate source tracking in root-cause determination or to determine risk associated with water nearby produce fields. The method enables the direct screening for pathogens in a timely manner, which avoids the need to rely on indicator or index organisms to evaluate food safety risks. Use of this method has the potential to decrease the risk of in-field fresh produce contamination.

Keywords
food safety, preharvest, produce, *Salmonella*, surface water.

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2013/1439: received 10 August 2012, revised 17 October 2012 and accepted 18 October 2012

doi:10.1111/lam.12016

Abstract

This study compares the use of tangential flow filtration (TFF), normal flow filtration and modified Moore swabs (MMS) for the concentration and detection of *Salmonella*, spiked at 1–760 CFU l^{-1}, from 10 l of surface water. Two immunomagnetic separation (IMS) methods, Pathatrix and Dynabeads, for further concentration of *Salmonella* were compared following filtration and overnight enrichment. Detection of *Salmonella* by PCR, qPCR or culture-based methods was compared. TFF and MMS preformed equally well in concentrating *Salmonella*. MMS was able to consistently concentrate *Escherichia coli* O157:H7 for culture-based detection; only at the higher concentrations tested was the TFF able to consistently concentrate *E. coli* O157:H7 for culture-based detection. *Salmonella*, at population densities <10 CFU l^{-1} in 10 l of spiked surface water, could be reliably (6/6) detected within 2 days by combining TFF or MMS, with IMS Pathatrix and qPCR. The theoretical limit of detection for *Salmonella* is considered to be sufficiently sensitive to meet all the practical screening purposes for surface waters in an agricultural setting intended for application to edible horticultural crops.

Introduction

Reliable screening of fresh produce for the presence of foodborne pathogens, including *Salmonella*, is difficult for multiple reasons. Challenges include collecting representative but manageable volumes of water for analysis, how and where to collect representative samples, and the time required for culture-based conformations of pathogens (i.e. *Salmonella*, up to 7 days; US FDA 1998). This interval may represent a significant portion of the shelf life of often highly perishable commodities, making test and hold screening financially and logistically untenable. Broadly viewed, the occurrence of pathogen contamination on produce items has been determined to be sporadic and heterogeneously distributed (Kase *et al.* 2012), rendering even a single composite sample an unreliable representation of the entire lot.

Testing preharvest water for *Salmonella* or *Escherichia coli* O157:H7 that comes into contact with, or is in close proximity to, produce production may be one component

of an effective strategy to implement a risk-based approach to enhance produce safety. Large-volume water samples can be concentrated and screened preseason and preharvest and may provide information for management decisions to reduce the risk of initial contamination. Current microbial water quality standards in the US produce industry rely on testing 100 ml of water for generic *E. coli* (Florida Department of Agriculture and Consumer Services 2007; Leafy Greens Marketing Agreement 2012) rather than pathogen populations. It is likely that pathogen contamination of surface water occurs at low rates (Rhodes and Kator 1988; Madsen 1994; Escartin *et al.* 2002; Haley *et al.* 2009; Ijabadeniyi *et al.* 2011); large-volume samples may be required to provide a more realistic representation of the risk of common pathogen presence in the body of water at levels relevant to the mode of application.

Various filtration methods can be used to concentrate large volumes of water. Normal flow filtration (NFF) is the standard water testing method of the American Public Health Association (APHA, American Public Health Association 1992), where 100 ml is passed through a sterile filter and then filter applied directly to an agar plate for the enumeration of coliforms. Alternative filtration methods include TFF where water flows across a filter membrane; Moore swabs (MS) where rolled cotton gauze is tied and allowed to sit in a body of water for at least 24 h; and modified Moore swabs (MMS) where pieces of pipe are filled with rolled cotton gauze, which traps bacteria as water is pushed through the pipe. Previously, up to 100 l of surface water has been concentrated using TFF (Mull and Hill 2009; Gibson and Schwab 2011), and MS has been used to isolate *Salmonella* (Spino 1966; Sears *et al.* 1984; Escartin *et al.* 2002), *E. coli* O157 (Ogden 2001), *Campylobacter* (Fernandez *et al.* 2003) and *Vibrio cholerae* (Barrett *et al.* 1980). MMS has been used to isolate *E. coli* O157 and *Salmonella* (Bisha *et al.* 2011).

