CENTER for PRODUCE SAFETY

CPS 2011 RFP – Pilot Project
FINAL PROJECT REPORT

Project Title

On-farm evaluation of the prevalence of human enteric bacterial pathogens during the production of melons in California

Project Period

March 1, 2012 – September 30, 2012

Principal Investigator

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Objectives

1. Assessment of the microbiological quality of melons, including the presence of human bacterial pathogens, *Escherichia coli* O157:H7, including other Shiga-toxin producing *E. coli*, *Salmonella enterica*, *Listeria* spp. (the standard indicator used in facility environmental testing), and *Listeria monocytogenes*.
2. Evaluation of a probe based *Enterococcus* quantification system as an improved fecal indicator bacteria and source-tracking tool for prevalence of fecal contamination.
3. Analysis of the correlation between the potential presence of human pathogens and fecal indicators with the geographical, seasonal, practice-specific inputs, and environmental conditions.

FINAL REPORT

Abstract

A Pilot Study was initiated under funding by the Center for Produce Safety to gather preliminary baseline data on the prevalence of shiga-toxin positive *E. coli* (both EHEC and STEC), *Salmonella enterica*, and *Listeria monocytogenes* in California cantaloupe production districts. This study was co-funded by the California Melon Research Board (CMRB). Evaluation of presumptive indicators including fecal coliform, *E. coli*, Enterococcus, and Listeria spp was included in what became a regional survey (San Joaquin Valley). The primary purpose of this study was to gather information on the probability of pathogen detection at shipping point towards a future, more extensive longitudinal survey and assessment of the practical validated of recommending routine product testing as a Best Practice. Approximately 200 fruit per lot and 1800 cantaloupe in total were tested between July and October 2012. At the time of this report, only one lot tested positive for *Salmonella enterica* and one positive for the indicator *Listeria innocua* but not *L. monocytogenes*. At the time of this report, no samples have resulted in a confirmed positive for pathogenic and toxigenic *E. coli*. All pre-enrichment inoculated rind controls, for internal pathogen detection capabilities per lot were positive once increased to 100 CFU/sample for *E. coli* O157:H7, *Salmonella enterica* sv. Typhimurium, and *L. monocytogenes*. All preliminary screens of composite enrichments, conducted collaboratively for Salmonella and Listeria spp. by Roka Biosciences, including the blind-coded inoculated controls were confirmed as positive by secondary enrichment qPCR and cultural methods. Outcomes indicate that a low level of environmental contamination, at least by Salmonella, may be present in California production lots but the infrequency would appear to preclude routine levels of fruit testing as a measure of product safety. It is highly recommended that an expanded survey be conducted that expands the regional and seasonal baseline for pathogen prevalence.

Background

In view of the anticipated and evolving expectations for industry performance in protecting public health through preventive controls, embodied by the Food Safety Modernization Act and the FDA Produce Safety Rule soon to be released, it is essential to begin to assess the prevalence of key pathogens across each supply-chain. One such industry and public health stakeholder-group, currently in the process of a detailed reassessment and re-design of food safety systems, are the cantaloupe and netted melon commodities. The natural

prevalence of foodborne pathogens on melons in the major production regions has been recently identified by the supply and retail industry as essential to the establishment of meaningful and verifiable industry-wide food safety standards to regain and enhance consumer confidence so badly shaken by the listeriosis tragedy of 2011. To address this knowledge gap, this pilot study was suggested by the California Melon Research Board and California Cantaloupe Advisory Board as a priority to expand the available survey-data for a pathogen prevalence baseline, to include EHEC/STEC and *Listeria monocytogenes*, and to assess the microbiological quality of melons produced in California, a major production area of the United States. The key objective was to initiate a preliminary data gathering effort on the prevalence of two foodborne pathogens that have been involved in major produce associated outbreaks on cantaloupe outside of California; *Salmonella enterica* and *L. monocytogenes.* Our goal was to acquire this timeframe-limited data set to use in future applications for a longer-term and longitudinal survey of pathogen prevalence in the farm-scape and specific frequency of detectable association with melons distributed into marketing channels.

