CENTER for PRODUCE SAFETY

CPS 2011 RFP
FINAL PROJECT REPORT

Project Title

Validation of testing methods for the detection and quantification of *Escherichia coli* O157:H7, *Salmonella* spp., fecal coliforms and non-pathogenic *Escherichia coli* in compost

Project Period

January 1, 2012 – December 31, 2012

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Objectives

1. Compare TMECC and USEPA methods for detecting and quantifying *E. coli* O157:H7, *Salmonella*, non-pathogenic *E. coli* and fecal coliforms in 'point of sale' compost to determine which method should be utilized by the compost industry as well as on-farm practitioners.
2. Determine whether soluble carbon profiles in finished compost samples can accurately predict the re-growth potential of *E. coli* O157:H7 and *Salmonella* in mature, finished 'point of sale' composts that have been inoculated with low population levels.

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FINAL REPORT

Abstract

Compost is well known for many beneficial properties as a soil amendment for both organic and conventional farming systems. Aerobic, thermophilic compost production processes are designed to achieve significant reductions in human and plant pathogens through time-temperature exposures. While compost is generally regarded as a safe product for unrestricted usage, it is gaining recognition as a potential source of foodborne pathogen contamination in the fresh produce industry. Recommended testing for pathogens in compost refer to US Environmental Protection Agency (EPA) methodology (which has not been evaluated for non-biosolids based composts) or other laboratory-certified/accredited methods like U.S. Composting Council Test Methods for the Examination of Composting and Compost (TMECC), which have not been validated for use across a wide variety of composts. In addition, we evaluated the use of two different immunomagnetic separation (IMS)-based methods for the detection of *E. coli* O157:H7 in composts to assess the use of current, rapid, sensitive microbiological detection technologies for compost pathogen testing. Our study included 29 different 'point of sale' mature composts, made from manure (n=4), biosolids (n=10), and yardwaste (n=15) feedstocks, collected from across the United States. Subsamples of these products were inoculated in our laboratory with relatively low concentrations of multiple strains of non-pathogenic *E. coli*, *E. coli* O157:H7, and *Salmonella* spp. The EPA and TMECC methods were compared for their specificity and sensitivity in recovering *E. coli* and *Salmonella* spp. (**Objective 1**). The EPA method recovered significantly ($p < 0.10$) greater levels of fecal coliforms and *E. coli* than TMECC methods ($p = 0.0003$). The EPA methods recovered significantly greater levels of *E. coli* from compost made from biosolids and manure feedstocks compared to TMECC methods. Both methods showed equivalent recoveries of *Salmonella* spp; however, the EPA method recovered significantly greater *Salmonella* levels from biosolids compost than did the TMECC method. Both immunomagnetic separation methods tested, direct plating (DP) and automated recirculating (AR), were equally effective in recovering low populations of *E. coli* O157:H7 from of 100% (29/29) of compost samples used in the study. Levels of total organic carbon and carbon /nitrogen ratios in composts examined were not correlated with the regrowth of *Salmonella* spp. and *E. coli* O157:H7 in point-of-sale finished composts, indicating that pathogen regrowth cannot be predicted by either of these chemical parameters (**Objective 2)**. Overall, EPA methods were superior or equivalent to the TMECC methods for recovery of fecal coliforms and *Salmonella* spp. from point-of-sale finished composts, and were effective in recovering pathogens from non-biosolids based composts.

Background

Compost usage has become an integral part of many conventional and organic farming practices. The composting process biologically transforms a variety of organic residuals from on- and off-farm sources into a stable soil amendment that can be easily stored, handled, and used to enhance soil quality while providing a small amount of slow-release nutrients for crops. In addition to on-farm composting, numerous centralized composting facilities located throughout the United States generate products from municipal, industrial, and agricultural by-products that ultimately are purchased and used by horticulturalists, produce growers, landscapers, and home gardeners. Recent survey information reported 14 municipal solid waste composting facilities, 250 biosolids, 3,260 yard trimmings and over 175 food residual composting sites across the United States.