Screening for the presence of pathogens is preferred to enumeration of indicator organisms in surface waters as no strong correlations between the presence and/or concentration of pathogens and indicator or index organisms have consistently been documented (Burton *et al.* 1987; Chandran and Hatha 2005; Pachepsky *et al.* 2011). The use of PCR-based methods for foodborne pathogen detection reduces testing times compared with cultural-based methods (Mafu and Sirois 2009). Real-time PCR (qPCR) has the added advantage of increased speed, sensitivity and minimizing post-PCR contamination (Balachandran *et al.* 2011; Delibato *et al.* 2011).

The objectives of this study were to compare (i) concentration and detection of *Salmonella* in large volumes (10 l) of surface water by TFF and NFF and (ii) concentration of *Salmonella* and *E. coli* O157:H7 by TFF and MMS.

Results and discussion

The public health impact of testing 100 ml water for generic *E. coli* concentrations currently used in US produce production is poorly established (Burton *et al.* 1987; Chandran and Hatha 2005; Erickson *et al.* 2010). This protocol originates with the US EPA standards for testing potable (direct consumption) and recreational (direct contact) waters, where potable water is required to have no detectable *E. coli* in 100 ml and in recreational waters, *E. coli* populations average 126 cells per 100 ml (rolling mean of five samples) and no one sample exceeds 235 cells per 100 ml (US Code of Federal Regulations 2012). The appropriateness of these standards for water used in produce production (indirect consumption) is unknown.

Concentration of Florida surface water (10 l) by NFF was not possible; the filter irreversibly clogged following *c.* 500 ml (data not shown) of the applied water sample passing through the filter under vacuum suction. Addition of filter aids (Whatman Grade 41 filter paper or diatomaceous earth) did not significantly increase the maximum volume of water filtered prior to irreversible clogging. No further efforts were made using NFF. While NFF is part of the APHA standard methods for screening surface water for various indicators and pathogens (APHA 1992), the recommended volume for screening is 100 ml. Screening higher volumes of water for pathogens may be necessary as pathogens likely are present at low levels (Rhodes and Kator 1988; Madsen 1994; Escartin *et al.* 2002; Haley *et al.* 2009; Ijabadeniyi *et al.* 2011).

The concentration and detection of *Salmonella* by TFF for surface water samples spiked with a five-strain cocktail of *Salmonella* are shown in Table 1. Detection of 1 CFU l^{-1} in a total of 10 l was consistently (6/6) obtained within 2 days when using the TFF, immunomagnetic separation (IMS) and qPCR method. The use of the modified conventional culture method following TFF concentration and overnight pre-enrichment detected *Salmonella* in five of six water samples spiked at 1 CFU l^{-1}. Plating IMS beads directly onto CHROMagar or XLT4 was not able to consistently detect *Salmonella* at spiked levels below 76 CFU l^{-1} (Table 1). Inconsistent frequency (3/6 at 1 CFU l^{-1} and 2/6 at 760 CFU l^{-1}) of detection was achieved when the IMS beads were screened by conventional PCR. Use of Dynabeads brand of IMS beads resulted in inconsistent rates of detection at all concentrations of *Salmonella* attempted. No *Salmonella* was detected when a 0·250-ml aliquot of the permeate was spread-plated on tryptic soy agar (TSA; Difco, Becton Dickinson, Sparks, MD, USA) supplemented with 50 mg l^{-1} nalidixic acid (TSAN; Sigma, St Louis, MO, USA) and incubated overnight, ensuring that the filter was not by-passed or unknowingly ruptured during usage.