One of the widely pronounced speculative “facts” regarding pathogen prevalence on cantaloupe, pronounced by various pundits, which has stuck in many public and private sectors in a position to dictate food policy, is the certainty of the ubiquitous prevalence of *Salmonella* and, especially *Listeria monocytogenes*, in production environments and on fruit. This provoked a number of ‘knee-jerk’ reactions in setting supplier requirements that may not actually be contributing to reducing food safety risk. No such scientific certainty exists that can be applied to all agro-ecoregions and primary melon shipping points. In fact, the available data on the prevalence of *Salmonell*a on cantaloupe, complied within the 2005-2009 reports from the USDA Microbiological Data Program (USDA MDP) disclose that the detectable level is less than 10 in 8,000 for all samples and, essentially, 1 in 1,800 samples from California shipping points, specifically. In contrast, little public information is available that adequately characterizes the association of *Listeria* spp. and more significantly, *L. monocytogenes* in melon production environments and on fruit shipped to commercial marketing channels. It seems critical to address this knowledge gap, at least in establishing a baseline of evidence, before rational policies and measures may be designed and are adopted as industry standards and Best Practice guidance or mandatory programs. This data gathering is viewed as vital to establish meaningful and verifiable industry-wide food safety standards regarding the microbiological quality during melon production and marketing to regain and enhance consumer confidence so badly shaken by the 2011 cantaloupe:listeriosis tragedy.

Thus, the anticipated outcomes of this Pilot Study will result in the beginning of a public information database to define the degree of risk exposure to consumers and direct focus on point and non-point sources of contamination. Alternatively, an outcome of regionally defined low risk exposure expectation also carries significant public benefit.

Research Methods and Results

Preliminary Methods Assessment: Prior to beginning this Pilot Study, sample cantaloupe composites were mixed with 0.1M sodium phosphate supplemented with 0.05 % Tween 20 (Fisher, Fair Lawn, NJ) at a 2:1 ratio (v/w). The suspension was gently shaken and then allowed to settle for 20 min (Gutierrez-Rodriguez et al. 2012; Lopez-Velasco et al. 2012). An aliquot of 10 mL of the supernatant was transferred to 90 mL of the following media: Listeria enrichment broth (LEB; Oxoid), LEB supplemented with Listeria broth (LIS; Oxoid) and LIS supplemented with 40 mg/L of nalidixic acid, 10 mg/L amphotericin B and 15 mg/L of acriflavine hydrochloride (Oxoid). In addition inoculated controls were prepared by adding 10^2 or 10^5 CFU of *Listeria monocytogenes* (strain PTVS 333) to 100 mL of the media described above.

For samples and controls containing the supplements, they were first incubated for 6 hours at 35^{o}C previous to the addition of the supplements. All samples and controls were incubated for up to 18 h at 35^{o}C. After enrichment samples were stored in FTA cards (Whatman ®) following manufacturer instructions, in addition a 200 mL of the sample was boiled at 95^{o}C for 10 min. Samples were also prepared to be analyzed through BAX Listeria system (Dupont^{TM}) thus the sample lysate protocol and detection were done following manufacturer instructions. Boiled samples, lysates obtained for BAX and FTA cards were analyzed by real time PCR to detect *Listeria spp*. according to Rodriguez-Lazaro et al. (2004) after 48 of sample enrichment. In addition FTA cards were re-analyzed to detect *Listeria spp*. after 30 days. LEB with supplements was chosen for cantaloupe prevalence studies (Table 1)

Sample collection

For this Pilot Study, commercially packed cantaloupe was obtained a shipping point to assess microbiological objectives of the project after all touch-points, contact-points, and postharvest handling practices had been completed prior to release to the distribution supply-chain. Packed cartons, 180-200 cantaloupes per time point, were collected on-site by the

regional cooperators or the PI and transported to UC Davis Mann Lab in covered vehicles within 12h. After arrival, melons were held at 5°C for a period no greater than 24 h before analysis.

Sample preparation and quantification of fecal indicators

Samples were enriched for qualitative pathogen detection; for ease of descriptive overview a schematic is depicted in Figures 1 and 2. A composite of three cantaloupe fruit per replicate were peeled, without washing, using a disinfected knife and transferred to a sterile bag containing 500 mL of double-strength Universal Preenrichment Broth (UPB) supplemented with 0.05% Tween 20 as a surfactant. Samples were vigorously massaged by hand for one minute to aide in detaching indicator bacteria. An aliquot of 2 mL was removed and transferred to a sterile micro-centrifuge tube for quantification of coliforms and *E. coli* using Chrom-ECC agar after plating and incubation at RT for 2h and 42^{o}C for 24 h (evaluated again at 48h for additional colony development). The remaining wash suspension, including the entire excised rind, was enriched for pathogen detection as described below.

