Concentration and Detection of Low Levels of *Escherichia coli* O157:H7, *Listeria monocytogenes* 4b, and *Salmonella enterica* Typhimurium in High Organic Load Lettuce Wash

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Foodborne illness traced to fruits and vegetables coupled with new draft guidelines for produce issued in the Food Modernization and Safety Act (FMSA) have increased attention and research on methods for ensuring end-product safety. For large volume samples, such as produce wash, many current food testing protocols utilize random sampling of small volumes. An alternative and novel method has been developed to increase the probability of finding low numbers of cells via collection and concentration of representative sub-samples of larger volumes for indirect testing of food-borne pathogens. Demonstrated here is a user friendly, low-cost semi-automated method used to detect *Escherichia coli* O157:H7, *Listeria monocytogenes*, and *Salmonella enterica* Typhimurium from 5% organic load lettuce wash. This method could be useful for measuring microbial load reduction levels after flume washing of fresh produce, such as demonstrated here on samples from a pilot-scale leafy greens processing line.

Key Words:

Pathogenic Bacteria

Produce Sanitation

Food Equipment Sanitation

Food Safety

Food Sanitation Techniques

Detection of Pathogens in High Volume Samples

Introduction

Testing for major bacterial pathogens in fresh produce is challenging for many reasons including the time required to detect viable levels of bacteria, the short shelf life of these products, and the magnitude and diversity of produce composition, life cycle handling, and geographic source. Ground soils and other environmental factors such as agricultural animal, wildlife, or insect grazing; irrigation source water and composition; human contact; and transport and production facility equipment can all expose raw produce to potentially deadly pathogens. According to the United States Center for Disease Control and Prevention, *Escherichia coli* O157:H7, *Listeria monocytogenes*, and *Salmonella enterica* are three of eight known pathogens that account for the vast majority of reported foodborne illness, hospitalizations, and deaths each year. Salmonellosis is the most commonly reported bacterial food-borne illness resulting in hospitalization and/or death, and listeriosis caused by *L. monocytogenes* has the third highest reported incidence of death. These pathogens are also characterized as possessing high persistence once established in food processing environments. *E. coli* O157:H7 is an enterohemorrhagic E. *coli* (EHEC), a group recognized as the primary cause of hemorrhagic colitis (bloody diarrhea) that can progress to fatal hemolytic uremic syndrome (HUS)[1].

Techniques such as pasteurization, cooking, freezing, washing with chlorinated or other sanitary rinse solutions, treating with novel anti-microbials, or irradiation are all designed to decrease or eliminate these kinds of pathogens from foods or food processing equipment. Hazard Analysis of Critical Control Point (HACCP) programs have been developed to minimize the presence of these bacteria through a series of preventative controls engaged throughout the farm-to-fork continuum. Screening for low levels of pathogens can help to better ensure the success of sanitation treatments.

Different methods exist for detecting food-borne pathogens, but an ongoing challenge in perishable food testing is the enrichment time required for pathogen analysis, up to 40+ hours depending on the organism according to generally accepted regulatory protocols. Methods designed to decrease the time to result, therefore, are particularly desirable for fresh produce.

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The Portable Multi-Use Automated Concentration System (PMACS) uses a proprietary dead-end ultrafiltration system designed to increase the probability of finding low numbers of pathogenic cells in larger, more representative samples[2]. Concentration of cells from larger volumes can potentially decrease the time to result through shorter enrichments. Proof-of-principle research supporting the ability of the device to achieve this goal has been undertaken using detection technologies of qPCR and electrochemiluminescence. Alternatively, absorbance-based ELISA offers a user friendly, low cost alternative that detects bacteria with high specificity, although requiring higher cell counts for sensitivity. Paired with the PMACS, however, standard ELISA affords more rapid detection of pathogens when low levels are concentrated as shown here in lettuce wash.

Limits of detection, media validation, and growth curve studies for all 3 bacteria in spiked lettuce wash with and without PMACS were determined for 3M™ Tecra™ Visual Immunoassay kits using 15 and 20 minute substrate incubations and a BioTek ELx50™ Microplate Strip Washer and BioTek ELx800™ Microplate Reader (data not shown). Sampling was then done comparing non-concentrated to concentrated 5% organic load lettuce wash using the same semi-automated ELISA protocol. The method developed was then applied to samples from a pilot-scale leafy green processing line using lettuce heads spiked with Green Fluorescent Protein (GFP) transformed *E. coli* O157:H7 (GFP-*E. coli* O157:H7) and rinsed with different concentrations of chlorinated wash water to assess sanitizer efficacy.