Table 1 Percentage of *Salmonella*-positive samples of 10 l inoculated surface water, concentrated using TFF and pre-enriched in lactose broth evaluated using different detection techniques (n = 6)

	Detection technique*						
Inoculum concentration (CFU l^{-1})	Culture (%)	DB/qPCR (%)	DB/PCR (%)	DB/culture (%)	PTX/culture (%)	PTX/qPCR (%)	PTX/PCR (%)
1	83	100	17	33	67	100	50
7·5	100	100	33	33	67	100	17
76	100	83	67	100	100	100	50
760	100	100	67	83	100	100	33

IMS, immunomagnetic separation; TFF, tangential flow filtration.

*Detection techniques included culture, TT broth and RV broth enrichment, streaking onto XLT4 and *Salmonella* Plus CHROMagar; DB/qPCR, Dynabeads for IMS, DNA extraction and detection by real-time PCR (Ct < 35); DB/PCR, Dynabeads for IMS, DNA extraction, detection by conventional PCR (band on agarose gel at expected size; Malorny *et al.* 2003); DB/Culture, Dynabeads for IMS, beads spread onto XLT4 and *Salmonella* Plus CHROMagar; PXT/Culture, Pathatrix used for IMS, beads spread to XLT4 and *Salmonella* Plus CHROMagar; PTX/qPCR, Pathatrix beads for IMS, DNA extraction, detection by real-time PCR; PTX/PCR, Pathatrix beads for IMS, DNA extraction, detection by conventional PCR.

Tangential flow filtration has been successfully used to screen large volumes of surface water for the presence of specific pathogens. A major advantage of TFF over NFF is that the flowing retentate removes the filter cake during the concentration run, increasing the volume before the filter clogs and allowing greater volumes of water to be processed (Gibson and Schwab 2011). Several previous studies have shown that concentration of large volumes of surface water, when coupled with downstream cultural or molecular detection methods, have led to sensitive recovery and detection of the target bacteria. For example, using 100 l surface water, a recovery efficiency rate of 70·5% for *E. coli* (starting concentration 10–100 CFU l^{-1}) coupled with a culture-based assay has been reported (Gibson and Schwab 2011). In another example, TFF, coupled with continuous centrifugation and IMS followed by qPCR, can detect 100% of *E. coli* O157:H7 at 50 MPN per 40 l of surface water (Mull and Hill 2009). A hollow-fibre ultrafiltration (similar to TFF, but with a dead end) recovered up to 93% when 3 log CFU of *Enterococcus faecalis* was inoculated into 100 l of tap or surface water with low turbidity (0·29 NTU; Smith and Hill 2009); recovery of *E. faecalis* decreased to 78% when the turbidity increased to 4·3 NTU.

Moore swabs have previously been used to isolate *Salmonella* from surface (Spino 1966; Sears *et al.* 1984) and fountain water (Escartin *et al.* 2002), *E. coli* O157 from soil and field water (Ogden 2001), *Campylobacter* from river water (Fernandez *et al.* 2003) and *V. cholerae* from sewage (Barrett *et al.* 1980). In these cases, MS was secured in the body of water for a set duration of time, rather than pumping a specified volume of water through the swab.

A comparison of MMS to TFF using nonsterile saline followed by the detection of the *Salmonella* cocktail of three fluorescent protein–expressing strains and similar *E. coli* O157:H7 cocktail (cocktail previously described by Bisha *et al.* (2011)) is shown in Table 2. MMS is consistently able to capture *E. coli* O157:H7 for subsequent detection. The TFF results were more variable, with only the highest concentration of *E. coli* O157:H7 detected in all samples. *Salmonella* was detected by TFF in samples containing $\geq$10 000 CFU l^{-1} in 10 l but in none of the samples containing >100 CFU l^{-1} in 10 l of *Salmonella*. Only at the highest concentration was MMS able to concentrate *Salmonella* to the level of detection in all samples. Under no conditions was the process of concentration by TFF followed by pathogen detection more effective than that by MMS. Similarly, Bisha *et al.* (2011) report detecting concentrations of 100 CFU l^{-1} of *E. coli* O157:H7 and *Salmonella* using MMS and continuous flow centrifugation on 10 l of irrigation water.