An additional volume of 500 ml 2XUPB was added to the rind sample with additional hand massaging. UPB non-selective pre-enrichments were incubated at 37°C for 18 hours to favor bacterial recovery after environmental stress. This procedure has been successfully implemented and tested in our laboratory and it allows us to analyze a single sample for three different pathogens.

After pre-enrichment, aliquots of 10 mL will be transferred 90 mL of tetrathionate broth (TBB), samples will be incubated for 6 h at 42^{o}C. After selective pre-enrichment, 20 mL of the TBB enrichment will be transferred to 180 mL of Bacto M broth and incubated at 37^{o}C for 18 hours. For *L. monocytogenes* 10 mL of enrichment was transferred to 90 mL of modified Listeria enrichment broth (mLEB) with supplements {40 mg/L of nalidixic acid, 50 mg/L cyclohexamide and 15 mg/L of acriflavine hydrochloride (Oxoid)} and incubated for 48 h at 35^{o}C. After enrichment, the resultant samples were composited by combining 1 mL of each sample, up to 5 samples. Composites are mixed and utilized for pathogen detection. We have applied this approach in previous studies for environmental risk assessment and, after pooling 10 negative samples with one known individual positive, we have been able to detect the environmental pathogens by qPCR screening, molecular confirmation, and/or culture confirmation. This approach has been utilized in our lab for various leafy greens as well as for on-farm samples of cantaloupes. In this way we seek to maximize the number of fruit tested per location in our

prevalence survey and efficiently using the resources allocated to the pathogen detection assays.

Preparation of positive controls

During each sampling event, a set of 2-3 composites was segregated for inoculations to be used as a positive control once all other sample processing had been completed. After addition of 1L of UPB 2X, approximately 100 CFU total of *Salmonella enterica* (strain PTVS177), 100 CFU total for *E. coli* O157:H7 (PTVS 155),and 100 CFU total of *Listeria monocytogenes* (strain PTVS 335) were added to the bags. The protocol for non-selective and selective enrichment as well as colony confirmation was followed as described above.

Colony confirmation and molecular detection

Preliminary screening for molecular detection of EHEC was conducted by in-house qPCR tests for target genes eaeA, stx1, stx2, rfbE, and hylA. Preliminary screening for molecular detection of both *Listeria spp.* and *Salmonella enterica* was performed by Roka Bioscience ® using the Atlas detection Assays ™ which utilize ribosomal RNA (rRNA) targeting for the detection of the pathogen, followed by transcription mediated amplification. Post-enrichment samples are stabilized in the Suslow lab and shipped within 2 days, following the prescribed methods, to Roka facilities for analysis. After analysis, samples that resulted in a positive detection are additionally analyzed using the BAX system (Dupont Qualicon) following manufacturer instructions and our in-house real time qPCR protocols using TaqMan probes. At the time of preparing this progress report, an analysis of 10% of all negative samples, to assess the potential for false negatives in the primary screening, from the various time-points is being conducted using qPCR-based and cultural methods in the Suslow lab.

For those samples with positive detection for EHEC or Salmonella, the enrichment stored in glycerol at -80^{o}C was partially thawed and 500µL are transferred to 10 mL of buffered peptone water (BPW) and incubated at 37^{o}C for 12 hours. After this time, 200µL of the sample are boiled at 95^{o}C for 10 min in preparation for detection of pathogenicity markers by real time PCR (RT-PCR) and a total of 5µL of the enriched sample are used for detection through the manufacturer-specified protocols for the BAX system. Additionally a loop that carries approximately 20µL of the enriched sample is streaked on Xylose Lactose Tergitol agar (XLT-4) and incubated at 37^{o}C for up to 48 h; typical black colonies are purified for further taxonomic confirmatory analysis. Using this technique, weak or non-black colonies may also be selected,

depending on the collective outcomes of PCR and cultural testing as non-H2S forming *Salmonella enterica* (eg. sv. Heidelberg and Anatum) have been recovered from these regions.

For samples in which *Listeria spp.* was detected through the Roka Atlas system, 100µL of the enrichment previously stored in glycerol at -80^{o}C are transferred to 10 mL of Tryptic soy broth (TSB) and incubated at 35^{o}C for 6 hours, after this time the complete 10 mL of the culture is transferred to 90 mL of LEB with antibiotic supplement and incubated for an additional 12-14 h at 35^{o}C. After incubation, 5µL of the sample are subjected to a lysis protocol following the technical instructions for BAX sample preparations. These lysates are analyzed through BAX and real time PCR to detect *Listeria spp.*. Additionally, a loop which carries approximately 20µL of the enriched sample is streaked onto Oxford agar supplemented with antibiotics and on R&F Listeria selective/differential media; typical colonies are purified by sequential re-streaking on selective media for further analysis.