Materials and Methods

Bacteria

- *Escherichia coli* O157:H7, ATCC 35150
- *Listeria monocytogenes*, ATCC 19115
- *Salmonella enterica* subsp. *enterica* serovar Typhimurium, ATCC 19585

Materials

- Brain Heart Infusion (BHI) broth and agar
- Buffered *Listeria* enrichment broth w/nalidixic acid, acriflavine, and cycloheximide (TLEB)
- Buffered tryptone soy broth w/novobiocin (BTSB+N)
- Chlorine based sanitizer (XY-12; 100, 30, 10 ppm) + T128 stablilizer (Smartwash)
- Dulbecco's Phosphate Buffered Saline (DPBS)
- Fraser broth (FB)
- Lactose broth (LB)
- Lettuce wash (5% organic load; see Method 1)
- Listeria enrichment broth (LEB)
- M broth (MB)
- Microcentrifuge tubes
- Modified Buffered Peptone Water w/pyruvate, acriflavine, cefsulodin, vancomycin (mBPWp+ACV)
- Oxford medium agar
- Rappaport-Vassiliadis medium (RV)
- Selective enrichment broth (SEL)[3]
- Sieving mesh (125 µm) and 5 µm prefilterSodium Polyphosphate Buffer (NaPPB)
- Sodium thiosulfate
- Sorbitol MacConkey with cefixime and tellurite (CTSMAC) agar
- Sterile 500 mL culture flasks
- Tetrathionate medium (TT)
- Tryptic soy agar (TSA)
- Tryptic soy broth (TSB)
- Xylose Lysine Deoxycholate (XLD) agar plates
- 3M™ TECRA™ *E. coli* O157, *Listeria*, and *Salmonella* Visual Immunoassay kits

Equipment

- BioTek ELx800™ Absorbance Microplate Reader
- BioTek ELx50™ Microplate Strip Washer
- Lettuce shredder
- Portable Multi-Use Automated Concentration System (PMACS)
- Step conveyer
- 3.3 m-long stainless steel flume tank w/overhead spray jets
- 890 L capacity water recirculation tank
- Shaker table

Method 1: Compare PMACS retentate to non-concentrated 5% organic load lettuce wash samples using two enrichment procedures and semi-automated ELISA for *E. coli* O157:H7, *Salmonella* Typhimurium, and *L. monocytogenes*.

Stock cultures of *E. coli* O157:H7 and *Salmonella* Typhimurium were each grown in TSB at 35 °C for 18-20 h. *Listeria monocytogenes* was grown in BHI broth at 35 °C for 18-20 h. One mL of each broth culture was centrifuged at 16,100 x *g* for 5 min at 4 °C. Pellets were washed 2 - 3 times with DPBS and then resuspended in 1 mL of DPBS. Samples were diluted ten-fold. Direct counts were performed to determine approximate cell concentrations followed by spread plating in triplicate on corresponding selective media (CTSMAC, Oxford,and XLD agar for *E. coli*, *Listeria*, and *Salmonella* respectively). Plates were incubated at 35 °C for 18-24 h after which the target Colony Forming Units (CFU) were counted (Table 1).

Experiment	Stock direct count cells/mL			Stock viable counts: TSA CFU/mL			Stock viable counts: CFU/mL, CFU % loss: CTSMAC			Amount spiked into 75 L (LW): CFU		
							CFU % loss			CFU		
							CTSMAC	Oxford	XLD	CFU/mL (CFU/75000)		
	0157:H7	*Listeria*	*Salmonella*	0157:H7	*Listeria*	*Salmonella*	0157:H7	*Listeria*	*Salmonella*	0157:H7	*Listeria*	*Salmonella*
1	1.71×10^9	7.60×10^8	1.25×10^9	2.61×10^9	1.55×10^9	2.16×10^9	1.69×10^9	1.35×10^9	1.69×10^9	3800	6060	3210
							21.8	12.9	21.8	0.05	0.08	0.07
2	1.49×10^9	8.20×10^8	1.32×10^9	2.28×10^9	1.76×10^9	1.63×10^9	1.41×10^9	1.65×10^9	1.34×10^9	5180	6440	3700
							38.2	6.3	17.8	0.07	0.09	0.05
3	1.29×10^9	1.13×10^9	1.22×10^9	2.19×10^9	4.00×10^9	1.45×10^9	2.03×10^9	3.44×10^9	1.27×10^9	5100	1060	3590
							7.3	14	12.4	0.07	0.14	0.05

Table 1. Method 1 initial stock counts and lettuce wash (LW) concentrations for *E. coli*, *Listeria*, and *Salmonella* respectively.

Five percent organic load LW was generated by adding 7.5 kg of blended lettuce into 150 L of dechlorinated tap water and splitting into two, 75 L batches. One batch was spiked with the DPBS cell samples of E. coli O157:H7, *L. monocytogenes*, and *Salmonella* Typhimurium to final concentrations of 1 - 2 CFU/25 mL each (LWB) while the other batch remained unspiked (LWA). Both batches were sieved with a 125 µm mesh and a 5 µm prefilter, followed by concentration of 40 L with the PMACS to obtain 400 mL lettuce wash retentate (LWR) samples (Figure 1).