Due to the poor detection of *Salmonella* below 10 000 CFU l^{-1} in 10 l when comparing MMS to TFF, a

Table 2 Percentage of *Salmonella*-positive samples of 10 l inoculated nonsterile saline, preconcentrated by TFF or MMS and detected by cultural means* (n = 3)

Pathogen	Inoculum concentration (CFU l^{-1})	MMS (%)	TFF (%)
Salmonella spp.	137	67	0
	1366	67	33
	13 660	67	100
	136 604	100	100
Escherichia coli O157:H7	86	100	33
	865	67	67
	8652	100	33
	86 524	100	100

MMS, modified Moore swabs; TFF, tangential flow filtration.

*Detection by cultural means involved the liquid retentate being passed through a normal filter, 0·45-μm pore-size, 47 mm diameter, the filter placed on TSAA and incubated for 24 $\pm$ 2 h at 35 $\pm$ 2°C. A positive sample is noted by the presence of a fluorescing colony that also gave positive results from the appropriate REVEAL test.

final set of experiments comparing MMS to TFF, using the optimized TFF approach for *Salmonella*, were carried out. When surface water was inoculated with the *Salmonella* cocktail – expressing fluorescent proteins, at 2 CFU l^{-1}, concentrated using either MMS or TFF, and coupled with the modified conventional FDA-BAM culture method and IMS using the Pathatrix beads followed by culture methods or qPCR – *Salmonella* was detected from all samples ($n = 6$, Table 1). Using more rigorous *Salmonella* detection methods, both MMS and TFF concentration protocols performed equally well in detecting low levels of *Salmonella* (<10 CFU l^{-1}) from spiked 10 l surface water samples. Replacing the TFF with MMS does not alter the detection of *Salmonella* spp. in the optimized method ($P = 0{\cdot}4795$, McNemar's). The use of MMS over TFF is advantageous for the produce industry as MMS are inexpensive, simple to use, can be used in the field rather than collecting and returning large volumes of water to the laboratory, and require minimal operator prerequisite skill base and training. Enrichment of the MMS rather than the liquid wrung out of the MMS, as performed here and by Bisha *et al.* (2011), may further increase the sensitivity.

Reproducible detection of *Salmonella* in 10 l of surface water was accomplished within 2 days using TFF (Table 1 and 2) or MMS (Table 2) concentration, pre-enrichment in lactose broth, further concentration by IMS and detection by qPCR. From the TFF methods attempted, the Pathatrix–qPCR method was significantly better, by McNemars, at *Salmonella* detection at all spiking levels than Pathatrix–conventional PCR ($P = 0{\cdot}0003$); Pathatrix–culture ($P = 0{\cdot}0077$); and Dynabeads–conventional PCR ($P = 0{\cdot}0009$). Levels as low as 1 CFU l^{-1} in 10 l were reliably detected in spiked surface waters (Table 1). *Salmonella* populations at levels below 1 CFU l^{-1} may be below a significance threshold level; the detection of *Salmonella* at low levels may provide a more substantial public health impact than screening for *E. coli* in 100-ml samples as required by current produce industry standards (Florida Department of Agriculture and Consumer Services 2007; Leafy Greens Marketing Agreement 2012).

The detection limit described here is believed to be low enough to meet all practical testing purposes for surface waters used in the produce industry, where the requirement is no detectable *E. coli* in 100 ml (Florida Department of Agriculture and Consumer Services 2007). The method described here for screening large volumes of surface water enables larger, more representative samples to be evaluated for *Salmonella* presence. Detection of *Salmonella* within 2 days following sampling is advantageous in determining food safety risks associated with surface waters and may be advantageous for the produce industry in avoiding preharvest contamination.