For additional real time PCR diagnostic screening, each 20µL reaction contains 10µL of a 2X Taqman ® Gene expression master mix (Applied Biosystems), 0.5µM of forward and reverse primers (Table 1) and 2 mL of enrichment that was prepared as previously described. Amplification is conducted in a thermocycler (7300 Real time PCR system, Applied Biosystems) with a protocol consisting in one cycle of 50ºC for 5 min, one cycle of denaturation at 95^{o}C for 5 min, followed by 40 cycles of 95^{o}C for 30 s and annealing at 60^{o}C for 1 min. Amplification greater or equal to the Ct value of a standard containing 5 copies of the target gene is classified as positive.

Outcomes and Accomplishments

Objective 1. – Since project initiation in July 2012, slightly over 1,800 cantaloupes have been tested for target human pathogens from main to late season production in California. Composite samples of three whole, peeled melons per enrichment (typically 55 composites per production lot) were initially screened for *Listeria spp*. and *Salmonella spp*. by collaborators at Roka BioSciences using the Atlas System. Included in each post-enrichment submission to Roka, in AOAC validated, stabilized transfer tubes, a set of replicate controls for each pathogen was also submitted. Controls were established by inoculating independent enrichments with whole peeled rind to a level of approximately 100 CFU/3 fruit rinds or 0.21 CFU/gm. All samples, including controls, were submitted as blind-coded sequential Lab Sample ID using the Roka barcode system. Due to the planned delay, a condition of melon industry cooperation in providing access

to fields and market-ready fruit, in final testing of enrichment cultures until at least 2 months after the end of shelf-life and contiguous ranch parcel harvest period for the specific field each melon lot was associated with, not all 2012 detection assays have been conducted so far. Of the approximate 1,600 melons from composite assays that the PD has requested for release, the following presumptive molecular results are known;

1. Six composites of 74 , at least six of approximately 1,800 cantaloupes, tested positive for Salmonella; all positives were from one lot
2. Two and four composites from among of approximately 1,800 cantaloupes tested positive for Listeria spp.; both positives were from the same lot. Testing the retained enrichment and a secondary enrichment confirmed the positives were for nonpathogenic *Listeria innocua* and not *L. monocytogenes*.
3. Many but not all of the three replicate controls submitted per lot were identified as positive by the Roka screen or by TaqMan PCR conducted in the Suslow Lab for EHEC/STEC, Salmonella, and Listeria.; part way through this project period a reassessment of the Internal Control Limit of Detection for Salmonella and Listeria and the inoculum level was increased to 100 CFU/sample (being a sample a total of 3 melon rinds) or 0.21 CFU/gm. All controls were subsequently positive using this approach. The bacterial background on cantaloupe rind, in particular seemed to suppress the threshold populations when using a lesser cell number of inoculated isolates. This may be due to the use of the non-selective pre-enrichment UPB to attempt to resuscitate all three targets within the same sample rather than using more optimized enrichments for each target on independent fruit samples or spit-samples. Increasing the concentration of UPB to a double strength significantly reduced the suspected nutrient competition and allowed consistent detection and recovery of applied bacteria in positive controls.

Objective 2. Due to delays in getting funding allocated and cooperators enrolled in this pilot study, we assayed for the Enterococcus indicator by cultural methods rather than qPCR (also a decision based on overall cost of this assay, including processing time). In general, the populations of indicator coliforms and *Enterococcus* on packed cantaloupe ranged between log 4 to log 7 CFU/fruit. However it is important to point out that although several cases in which 100% of the samples contained indicator populations, within the same lot we often obtain fruits that contain populations of coliforms and *Enterococcus* below the limit of detection (log 2.17CFU/fruit). None of the *Listeria spp*. or *Salmonella* positive fruit was associated with the

greatest quantitative recovery of either coliforms or Enterococcus spp. We have not detected generic *E. coli* in any of these analyzed samples.

Objective 3. - A partial environmental assessment was conducted at one study site with the resources available. A cantaloupe field in the San Joaquin Valley with a presumptive adjacent point-source risk factor that was made available to the PI for a more detailed analysis of irrigation water and fruit collected over two commercial harvest time-points.