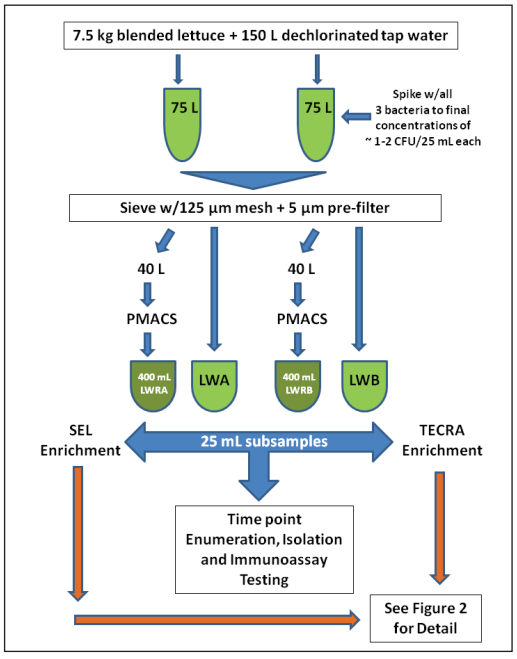

Figure 1. Method 1 workflow.

Twenty-five mL of non-concentrated and retentate samples from three individual experiments were enriched using two procedures: (1) TECRA VIA enrichment instructions in the kit insert; and, (2) *E. coli*, *Salmonella*, and *Listeria* all-in-one selective enrichment broth (SEL)[3]. Figure 2 details the comparative enrichment workflow. Sub-samples were removed at various time points for target enumeration (spread plating), viable isolation (isolation streaks), and semi-automated VIA testing according to the kit inserts and Figure 3 instrument settings. Each sub-sample was tested in triplicate with the VIA and determined to be positive if mean absorbance was ≥0.200 or negative if mean absorbance was <0.200.

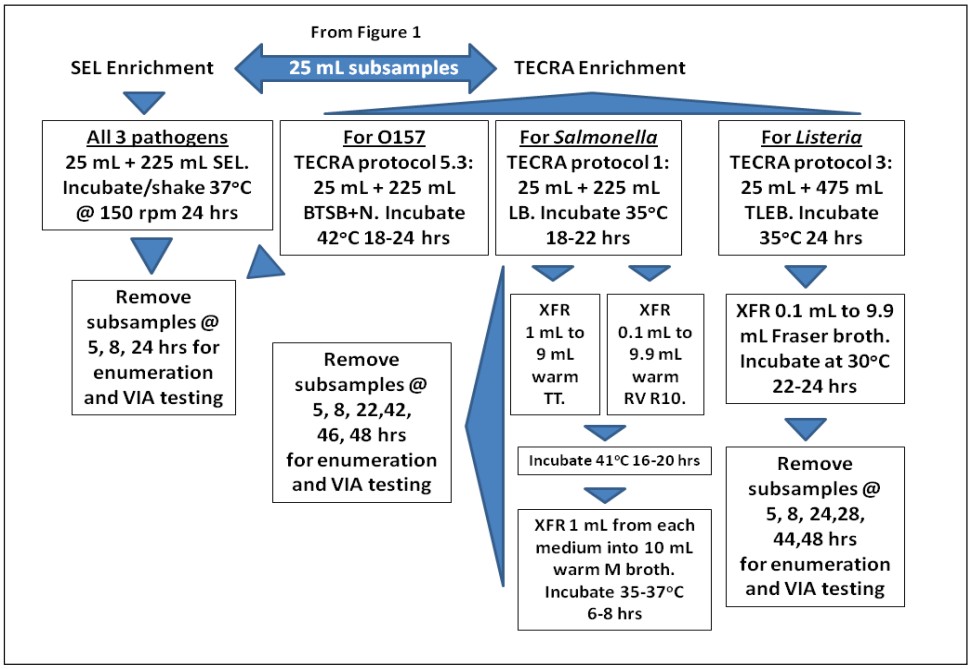

Figure 2. Method 1 enrichment workflow detail.

ELx50 Microplate Strip Washer Onboard Software	
Prime Before Start	
Volume	5 mL
Flow Rate	5
Number of cycles	3, No Soak
Plate or Strip format	Plate
Dispense Parameters	
Dispense Volume (per well)	550 µL
Flow Rate	7
Height	128
Horizontal Position	0
Aspirate Parameters	
Height	32
Horizontal Position	-15
Rate	2
Delay	0
Final Aspirate?	No

ELx800 Microplate Absorbance Reader Onboard Software			
Wavelength	Dual	Meas	Ref
		405	490
Data Analysis			
Control Validation		NC < 0.200	
		PC >= 1.000	
Cutoff		0.200	
Greyzone		0.000	
Pos Samples		>= Cutoff	

Figure 3. Settings for automated washing and detection of 3M™ TECRA™ VIAs.

Method 2: Compare semi-automated TECRA VIA detection of spiked GFP-*E. coli* O157:H7 in PMACS retentate and non-concentrated 5% organic load lettuce wash samples obtained from a pilot-scale leafy greens processing line.