Materials and methods

Bacterial strains and preparation of inoculum

Two different *Salmonella* cocktails were used in the experiments. NFF and TFF optimization was carried out using *Salmonella* serovars Saintpaul (BAC 133), Newport (C4.2), Anatum (LRB6802), Montevideo (LJH0654) and Typhimurium (LJH0738) resistant to nalidixic acid (50 mg l^{-1}). For the comparison of MMS and TFF, strains identical to those used by Bisha *et al.* (2011) were used, including *Salmonella* serovars Montevideo (MDD 22 pGFP), Poona (MDD 237 pDsRed) and Newport (MDD 314 pAMCyan) and three *E. coli* O157:H7 stains (PVTS 016 pGFP, PVTS 087 pDsRed and PVTS 088 pAMCyan), all transformed to express a differential fluorescent protein moiety and selectable ampicillin resistance at 20 mg l^{-1}, as previously described (Bisha *et al.* 2011). All isolates are available from the culture collection of Dr Michelle Danyluk.

Frozen stocks, stored at −80°C in tryptic soy broth (TSB; Difco, Becton Dickinson) with 15% glycerol, were streaked onto TSAN or TSA with 20 mg l^{-1} ampicillin (TSAA; Sigma) and incubated for 24 ± 2 h at 35 ± 2°C. An isolated colony was transferred into TSB supplemented with nalidixic acid (TSBN) or ampicillin (TSBA) and incubated for 24 ± 2 h at 35 ± 2°C. A 10-μl loop of the culture was transferred to TSBN or TSBA and incubated for 24 ± 2 h at 35 ± 2°C. Cultures were combined in equal parts and diluted in 0·1% peptone water (Difco, Becton Dickinson). Following addition to the 10-l water sample, the inoculum was distributed through the water by swirling in a 10-l carboy in a 30-cm circle for 30 s.

Inoculum concentrations were determined by serial dilutions in 0·1% peptone water, plated onto TSAN or TSAA and incubated for 24 ± 2 h at 35 ± 2°C. Target concentrations were 10^0–10^3 CFU l^{-1} for NFF and TFF optimization, 10^2–10^5 CFU l^{-1} for MMS and TFF comparison, and 10^0 CFU l^{-1} for MMS validation.

Collection of water samples

For NFF and TFF optimization and MMS validation, surface water (10 l) was collected from Lake Swoope, in the town of Lake Alfred, Florida, USA, and stored for no longer than 24 h at 4°C prior to use. For MMS and TFF comparison, 10 l of nonsterile 0·85% saline was used to emulate the methodology of Bisha *et al.* (2011).

Normal filtration

Vacuum filtration was used to attempt passage of 10 l of water, through a 0·45-μm pore-size filter (Millipore, Billerica, MA, USA). Whatman Grade 41 (Fisher Scientific,

Bridgewater, NJ, USA) filter paper and diatomaceous earth (1 g; Fisher Scientific) were evaluated separately as filtration aids.

Tangential flow filtration

The tangential flow filter used was a Mini Kros Plus Tangential Flow Filter Module (Spectrum Labs, Rancho Dominguez, CA, USA) composed of polyethersulfone with a nominal pore rating of 0·2 μm and a surface area of 1050 cm^2. Filter and tubing sets were sterilized prior to each experiment. All tubing was L/S 17, constructed of silicone with platinum coating (Cole-Palmer, Vernon Hills, IL, USA). A KrosFlo Research II Pump was used in combination with Masterflex easy-load pump head (Spectrum Labs). The inlet flow rate was standardized at 1000 ml min^{-1}. The TFF was run at a transmembrane pressure of 67 Pa. The initial 10 l of water was concentrated to a final retentate volume of ca. 250 ml.

Modified Moore swabs

The MMS was constructed out of PVC pipe (12 × 4·4 cm diameter; Home Depot, Winter Haven, FL, USA) with 2·54-cm PVC connector to a 2·54-cm male-to-male coupler on each end. Inside the PVC pipe was presterilized 80 × 22 cm cheesecloth (Fisher Scientific; Grade No. 90) folded lengthwise and rolled. A 10-l water sample was passed through the MMS at a flow rate of 300 ml min^{-1}. The use of MMS here was introduced as a proof of concept of another concentration means for pathogens, *Salmonella* and *E. coli* O157:H7, from water; further experiments were not performed using *E. coli* O157:H7.