Six 1L irrigation ditch water samples were taken prior to the date of projected commercial maturity. Cantaloupe fruit were hand harvested on 8/13/12 and 8/20/12 Full-slip fruit were taken from beds adjacent to but in advance of the commercial harvest crews working a particular section of the approximately 38 ha ranch parcel. Fruit were collected into large poly-bags with multiple slits to reduce condensation from fruit respiration. Melons were taken from 12 quadrants with 3 fruit per replication and two replicates per quadrant. Sterile nitrile gloves were worn and changed between each quadrant. Bagged fruit were placed in labeled cartons and transported to the lab for analysis within 2h. Fruit were held overnight at 4C prior to processing for indicators and pathogens, as described above. For these two dates, PCR screening for E. coli O157:H7 and non-O157 EHEC was included due to the nature of the potential risk factor.

In general, fecal indicator populations on fruit were no different than typically observed and widely variable among composite replicates. No target pathogens were detected from these samples on either date representing a total of 144 cantaloupes (Table 2).

Summary of Findings and Recommendations

The outcomes of this Pilot Study, in combination with our parallel prevalence study funded by ILSI for Salmonella and Listeria on cantaloupe, honeydew, and watermelon in California and Arizona (collaborator Dr. S. Ravishankar, University of Arizona) indicate that intensive sampling of a given lot of packed fruit may result in positive detection of pathogens of concern. Based on the sampling completed to date, the occurrence of pathogens on fruit is low and a typical compliance sampling regime would not appear likely to detect these infrequent events. However, one lot was associated with a more uniform distribution of Salmonella on fruit. We are currently reviewing the location-specific data with the cooperating handler and preliminary assessments indicate a field location and weather-related factor may have been involved.

This project was highly instructive in refining laboratory methodologies for sample processing, detection, and confirmation protocols. Due to the planned delay in confirmation assays we do not, at this time, have all pathogen cultural test data and will provide a Supplemental Report as soon as completed. We feel that it will be important to expand the prevalence survey and believe this pilot data will be an essential component of a future application for funding.

Citations for Methodology

E. Gutierrez-Rodriguez, A. Gundersen, A.O. Sbodio, T.V. Suslow. (2012). Variable agronomic practices, cultivar, strain source and initial contamination dose differentially affect survival of *Escherichia coli* on spinach. Journal of Applied Microbiology (112): 109-118.

G. Lopez-Velasco, A. Tomas-Callejas, A.O. Sbodio, P. Wei, T.V. Suslow. (2012). Assessment of root uptake and systemic vine-transport of *Salmonella enterica* sv. Typhimurium by melon (Cucumis melo) during field production. International Journal of Food Microbiology. (158): 65-72.

D. Rodriguez-Lazaro, M. Hernandez, M. Pla. (2004). Simultaneous quantitative detection of *Listeria spp.* and *Listeria monocytogenes* using a duplex real-time PCR-based assay. FEMS Microbiology Letters. (233): 257-267.

USDA Microbiological Data Program (MDP) 2002-2009. http://www.ams.usda.gov/AMSv1.0/ams.fetchTemplateData.do?template=TemplateO&topNav=&leftNav=ScienceandLaboratories&page=MDPProgramReports&description=MDP+Program+Reports&acct=microbiodataprg. Accessed on February 2012.

APPENDICES

Publications and Presentations
There have been no publications or public disclosure of this data thus far.

Budget Summary
All appropriated funds for this project were utilized in its execution. The co-funding by the CMRB was an essential component and the in-kind funding provided by Roka Biosciences in pre-screening enrichments for 2 of the 3 targets allowed us to maximize the availability of resources to sample acquisition and processing as well as partially fund the special-site opportunity that was unplanned.

Tables and Figures
Table 1. Comparison of the detection of Listeria spp. through real time PCR after stabilization of enrichment on FTA cards.