When appropriate process and management controls are implemented, composting operations are able to produce high quality, stabilized composts with a reduced risk of pathogen content. Recent surveys, however, show that some commercially- available compost products may introduce *Salmonella* spp., *Escherichia coli* O157:H7 and other pathogens into field soils used for production of fresh fruits and vegetables. The adulterated composts either were inadequately treated as required for thermal disinfection of pathogenic bacteria or improperly stored or cross-contaminated by contact with unsanitized equipment. Improper storage could expose a high quality compost product to pathogen recontamination through bioaerosols, direct contact with raw feedstock materials or animal vectors. However, one study postulated that adequate thermal processing alone may not be adequate to ensure pathogen-free compost. A region-specific microbiological survey on a variety of non-sludge based composts (n=94) produced with a variety of technologies (e.g., windrow, aerated static pile and turned static pile) in California, Washington and Oregon found that 1% contained *Salmonella,* 28% contained fecal coliforms above the populations stated in the US Environmental Protection Agency (EPA) Part 503 regulations, and 6% contained *E. coli* O157:H7. Another survey of 16 biosolids-based composting facilities in Massachusetts determined that one third of finished compost products exceeded the EPA Part 503 standards for fecal coliform content. A survey of 'point-of-sale' composts from fifteen commercial operations across the U.S. showed that 9%, 33% and 11% of these facilities failed to meet the EPA standards for fecal coliforms in March, July and November, respectively, and 7% of the facilities exceeded the EPA standards for *Salmonella* populations.

Current federal regulations (Title 40 CFR Part 503) require that only biosolids-based compost be tested to meet specific populations of fecal coliforms (< 1000 MPN/g) and *Salmonella* (< 3 MPN/4g) prior to unrestricted distribution to the public. Many states have proposed their own microbiological standards for 'point-of-sale' composting operations, but these requirements are vague in terms of specific sampling and testing methods required for compliance. Most state regulations defer to EPA rules in terms of pathogen limits, sampling and microbiological testing methods, regardless of compost feedstock composition; however the EPA test methods have not been evaluated for efficacy in non-biosolids-based composts. In California (CCR Title 14), for example, compost operations are required to provide a single composite sample at the 'point-of-sale' for verification of pathogen reduction at the same regulated concentrations imposed by the EPA part 503 rule for fecal coliform and *Salmonella* content. There are no recommendations, however, concerning the microbiological testing methods that should be used. The California Leafy Green Marketing Agreement (LGMA) requires that all compost applied to leafy greens production environments must also comply with the California state regulations (CCR Title 14), but with the added testing for *E. coli* O157:H7. However, no specific testing method for this pathogen is validated for these matrices. The Food Safety Leadership Council (FSLC) has also issued guidelines that are more stringent than the EPA or LGMA standards by including limits for generic *E. coli* (< 10 MPN/g) as well as setting a zero tolerance precedent for *Salmonella*, *E. coli* O157:H7, and *Shigella* in compost. A summary of microbiological requirements for 'point of sale' composts are listed in

Table 1. Microbiological requirements for 'point of sale' composts used in agriculture as specified by various regulatory agencies

Test Parameter	U.S. EPA[1]	CCR Title 14[2]	FSLC[3]	LGMA[4]
E. coli O157:H7	na[5]	na	Negative	< 1/30g
Salmonella spp.	< 3 MPN/4g	< 3 MPN/4g	Negative	< 1/30g
Shigella spp.	Na	na	Negative	na

E. coli	Na	na	< 10 MPN/g	na
Fecal Coliforms	< 1000 MPN/g	< 1000 MPN/g	na	< 1000 MPN/g

[1] U.S. Environmental Protection Agency, Code of Federal Regulations, Title 40, Part 503.32 *(5)*
[2] California Code of Regulations, CCR title 14, chapter 3.1, article 7 *(3)*
[3] Food Safety Leadership Council *(7)*
[4] Leafy Greens Marketing Agreement *(4)*
[5] Not applicable – testing parameters are not specified in regulation

Standardized guidelines and recommended methods for microbiological testing are needed to ensure that accurate measurements are made for 'point of sale' products. These gaps within the 'science-based' regulations are a major limitation in ensuring that pathogen reduction regulations are met and encumber producers with the added task of deciding which methods are best suited for their operations. In addition, the lack of standardized methods for pathogen detection hampers efforts by regulators and others when comparing reported data. As such, it is difficult to obtain accurate data concerning the status of pathogen populations in 'point-of-sale' composts in the United States.