Five percent organic load lettuce washes were generated by Gordon Davidson and assistants at Dr. Elliot Ryser's laboratory [Department of Food Science and Human Nutrition, Michigan State University (MSU)].[4]

Lettuce heads were spiked with attenuated GFP labeled strains of *E. coli* O157:H7[5], and then processed in a pilot-scale leafy green line consisting of a lettuce shredder, step conveyer, 3.3 m-long stainless steel flume tank with overhead spray jets, 890-L capacity water recirculation tank, and a shaker table [5,6]. The recirculation tank was filled with 5% organic load lettuce wash water with varying chlorine (Cl) concentrations and a stabilizer. The line was primed with uninoculated lettuce followed by processing of spiked lettuce heads. Once processing of lettuce was completed, 80 L of wash water were collected from the recirculation tank and neutralized with sodium thiosulfate. Wash water was then sieved and concentrated following the PMACS method. Non-concentrated and retentate samples were enriched following the FDA BAM *E. coli* O157:H7 procedure [7]. Sub-samples were removed at various time points for enumeration (spread plating) and immunoassay testing. Enumeration of *E. coli* O157:H7 was done by plating samples on TSA with 0.6% yeast extract and 100 ppm ampicillin (TSAYE) and CTSMAC. Plates were incubated at 35 °C for 18 - 24 h and then counted. Plates were further incubated for 24 h at 35 °C for confirmation of the target pathogen.

Results and Discussion

Method 1

Table 2 and Figure 4 show representative results for detection of low-level spiked (B) and non-spiked (A) *E. coli* O157:H7 in non-concentrated and concentrated 5% organic load lettuce wash samples. Spiked lettuce wash concentrated with PMACS (LWRB) and enriched using BTSB+N (green) showed detection for *E. coli* O157:H7 in all 3 samples after just 5 h of enrichment whereas non-concentrated samples in the same broth needed 8 h. *E. coli* positive for these methods was confirmed via isolation streak performed on undiluted enriched sample. Spiked samples (LWB, LWRB) enriched using SEL (blue, Table 2) were *E. coli* O157:H7 negative for isolation streaks but did not correlate with ELISA results for the same samples. This was likely due to an inhibitory effect from SEL on CTSMAC[8,9], as a lack of false positives for non-spiked samples indicate that background did not influence ODs in spiked samples. Concentrations for samples with no isolated *E. coli* O157:H7 CFU were calculated as less than (<) the lowest CFU possible based on sample dilution and volume (mL) plated.

Sample	Enrichment Time (h)	E. coli O157:H7					
		CTSMAC				ELISA	
		CFU/mL		Isolation Streak		# Positive/Total # Tested	
		SEL	BTSB+N	SEL	BTSB+N	SEL	BTSB+N
Lettuce Wash non-spiked (LWA)	24	< 500	< 500	Neg	Neg	0/2	0/3
		< 50	< 50				
		< 50	< 50				
Lettuce Wash PMACS_Retentate non-spiked (LWRA)	24	< 500	< 500	Neg	Neg	0/2	0/3
		< 500	< 500				
		< 500	< 500				
Lettuce Wash spiked (LWB)	8	50	2.39×10^3			2/3	3/3
		1.90×10^3	8.20×10^3				
		< 50	3.05×10^4				
	24	$< 5.0 \times 10^3$	5.00×10^4	Neg	Pos	2/3	3/3
		< 50	$>=1.0 \times 10^5$				
		< 50	1.60×10^7				
Lettuce Wash PMACS_Retentate spiked (LWRB)	5	Nd	2.00×10^3				3/3
			3.45×10^3				
			5.80×10^3				
	8	< 500	1.05×10^4			2/3	3/3
		2.40×10^3	5.62×10^5				
		< 50	7.10×10^5				
	24	$< 5.0 \times 10^3$	4.65×10^5	Neg	Pos	2/3	3/3
		< 500	3.29×10^7				
		< 500	1.00×10^7				

Table 2. Representative results of comparative enrichment methods on *E. coli* O157:H7 detection from PMACS retentate (R) and non-concentrated lettuce wash samples