Conventional *Salmonella* detection using NFF and TFF

A modified conventional *Salmonella* isolation method (US FDA 1998) was used. For NFF, the finished filter was placed into 50 ml of lactose broth (Difco, Becton Dickinson), and for TFF, a 1 : 1 volume of double-strength lactose broth was added to the retentate. The inoculated lactose broth was allowed to incubate for 1 h at room temperature, before the addition of 50 μg l^{-1} nalidixic acid or 20 μg l^{-1} of ampicillin and incubation for 24 ± 2 h at 35 ± 2°C. A 1-ml aliquot of the incubated lactose pre-enrichment was transferred to tetrathionate broth (TT broth; Difco, Becton Dickinson) and Rappaport Vassiliadis R10 broth (RV broth; Difco, Becton Dickinson) and incubated at 35 ± 2°C for 24 ± 2 h and 41 ± 2°C for 48 ± 2 h, respectively. Following incubation, 10 μl was streaked onto Xylose Lysine Tergitol-4 agar (XLT4; Difco, Becton Dickinson) and CHROMagar *Salmonella* Plus (DRG International Inc, Mountainside, NJ, USA). Both media were incubated for 24 ± 2 h at 35 ± 2°C; colonies were confirmed on lysine iron agar slants (LIA; Difco, Becton Dickinson) and triple sugar iron agar slants (TSI; Difco, Becton Dickinson).

Immunomagnetic separation of *Salmonella* during TFF optimization

Salmonella was captured on antibody-coated paramagnetic beads using either Pathatrix (Matrix MicroScience, Golden, CO, USA) or Dynabeads (Invitrogen, Grand Island, NY, USA) microspheres. The entire lactose broth pre-enrichment was used in the Pathatrix by replacing the 100-ml conical tube that came with the purchased set-up, with a sterile plastic stomacher bag. Either Pathatrix or Dynabeads (50 μl) were added and used according to the manufacturers' instructions on the pre-set 30-min circulation cycle. Following IMS, beads (10 μl, each) were plated in duplicate onto XLT4 and CHROMagar *Salmonella* Plus; colonies were confirmed on LIA and TSI as described above.

Detection of *Salmonella* during TFF optimization

DNA was extracted from the remaining IMS beads (50 μl) using the MoBio UltraClean DNA kit (MoBio, Carlsbad, CA, USA). DNA was used to detect *Salmonella* by PCR (*invA* and *oriC* genes) or by qPCR, using Applied Biosystems' MicroSEQ *Salmonella* spp. detection kit (Applied Biosystems, Carlsbad, CA, USA) according to the manufacturer's instructions in a Bio-Rad CFX96 Real-time thermocycler.

The *invA* and *oriC* primer sets were used as described previously by Malorny *et al.* (2003). The conventional PCR was optimized using overnight cultures of the *Salmonella* cocktail used in the TFF optimization approach. All reagents were obtained from the Fisher exACTGene Complete PCR kit (Fisher Scientific). Primers were used at a concentration of 20 μmol l^{-1}. The PCR mix was as follows: 34·75 μl water, 5 μl 10× PCR buffer, 1 μl of each primer, 0·25 μl Taq DNA polymerase and 5 μl template DNA. The optimized PCR conditions were as follows: melting for 3 min at 94°C, followed by 30 cycles of 30 s at 94°C, 30 s at 58°C, 1 min at 72°C and a final elongation of 5 min at 72°C. Gel electrophoresis was carried out using a 1·8% agarose gel with 0·5× TBE buffer. DNA was stained with ethidium bromide (1 μg ml^{-1}), and amplicons were visualized and imaged under UV light (MultiDoc-It Digital Imaging System; UVP, Upland, CA, USA). PCR amplicon size was determined by comparison with molecular size markers (Hyperladder V; Bioline USA Inc., Randolph, MA, USA).

Detection of pathogens from MMS

Following MMS filtration, the cheesecloth was removed and placed in a stomacher bag. Excess liquid (*c.* 25–35 ml) was wrung out of the cheesecloth and passed through a 0·45-μm pore-size, 47-mm-diameter filter using NFF vacuum filtration. The filter was placed on TSAA and incubated for 24 ± 2 h at 35 ± 2°C. Colonies were checked for fluorescence and confirmed by REVEAL *Salmonella* or *E. coli* O157:H7 lateral flow device (Neogen Corp., Lansing, MI, USA).