The United States Composting Council has developed and endorsed microbiological testing protocols, called the Test Methods for the Examination of Compost and Composting (TMECC) that includes sampling schemes and laboratory procedures for the quantification of total coliforms, fecal coliforms*, E. coli,* and *Salmonella*. These methods were developed, in part, to begin the standardization of practices involved in the creation of good quality compost through uniform monitoring of the physical, chemical and microbiological attributes of all compost. To our knowledge, neither EPA nor TMECC microbiological testing procedures have been evaluated for the recovery of pathogens from non-biosolids based compost that produce growers utilize across the United States. Identifying the most sensitive and accurate method to determine the presence of pathogens in compost will provide certainty with regard to microbiological testing for compost producers, and provide produce growers another tool to ensure produce safety. In addition, neither the EPA nor the TMECC methods take advantage of rapid, sensitive microbiological detection technologies currently available. Updating and validating the detection/enumeration methodology to include rapid methods for *E. coli* O157:H7 detection will also provide improved efficiency and reliability of compost test methods for compost producers, purchasers, and regulators. This will further support confidence in the third-party audit programs that include compost product testing as part of their on-farm checklist activities designed to prevent the dissemination of foodborne pathogens.

The use of a non-microbiological indicator in finished compost may also have some benefit. Chemical or physical determinants in compost may indicate the presence of pathogens in point of sale material. Some of these parameters (moisture, water activity, electrical conductivity, soluble carbon, pH and carbon:nitrogen ratios) have been previously associated with pathogen re-growth potential in biosolids-based and dairy manure compost. Further investigation is needed, however, to determine which of these parameters are important for pathogen re-growth in finished compost products from a variety of feedstocks and locations across the U.S. Our previous studies involving pathogen re-growth in compost, compost teas, and irrigation water suggests that the concentration of soluble carbon (glucose, in particular) in finished composts may be a significant factor in predicting the potential for *E. coli* O157:H7 and *Salmonella* to effectively and rapidly re-populate composts that have previously been determined to be pathogen-free.

Research Objectives

1. Compare a) TMECC and USEPA methods for detecting and quantifying fecal coliforms, *Escherichia coli*, and *Salmonella* in 'point of sale' compost to determine which method should be utilized by the compost industry as well as on-farm practitioners; b) compare two immunomagnetic separation techniques – automated recirculation (AR) and Direct Plating (DP) for the detection of *E. coli* O157:H7 in same point of sale composts.
2. Determine whether soluble carbon profiles in finished compost samples can accurately predict the re-growth potential of *E. coli* O157:H7 and *Salmonella* in mature, finished 'point of sale' composts that have been inoculated with low population levels.

Research Methods and Results

Methods

Compost samples. Twenty-nine compost samples were obtained from across the United States (please see Table 1 in the in Appendix for Tables and Graphs for type and geographic location of each sample). Twenty-four of these samples are certified by the Seal of Testing Assurance (STA), a program established by the U.S. Composting Council (USCC) for their quality. Samples of compost were tested for microbial background and physicochemical properties (listed in Table 2).

Table 2. List of physicochemical tests and methods used on compost samples.

Test Performed	Brief Description of Method
Total Organic Carbon (TOC)	Phoenix 8000 infrared spectroscopy
Carbon to Nitrogen Ratio (C:N)	Elementar Vario Max CN
Compost Maturity	Used Solvita maturity kits (CO_2 and NH_3 panels)
pH	pH probe
EC (electrical conductivity)	EC probe
% Moisture	Drying oven (% = wet (mg) - dry (mg)/wet (mg)
% Organic Matter	Ashing oven (% OM determined = dessicated - ashed / dessicated)