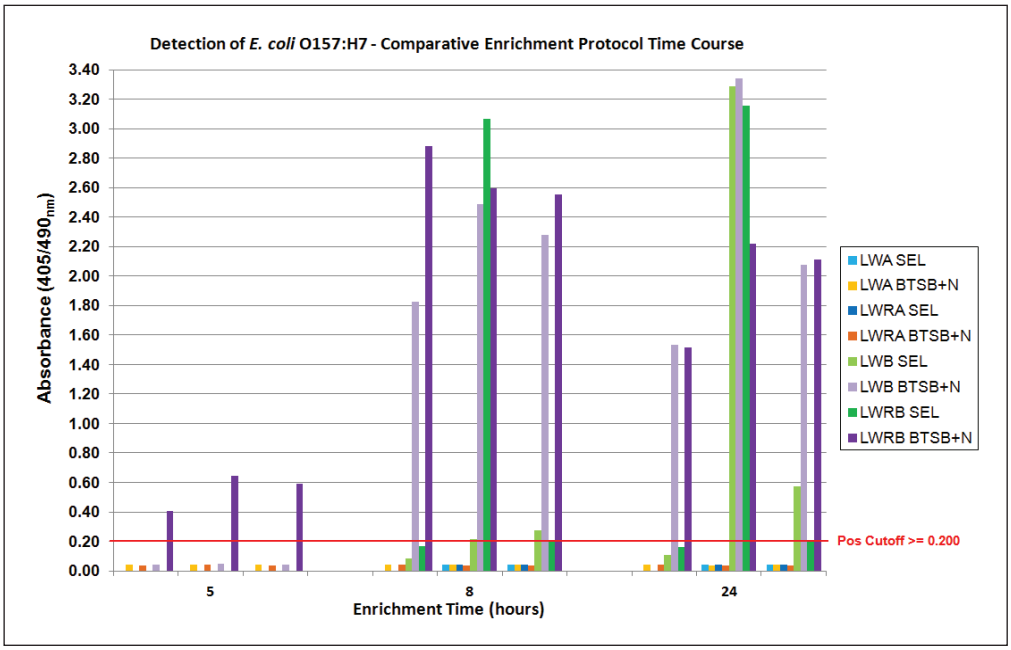

Figure 4. Comparison of *E. coli* O157:H7 ELISA detection from samples (experiments 1-3) enriched in SEL and BTSB+N and tested at 5, 8, and 24 h of enrichment. Each sample (column) was VIA tested in triplicate and determined to be positive if mean absorbance was ≥0.200 or negative if <0.200.

Table 3 and Figure 5 illustrate detection of low-level spiked (B) and non-spiked (A) *Salmonella* Typhimurium in concentrated and non-concentrated 5% organic load lettuce wash samples. *Salmonella* Typhimurium was detected (3/3 times) in TT (42) and RV (44) enrichments 6 and 4 h earlier for spiked retentate samples (LWRB) than for the non-concentrated spiked samples (LWB). SEL enrichment resulted in 1/3 positive samples at 24 h for both LWB and LWRB samples. Concentrations for samples with no isolated *Salmonella* CFU on XLD were calculated as less than (<) the lowest CFU possible based on sample dilution and volume (mL) plated. Isolation streaks were performed from undiluted enriched samples. Interference of microbial background was an issue during enumeration and enrichment of *Salmonella* Typhimurium at such low levels. Enrichment in Lactose broth is considered a pre-enrichment step which allows for repair of cell damage and an opportunity to increase the ratio of *Salmonella* to non-*Salmonella* due to a bacteriostatic effect - non-*Salmonella* cells ferment lactose decreasing the pH of the broth allowing *Salmonella*, that survives and grows in the lower pH environment, to outcompete them. In these experiments some background microorganism(s) still grew along with *Salmonella* as observed on the XLD plates. XLD agar inhibits Gram-positive organisms and allows growth of most enteric bacteria while differentiating *Salmonella* and *Shigella*. If the background population is less than *Salmonella* this method will work, however alternatives suggested by the Difco Manual for adding brilliant green to XLD to inhibit coliforms and *Shigella*, or using Bismuth Sulfite (includes brilliant green) agar instead of XLD may produce better results[10].

Enrichment Media - Time (h) [Total enrichment time]	*Salmonella enterica* Typhimurium											
	XLD								ELISA			
	CFU/mL				Isolation Streak				# Positive/Total # Tested			
	LWA	LWRA	LWB	LWRB	LWA	LWRA	LWB	LWRB	LWA	LWRA	LWB	LWRB
SEL-24 [24]	< 5.0 x 10^3	< 5.0 x 10^4	< 5.0 x 10^3	< 5.0 x 10^4	Neg	Neg	Neg	Neg	0/3	0/3	1/3	1/3
	< 5.0 x 10^4	< 5.0 x 10^4	< 5.0 x 10^5	< 5.0 x 10^5								
	< 5.0 x 10^4	< 5.0 x 10^4	< 5.0 x 10^5	< 5.0 x 10^5								
Lac-22 [22]	< 5.0 x 10^3	< 5.0 x 10^3	< 5.0 x 10^4	< 5.0 x 10^4					0/3	0/3	0/3	1/3
	< 5.0 x 10^4	< 5.0 x 10^4	< 5.0 x 10^4	2.20 x 10^7								
	< 5.0 x 10^4	< 5.0 x 10^4	< 5.0 x 10^4	< 5.0 x 10^5								
RV-20 [42]	< 500	< 5.0 x 10^4	< 5.0 x 10^3	< 5.0 x 10^4					0/3	0/3	0/3	1/3
	< 50	< 500	8.95 x 10^3	3.07 x 10^6								
	< 50	< 5.0 x 10^3	>= 3.0 x 10^5	7.55 x 10^5								
TT-20 [42]	< 5.0 x 10^3	< 5.0 x 10^5	< 5.0 x 10^3	9.50 x 10^5					0/3	0/3	2/3	3/3
	< 5.0 x 10^3	< 5.0 x 10^3	4.20 x 10^6	8.05 x 10^6								
	< 5.0 x 10^3	< 5.0 x 10^3	6.35 x 10^5	2.73 x 10^6								
RM-6 [48]	< 500	< 5.0 x 10^4	< 5.0 x 10^3	9.10 x 10^8	Neg	Neg	Neg	Pos	0/2	0/1	2/3	2/2
	< 5.0 x 10^4	< 5.0 x 10^4	5.50 x 10^6	3.50 x 10^8			Pos					
	< 5.0 x 10^4	< 5.0 x 10^5	6.40 x 10^7	8.85 x 10^8			Pos					
TM-6 [48]	< 5.0 x 10^4	< 5.0 x 10^6	>= 1.0 x 10^7	>= 1.0 x 10^7	Neg	Neg	Neg	Pos	0/1	0/2	3/3	1/1
	< 5.0 x 10^5	< 5.0 x 10^5	4.50 x 10^6	5.00 x 10^6			Pos					
	< 5.0 x 10^5	< 5.0 x 10^5	1.65 x 10^7	8.10 x 10^7			Pos					

Table 3. Representative results of comparative enrichment methods on *Salmonella* Typhimurium detection from PMACS retentate (R) and non-concentrated lettuce wash samples.