Validation of MMS concentration

Modified Moore swabs and TFF were used to concentrate a 10^0 CFU l^{-1} *Salmonella* spiked 10-l water sample, and retentates pre-enriched in lactose broth. The entire pre-enrichment was used in the Pathatrix system with the Pathatrix beads (50 μl); beads (10 μl) were plated to XLT4 and CHROMagar *Salmonella* Plus and typical colonies confirmed. Remaining beads (40 μl) were used in DNA extraction, and qPCR was performed, all as described above.

Data analysis

Experiments evaluating TFF and detection methods were repeated six times for each dilution reported. Experiments comparing MMS with TFF were repeated in triplicate. Data were analysed using McNemar's test using GraphPad (GraphPad Software Inc., LaJolla, CA, USA).

Acknowledgements

This work was funded by USDA NIFA SCRI 2008-51180-04846 and a UF Alumni Graduate Award. The technical assistance of Loretta Friedrich, Gwen Lundy, Luis Martinez and Lisseth Proano is appreciated.

References

APHA, American Public Health Association. (1992) *Standard Methods for the Examination of Water and Wastewater*, 18th edn. Washington, DC: American Public Health Association.

Balachandran, P., Cao, Y., Wong, L., Furtado, M.R., Petrauskene, O.V. and Tebbs, R.S. (2011) Evaluation of applied biosystems MicroSEQ (R) real-time PCR system for detection of *Salmonella* spp. in food. *J AOAC Int* **94**, 1106–1116.

Barrett, T.J., Blake, P.A., Morris, G.K., Puhr, N.D., Bradford, H.B. and Wells, J.G. (1980) Use of Moore swabs for isolating *Vibrio cholerae* from sewage. *J Clini Microbiol* **11**, 385–388.

Bisha, B., Perez-Mendez, A., Danyluk, M.D. and Goodridge, L. D. (2011) Evaluation of modified Moore swabs and continuous flow centrifugation for concentration of *Salmonella* and *Escherichia coli* O157:h7 from large volumes of water. *J Food Prot* **74**, 1934–1937.

Burton, G.A., Gunnison, D. and Lanza, G.R. (1987) Survival of pathogenic bacteria in various freshwater sediments. *Appl Environ Microbiol* **53**, 633–638.

Chandran, A. and Hatha, A.A.M. (2005) Relative survival of *Escherichia coli* and *Salmonella typhimurium* in a tropical estuary. *Water Res* **39**, 1397–1403.

Delibato, E., Fiore, A., Anniballi, F., Auricchio, B., Filetici, E., Orefice, L., Losio, M.N. and De Medici, D. (2011) Comparison between two standardized cultural methods and 24 hour Duplex SYBR Green Real-Time PCR assay for *Salmonella* detection in meat samples. *New Microbiol* **34**, 299–305.

Erickson, M.C., Webb, C.C., Diaz-Perez, J.C., Phatak, S.C., Silvoy, J.J., Davey, L., Payton, A.S., Liao, J. *et al.* (2010) Surface and internalized *Escherichia coli* O157:H7 on field-grown spinach and lettuce treated with spray-contaminated irrigation water. *J Food Prot* **73**, 1023–1029.

Escartin, E.F., Lozano, J.S., Garcia, O.R. and Cliver, D.O. (2002) Potential *Salmonella* transmission from ornamental fountains. *J Environ Health* **65**, 9–12.

Fernandez, H., Otth, L. and Wilson, M. (2003) Isolation of thermotolerant species of *Campylobacter* from river water using two collection methods. *Arch Med Vet*, **35**, 95–97.

Florida Department of Agriculture and Consumer Services. (2007) Tomato best practices manual. A guide to tomato good agricultural practices (T-GAP) and tomato best management practices (T-BMP). Available at http://www.freshfromflorida.com/fs/TomatoBestPractices.pdf (accessed 10 Jun 2012).