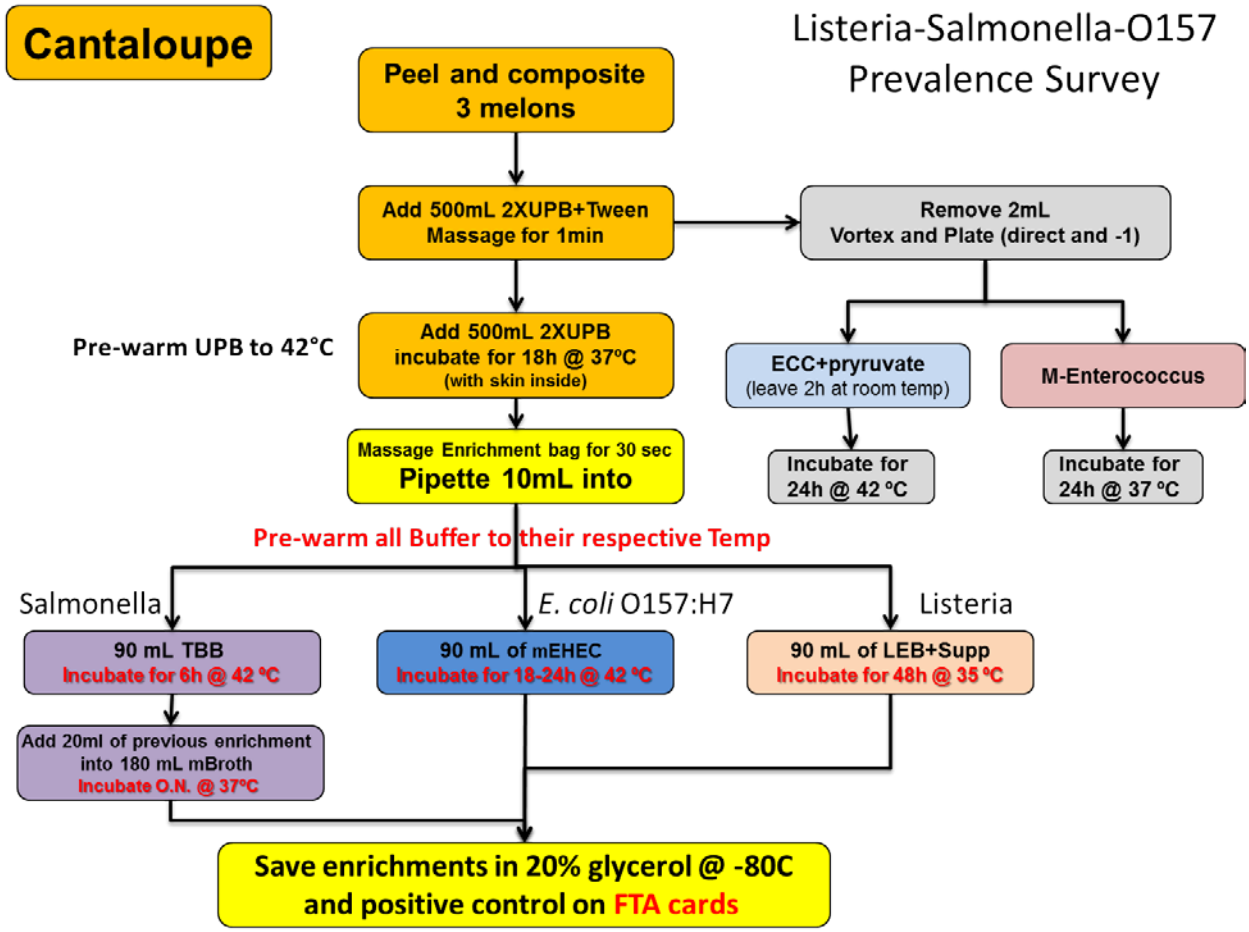
			Taqman detection (qRT-PCR)			
Type of sample	**Enrichment media**	**BAX listeria**	**Boiled**	**Lysed and boiled**	**FTA cards (48 h after collection)**	**FTA cards (30 days after collection)**
Pure culture (planktonic growth)	LEB[A]	+ve	-ve	+ve	+ve	+ve
	LEB[B]	+ve	-ve	+ve	+ve	+ve
	LEBsup[A]	+ve	-ve	+ve	+ve	+ve
	LEBsup[B]	+ve	-ve	+ve	+ve	+ve
	LIS[A]	+ve	+ve	+ve	+ve	+ve
	LIS[B]	+ve	-ve	+ve	+ve	+ve
	LISsup[A]	+ve	+ve	+ve	+ve	+ve
	LISsup[B]	+ve	+ve	+ve	+ve	+ve
Soil composited samples collected at a melon field in close proximity with an animal operation	LEB composite1	-ve	-ve	-ve	-ve	-ve
	LEB composite2	+ve	-ve	+ve	+ve	+ve
	LEBsup composite1	-ve	-ve	-ve	-ve	-ve
	LEBsup composite2	+ve	-ve	+ve	+ve	+ve
	LIS composite1	-ve	-ve	-ve	-ve	-ve
	LIS composite2	-ve	-ve	-ve	-ve	-ve
	LISsup composite1	-ve	-ve	-ve	-ve	-ve
	LISsup composite2	+ve	-ve	+ve	+ve	+ve