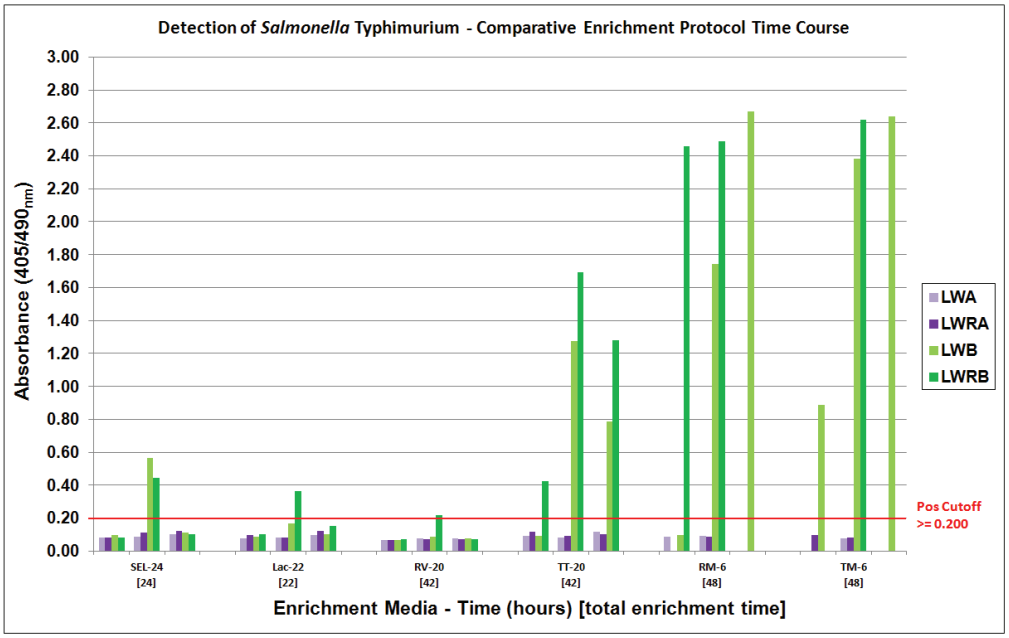

Figure 5. Comparison of *Salmonella* Typhimurium VIA detection from samples (experiments 1-3) enriched in different media and tested at various time-points. Each sample (column) was VIA tested in triplicate and determined to be positive if mean absorbance was ≥0.200 or negative if <0.200.

Table 4 and Figure 6 illustrate detection of low-level spiked (B) and non-spiked (A) *Listeria monocytogenes* in concentrated and non-concentrated 5% organic load lettuce wash samples. *Listeria* was detected (3/3 times) within 24 h using TLEB and 25 h using Fraser broth for spiked, concentrated samples (LWRB), whereas almost 2x longer (44 h) was needed for the non-concentrated, spiked samples (LWB). *Listeria* was not detected in the LWB SEL at 24 h enrichment, but was detected 2/3 times in the LWRB samples. Concentrations for samples with no *L. monocytogenes* colonies on Oxford were calculated as less than (<) the lowest CFU possible based on sample dilution and volume (mL) plated. Isolation streaks were performed from undiluted enriched samples. Although *Listeria* was detected in TLEB and SEL enriched samples and confirmed by isolation streak, these methods should be subjected to repeatability testing as the 3M™ TECRA™ VIA targets *Listeria* flagella that may be not be produced when the bacteria is grown at a temperature >30 °C (data not shown).

Enrichment Media - Time (h)	*Listeria monocytogenes*											
	Oxford								ELISA			
	CFU/mL				Isolation Streak				# Positive/Total # Tested			
	LWA	LWRA	LWB	LWRB	LWA	LWRA	LWB	LWRB	LWA	LWRA	LWB	LWRB
SEL-24	< 500	< 500	2.30×10^6	1.35×10^7	Neg	Neg	Pos	Pos	0/1	0/1	0/3	2/3
	$< 5.0 \times 10^3$	$< 5.0 \times 10^3$	5.85×10^6	3.35×10^7								
	< 500	$< 5.0 \times 10^3$	2.00×10^5	$< 5.00 \times 10^4$								
TLEB-24			2.45×10^6	2.45×10^6							2/3	3/3
			1.16×10^6	3.30×10^8								
			1.00×10^4	1.20×10^7								
FB-28				8.65×10^6								3/3
				2.40×10^7								
				5.95×10^6								
FB-44	$< 5.0 \times 10^3$	$< 5.0 \times 10^4$	3.79×10^8						0/3	0/3	3/3	
	$< 5.0 \times 10^4$	$< 5.0 \times 10^5$	5.50×10^8									
	$< 5.0 \times 10^3$	$< 5.0 \times 10^4$	1.10×10^7									
FB-48	$< 5.0 \times 10^3$	$< 5.0 \times 10^3$	5.60×10^8	7.20×10^8	Neg	Neg	Pos	Pos				
	$< 5.0 \times 10^4$	$< 5.0 \times 10^4$	4.95×10^8	6.50×10^8								
	$< 5.0 \times 10^4$	$< 5.0 \times 10^4$	5.00×10^7	6.30×10^8								