Gibson, K.E. and Schwab, K.J. (2011) Tangential-flow ultrafiltration with integrated inhibition detection for recovery of surrogates and human pathogens from large-volume source water and finished drinking water. *Appl Environ Microbiol* **77**, 385–391.

Haley, B.J., Cole, D.J. and Lipp, E.K. (2009) Distribution, diversity, and seasonality of waterborne *Salmonellae* in a rural watershed. *Appl Environ Microbiol* **75**, 1248–1255.

Ijabadeniyi, O.A., Debusho, L.K., Vanderlinde, M. and Buys, E. M. (2011) Irrigation water as a potential preharvest source of bacterial contamination of vegetables. *J Food Saf* **31**, 452–461.

Kase, J.A., Borenstein, S., Blodgett, R.J. and Feng, P.C.H. (2012) Microbial quality of bagged baby spinach and romaine lettuce: effects of top versus bottom sampling. *J Food Prot* **75**, 132–136.

Leafy Greens Marketing Agreement. (2012) Commodity specific food safety guidelines for the production and harvest of lettuce and leafy greens. Available at http://www.caleafygreens.ca.gov/sites/default/files/01.20.12%20CALGMA%20GAPs%20-%20metrics.pdf (accessed 13 Jun 2012).

Madsen, M. (1994) Enumeration of *Salmonellae* in crocodile pond water by direct plate counts and by the MPN technique. *Water Res* **28**, 2035–2037.

Mafu, A.A. and Sirois, S. (2009) Real-time PCR as a tool for detection of pathogenic bacteria on contaminated food contact surfaces by using a single enrichment medium. *J Food Prot* **72**, 1310–1314.

Malorny, B., Hoorfar, J., Bunge, C. and Helmuth, R. (2003) Multicenter validation of the analytical accuracy of *Salmonella* PCR: towards an international standard. *Appl Environ Microbiol* **69**, 290–296.

Mull, B. and Hill, V.R. (2009) Recovery and detection of *Escherichia coli* O157:H7 in surface water, using ultrafiltration and real-time PCR. *Appl Environ Microbiol* **75**, 3593–3597.

Ogden, I.D. (2001) The fate of *Escherichia coli* O157 in soil and its potential to contaminate drinking water. *Int J Food Microbiol* **66**, 111–117.

Pachepsky, Y., Shelton, D.R., McLain, J.E.T., Patel, J. and Mandrell, R.E. (2011) Irrigation waters as a source of pathogenic microorganisms in produce: a review. *Adv Agron* **113**, 73–138.

Rhodes, M.W. and Kator, H. (1988) Survival of *Escherichia coli* and *Salmonella* spp. in estuarine environments. *Appl Environ Microbiol* **54**, 2902–2907.

Sears, S.D., Ferreccio, C., Levine, M.M., Cordano, A.M., Monreal, J., Black, R.E., D'Ottone, K. and Rowe, B. (1984) The use of Moore swabs for isolation of *Salmonella* typhi from irrigation water in Santiago, Chile. *J Infect Dis* **149**, 640–642.

Smith, C.M. and Hill, V.R. (2009) Dead-end hollow-fiber ultrafiltration for recovery of diverse microbes from water. *Appl Environ Microbiol* **75**, 5284–5289.

Spino, D.F. (1966) Elevated-temperature technique for the isolation of *Salmonella* from streams. *Appl Microbiol* **14**, 591–596.

US Code of Federal Regulations. (2012) Title 40 Protection of the Environment. Available at http://ecfr.gpoaccess.gov/cgi/t/text/text-idx?c=ecfr&sid=4069b93e5cf3d85662170ccf95ca91b2&rgn=div8&view=text&node=40:23.0.1.1.18.4.16.11&idno=40 (accessed 13 July 2012).

US FDA, United States Food and Drug Administration. (1998) *Salmonella*. In: Bacteriological Analytical Manual, Chapter 5. Available at http://www.fda.gov/Food/ScienceResearch/LaboratoryMethods/BacteriologicalAnalyticalManualBAM/ucm070149.htm. (accessed 12 Dec 2011).