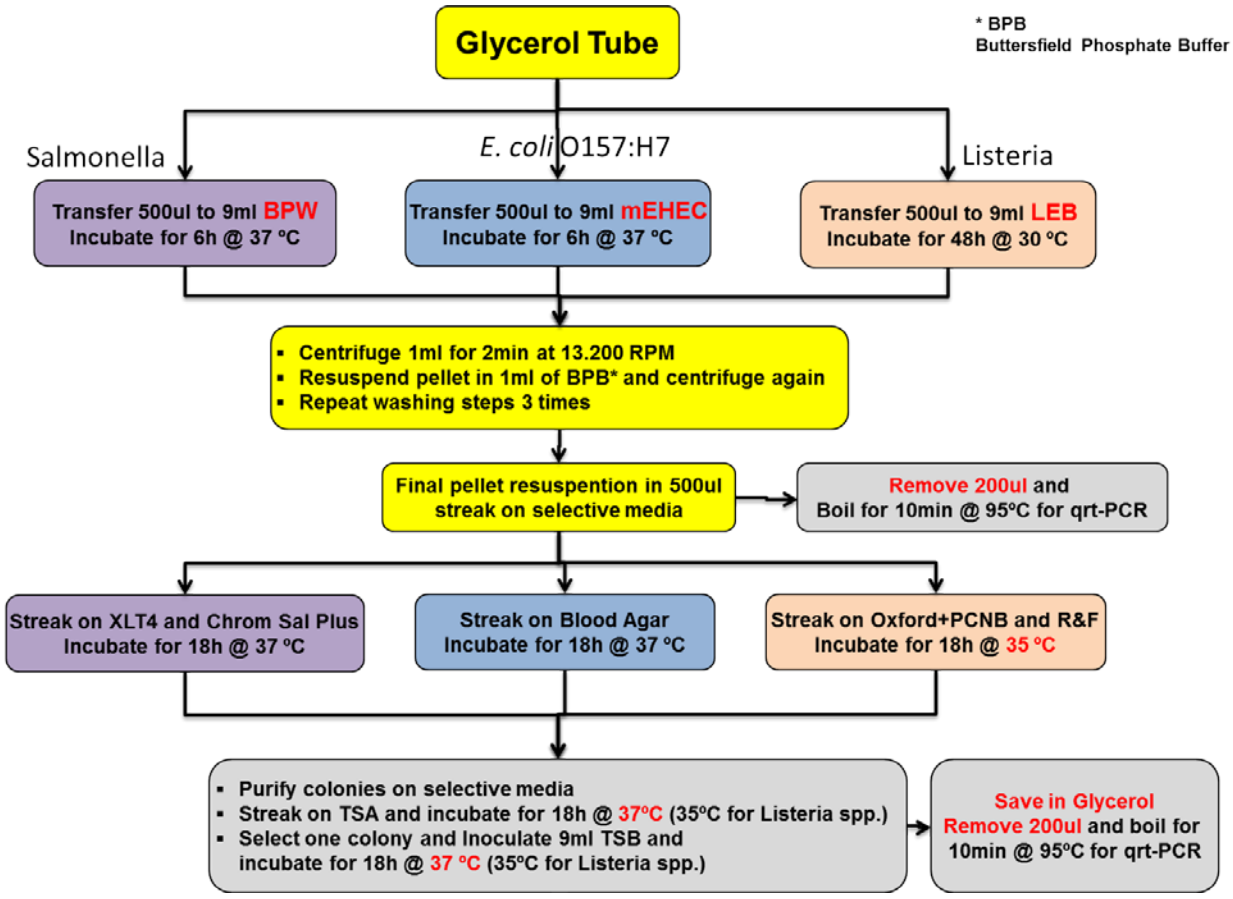
[A]Low inoculum dose 10^5 CFU/100 mL
[B]Low inoculum dose 10^2 CFU/100 mL
(LEB) Listeria enrichment broth (Oxoid)
(LIS) Listeria broth (Oxoid)
(sup) Selective supplement
(+ve and –ve) Positive and negative detection respectively