Table 4. Representative results of comparative enrichment methods on *Listeria monocytogenes* detection from PMACS retentate (R) and non-concentrated lettuce wash samples.

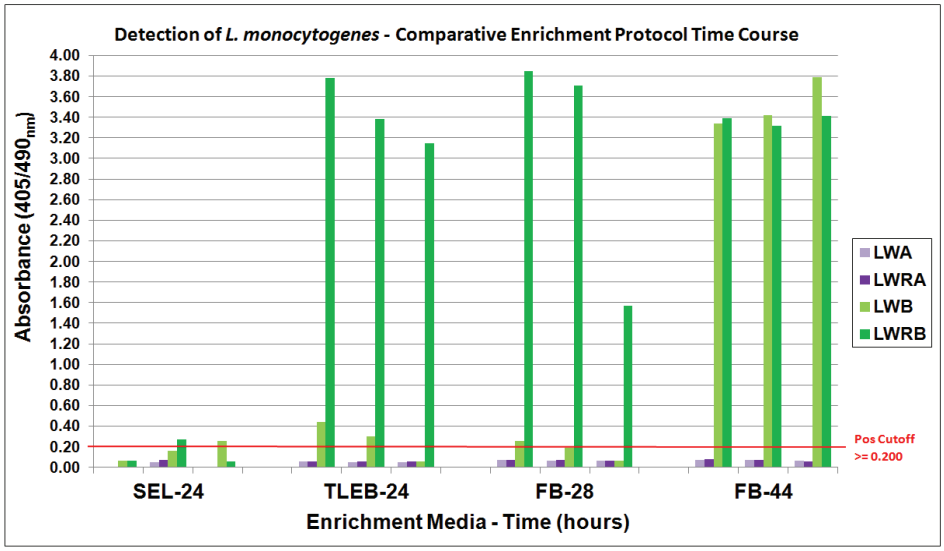

Figure 6. Comparison of *L. monocytogenes* VIA detection from samples (experiments 1-3) enriched in different media and tested at various time-points. Each sample (column) was VIA tested in triplicate and determined to be positive if mean absorbance was ≥0.200 or negative if <0.200.

Method 2

Table 5, Figure 7, and Figure 8 illustrate results from detection of spiked GFP-*E. coli* O157:H7 in chlorinated, non-concentrated and concentrated (R) 5% organic load lettuce wash samples obtained from a pilot-scale leafy greens processing line. *E. coli* O157:H7 was detected 3/3 times in the concentrated samples (LWR) without any enrichment for both 10 ppm and 30 ppm Cl, whereas enrichment times of 24 h at 30 ppm and 5 h at 10 ppm were required to detect *E. coli* O157:H7 in non-concentrated samples. Estimates of lettuce wash sample concentrations were 10^3-10^4 CFU/mL of *E. coli* O157:H7 (dead and viable).

Cl (ppm)	Enrichment Time (h)	GFP-*E. coli* O157:H7					
		CFU/mL				ELISA	
		TSAYE		CTSMAC		# Positive/Total # Tested	
		LW	LWR	LW	LWR	LW	LWR
30	0 (D)	< 5	15	< 5	<0.5	0/3	3/3
		< 5	5	< 5	5		
		2.5	75	0.45	65		
	5	< 50	3.00×10^3	< 50	<500	0/3	3/3
		5	8.50×10^3	< 5	250		
		350	1.90×10^4	30	3.05×10^3		
	24			$< 5.0 \times 10^3$	6.50×10^6	2/3	3/3
				$>= 5.0 \times 10^7$	8.00×10^6		
				$< 5.0 \times 10^5$	6.00×10^7		
10	0 (D)	585	2.75×10^4	275	1.30×10^4	0/3	3/3
		950	9.40×10^4	250	1.10×10^4		
		2100	1.40×10^5	230	7.50×10^3		
	0	293	1.40×10^4	138	6.30×10^3	0/3	3/3
		475	4.70×10^4	125	5.50×10^3		
		1050	7.00×10^4	115	3.80×10^3		
	5	2.00×10^4	1.30×10^6	$>= 3.0 \times 10^3$	$>= 9.65 \times 10^4$	3/3	3/3
		2.50×10^4	3.55×10^6	4.00×10^3	7.50×10^4		
		1.55×10^4	7.35×10^6	5.50×10^3	8.00×10^4		
	24			3.50×10^5	5.00×10^4	3/3	3/3
				$< 5.0 \times 10^4$	$<5.00 \times 10^4$		
				1.10×10^6	2.70×10^5		

Table 5. Representative results of GFP-*E.coli* O157:H7 detection from PMACS retentate (R) and non-concentrated 5% organic load lettuce wash after treatment with comparative chlorination levels in a pilot scale leafy greens processing line.