Strains used. Compost samples (400g) were inoculated simultaneously with non-pathogenic *E. coli*, *Salmonella* spp., and *E. coli* O157:H7. Three strains of non-pathogenic *E. coli*, isolated from agricultural environments in California, and adapted for rifampicin resistance (80 µg/ml). *Salmonella* serotypes used in the study were *S.* St. Paul, isolated from jalapeno peppers, and *S.* Newport, isolated creek sediment from Virginia, were adapted to nalidixic acid (50 µg/ml). Two isolates of *E. coli* O157:H7: RM 4407, isolated from spinach, and RM 5279, isolated from lettuce, and adapted to nalidixic acid (50 µg/ml). All strains were grown separately in milorganite extract before being combined into a single bacterial inoculum. All strains were inoculated at between 1 -2 $\log_{10}$ CFU/g, homogenized thoroughly to disperse the inoculum throughout each compost. Inoculated compost was then hand massaged for 2 min to thoroughly mix bacterial inoculum. Thereafter, inoculated compost samples were analyzed by standardized procedures.

Microbiological analysis. Inoculated compost was analyzed by the standard microbiological procedures - either Environmental Protection Agency (EPA) methods or U.S. Composting Council Test Methods for the Examination of Composting and Compost (TMECC). Flowcharts of the procedures used, listed below in the table, are included in the appendices. Minor modifications were made to these procedures at the last step of presumptive identification of *Salmonella* and *E. coli* isolates. Because the strains used were resistant to antibiotics, isolation of presumptive *E. coli* and *Salmonella* spp. were performed on MacConkey agar supplemented with 50 µg/ml nalidixic acid (MACN) and Xylose Lysine Desoxycholate supplemented with 80 µg/ml rifampicin (XLDR) to calculate the recovery percentage of the specific target pathogen. A minor modification was made to the EPA 1680 method. After the final step (turbidity and gas formation in tubes), aliquots from the tubes with positive responses for both these parameters were streaked for isolation onto MACN.

Table 3. EPA and TMECC Method used to examine the recovery of fecal coliforms, *E. coli* and *Salmonella* from compost.

	Method of Analysis	
Target organism	**EPA**	**TMECC**
Fecal coliforms	1680	7.01B
Escherichia coli	-	7.01C
Salmonella spp.	1682	7.02

Two immunomagnetic separation (IMS)-based methods for the detection of *E. coli* O157:H7 were also evaluated. An automated recirculating (AR) method was performed by incubating 25 g of compost inoculated with *E. coli* O157:H7 in 225 mL in mEHEC (BioControl) at 37^{o}C for 5 h. Samples were then placed at 4^{o}C overnight, before placement in a Pathatrix automated recirculation system. Aliquots (50 µl) of immunomagnetic beads coated with O157-antibodies (supplier) were added to the enriched compost sample and recirculated. After 30 min, beads with bound *E. coli* O157:H7 cells were removed from the system by magnetic separation, washed, and then frozen until DNA was extracted using InstaGene matrix (BioRad) according to recommended procedures. This step was followed by sequential purification with a) DNA Clean and Concentrate kit and b) One-step PCR inhibitor removal kit (Zymo Research). Real-time PCR, using a BioRad iCycler, was then performed using primers and probes for the shiga-toxin 2 gene (*stxII)* as described by Rashid et al, 2006. [Infect. Immun. 74: 4142-4148]. Detection of the stxII gene in samples indicated the presence of *E. coli* O157:H7 in compost samples. The second IMS method, direct plating (DP), was performed by diluting 10 g of compost into 90 mL of Tryptic Soy Broth (TSB) and making serial dilutions (1:10 to 1:1000) of the inoculated sample in buffered peptone water, and then incubating at room temperature for 2 h, followed by incubation at 42^{o}C for 6 h before being placed at 4^{o}C overnight. The O157-specific immunomagnetic beads were added to serial dilutions and incubated at room temperature for 10 min before being magnetically removed from dilutions, washed, and spiral plated, 50 µl, in duplicate, to chromO157 agar, for culture detection of *E. coli* O157:H7.

For Objective 2, to determine the regrowth of *Salmonella* spp., EPA method 1682 was used to recover and quantify *Salmonella* from inoculated compost samples stored at 25^{o}C for 3 days. To determine regrowth of *E. coli* O157:H7 in inoculated compost over 3 days, a modified direct plating system was used where 10 g of compost was added to 90 ml TSB, homogenized, and serial dilutions were then plated directly to chromO157 agar.
Three replicates of each microbiological analysis were performed on each compost sample.