Table 2. Assessment of indicator and pathogen prevalence at sequential sample site CA12-2 during 8/20/12

Cantaloupe Composite Group[a]	Fecal Coliform[b]	*E. coli*[b]	*E. coli* EHEC	*E. coli* O15:H7	*Listeria spp.*	*Listeria monocytogenes*	*Salmonella*
1	< 2.0	< 2.0	N	N	N	N	N
2	< 2.0	< 2.0	N	N	N	N	N
3	< 2.0	< 2.0	N	N	N	N	N
4	< 2.0	< 2.0	N	N	N	N	N
6	< 2.0	< 2.0	N	N	N	N	N
7	< 2.0	< 2.0	N	N	N	N	N
8	< 2.0	< 2.0	N	N	N	N	N
9	< 2.0	< 2.0	N	N	N	N	N
10	< 2.0	< 2.0	N	N	N	N	N
11	< 2.0	< 2.0	N	N	N	N	N
12	< 2.0	< 2.0	N	N	N	N	N
13	< 2.0	< 2.0	N	N	N	N	N
14	< 2.0	< 2.0	N	N	N	N	N
15	< 2.0	< 2.0	N	N	N	N	N
16	< 2.0	< 2.0	N	N	N	N	N
17	< 2.0	< 2.0	N	N	N	N	N
18	93.3	93.3	N	N	N	N	N
19	3.6	< 2.0	N	N	N	N	N
20	< 2.0	< 2.0	N	N	N	N	N
21	< 2.0	< 2.0	N	N	N	N	N
22	< 2.0	< 2.0	N	N	N	N	N
23	3.6	<2.0	N	N	N	N	N
24	3.6	3.6	N	N	N	N	N

(a) Each group was a composite of 3 cantaloupes
(b) Fecal coliforms and *E. coli* were determined at 42.5 $\pm$ 0.5 ^{o}C utilizing the QuantiTray System, thus results represent MPN/mL that was obtained from 500 mL of bacterial suspension that came from a composite of 3 melon rinds
(N) and (P) indicate negative and positive pathogen detection respectively


Cantaloupe
Listeria-Salmonella-O157
Prevalence Survey
Peel and composite
3 melons
Add 500mL 2XUPB+Tween
Massage for 1min
Remove 2mL
Vortex and Plate (direct and -1)
Pre-warm UPB to 42°C
Add 500mL 2XUPB
incubate for 18h @ 37°C
(with skin inside)
ECC+pryruvate
(leave 2h at room temp)
M-Enterococcus
Massage Enrichment bag for 30 sec
Pipette 10mL into
Incubate for
24h @ 42 °C
Incubate for
24h @ 37 °C
Pre-warm all Buffer to their respective Temp
Salmonella
E. coli O157:H7
Listeria
90 mL TBB
Incubate for 6h @ 42 °C
90 mL of mEHEC
Incubate for 18-24h @ 42 °C
90 mL of LEB+Supp
Incubate for 48h @ 35 °C
Add 20ml of previous enrichment
into 180 mL mBroth
Incubate O.N. @ 37°C
Save enrichments in 20% glycerol @ -80C
and positive control on FTA cards


Glycerol Tube
* BPB
Buttersfield Phosphate Buffer
Salmonella
E. coli O157:H7
Listeria
Transfer 500ul to 9ml BPW
Incubate for 6h @ 37 °C
Transfer 500ul to 9ml mEHEC
Incubate for 6h @ 37 °C
Transfer 500ul to 9ml LEB
Incubate for 48h @ 30 °C
Centrifuge 1ml for 2min at 13.200 RPM
Resuspend pellet in 1ml of BPB* and centrifuge again
Repeat washing steps 3 times
Final pellet resuspention in 500ul
streak on selective media
Remove 200ul and
Boil for 10min @ 95°C for qrt-PCR
Streak on XLT4 and Chrom Sal Plus
Incubate for 18h @ 37 °C
Streak on Blood Agar
Incubate for 18h @ 37 °C
Streak on Oxford+PCNB and R&F
Incubate for 18h @ 35 °C
Purify colonies on selective media
Streak on TSA and incubate for 18h @ 37°C (35°C for Listeria spp.)
Select one colony and Inoculate 9ml TSB and
incubate for 18h @ 37 °C (35°C for Listeria spp.)
Save in Glycerol
Remove 200ul and boil for
10min @ 95°C for qrt-PCR