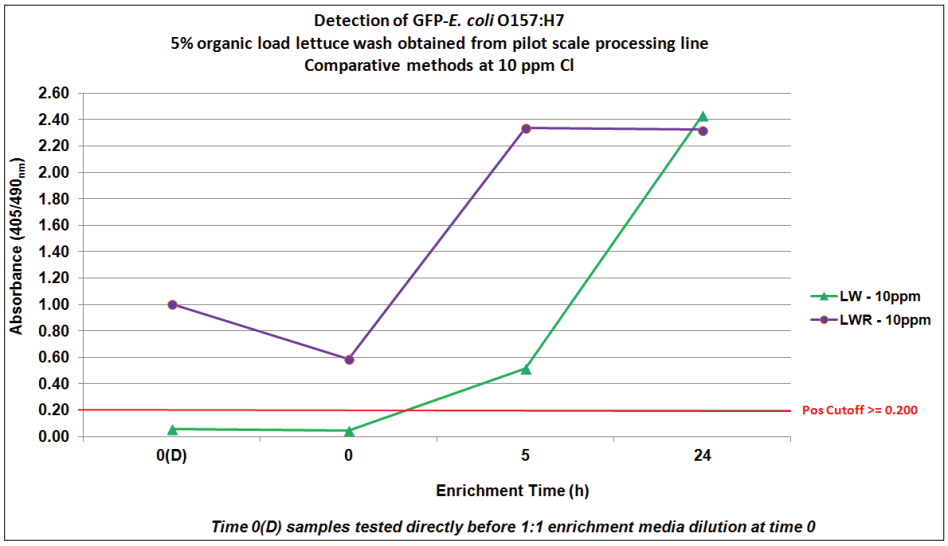

Figure 7. Comparison of *E. coli* O157:H7 VIA detection from chlorinated samples enriched according to FDA-BAM method and tested at different time-points. Each sample (point) was VIA tested in triplicate and determined to be positive if mean absorbance was ≥0.200 or negative if <0.200. Time point 0(D) samples were tested directly, then diluted 1:1 in enrichment media and tested again (time point 0) resulting in decreased absorbance values due to incorporation of the dilution factor.

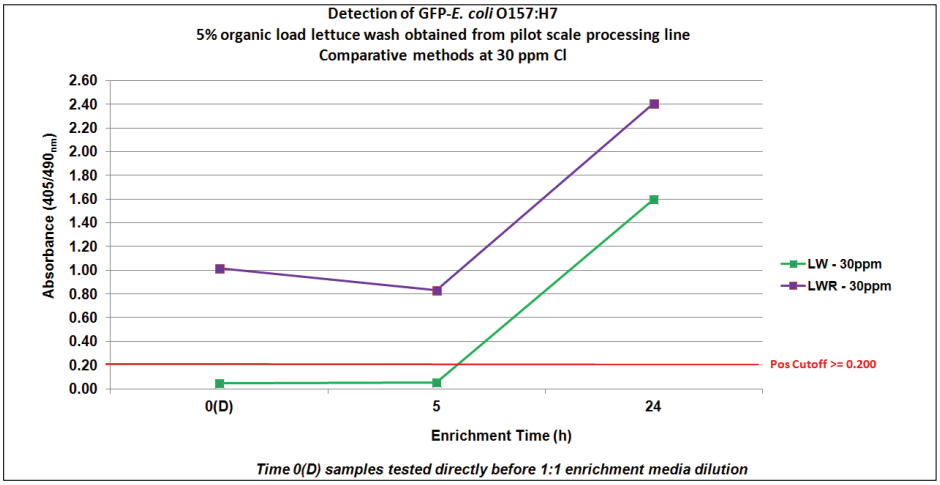

Figure 8. Comparison of *E. coli* O157:H7 VIA detection from chlorinated samples enriched according to FDA-BAM method and tested at different time-points. Each sample (point) was VIA tested in triplicate and determined to be positive if mean absorbance was ≥ 0.200 or negative if < 0.200. Time point 0(D) samples were tested directly before a 1:1 in enrichment media.

Conclusions

PMACS concentration helped to decrease the enrichment time needed to detect 1-2 CFU/25 mL of *E. coli* O157:H7, *L. monocytogenes*, and *Salmonella* Typhimurium in spiked lettuce wash samples (Method 1) and eliminated the enrichment time needed to detect *E. coli* O157:H7 in chlorinated, spiked lettuce wash samples using the 3M™ TECRA™ visual immunoassays semi-automated with a BioTek ELx50™ Microplate Strip Washer and BioTek ELx800™ Absorbance Microplate Reader. 3M™ TECRA™ VIAs have been certified Performance Tested by AOAC for use in food testing. Methods described here may be beneficial as a value added way of qualitatively screening for pathogenic bacterial loads in large volume zero tolerance sanitation routines used for fresh produce and processing equipment.

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