Statistical Analysis

For the analysis of objective 1 and the comparison of EPA and TMECC methods for the recovery of fecal coliforms, *E. coli* and *Salmonella*, a two-way analysis of variance (ANOVA) based on a beta-distribution of the recovery percentages of each pathogen in each compost type was used at an alpha level of 0.10. For objective 2, a linear regression model was constructed to predict regrowth of *Salmonella* spp. or *E. coli* O157:H7 based on total organic carbon or the carbon/nitrogen ratio.

Results

Recovery percentages for fecal coliforms, *E. coli* and *Salmonella* are listed in Table 2 in the Tables and Graphs Appendix. EPA methods recovered significantly ($p < 0.10$) greater levels of fecal coliforms and *E. coli* when compared TMECC methods ($p = 0.0003$) for all compost type (biosolids, manure, and yardwaste). EPA methods also recovered significantly greater levels of *E. coli* from compost made from biosolids and manure feedstocks compared to TMECC methods. Both methods showed equivalent recoveries of *Salmonella*; however, EPA methods recovered significantly higher ($p = 0.0596$) *Salmonella* levels from compost with a biosolid feedstock compared to TMECC methods. Both immunomagnetic separation methods (direct plating and automated recirculating) were equally effective in recovering low population of *E. coli* O157:H7 from inoculated compost samples. Levels of total organic carbon and carbon / nitrogen ratios were not correlated to the regrowth of *Salmonella* spp and *E. coli* O157:H7 in point of sale finished composts, indicating that pathogen regrowth cannot be predicted by either of these chemical parameters. However, composts containing biosolids feedstocks were more likely to have maturity levels less > 5, potentially indicating that they may require longer times to reach suitable maturity.

Outcomes and Accomplishments

Objectives 1 and 2 were accomplished by completing this project. This the first study to actively compare EPA and TMECC methods for the recovery of pathogens from compost, and to attempt to correlate pathogen regrowth to physicochemical factors.

Summary of Findings and Recommendations

1. EPA method 1680 recovered a significantly higher percentage of fecal coliforms than TMECC method 7.01B from inoculated composts.

2. EPA method 1682 and TMECC method 7.02 were statistically equivalent in the percentage of *Salmonella* spp. recovered from inoculated composts.

3. EPA methods seem to recover higher or equivalent percentages of fecal coliforms and *Salmonella* spp. compared to TMECC methods.

4. Immunomagnetic methods – either direct plating or automated recirculation – are effective and relatively rapid in recovering low levels of *E. coli* O157:H7 from compost.

5. Total organic carbon and the carbon/nitrogen ratio were not correlated to the ability of *Salmonella* spp. and *E. coli* O157:H7 to regrow in point of sale finished composts; however

the interaction of all physicochemical factors evaluated and their effect on pathogen regrowth deserves more attention.

6. Composts made from biosolids feedstocks were significantly more likely to score lower on the maturity index compared to composts made from yard waste and manure; it is unclear from the data gathered in this study how this finding may be related to pathogen regrowth in finished composts.

7. When possible, the greater volumes of sample used in the analysis of primary dilutions in the MPN series outline in the EPA methods 1680 and 1682 should be used to recover fecal coliforms and *Salmonella* spp. from compost.

APPENDICES

Publications and Presentations (required)

No publications or presentations have been made to this point.

Budget Summary (required)

Item	**Amount**
Salaries	56023.13
Benefits	3138.31
Supplies	49752.06
Indirect Costs	2958.07
Travel (to attend CPS 2013 meeting)	1890
TOTAL	**113761.60**

Tables and Figures

Table 1. Source of point of sale, finished composts.

Sample[1]	Class[2]	State
1	B	GA
2	M	CA
3	Y	CA
4	M	CA
5	Y	MD
6	B	MD
7	B	IA
8	Y	VA
9	Y	MD
10	B	VA
11	Y	NJ
12	Y	DE
13	Y	MA
14	B	MA
15	Y	MA
16	B	VA
17	Y	NC
18	M	PA
19	B	CA
20	B	CO
21	B	NH
22	Y	OR
23	M	KA
24	Y	KY
25	Y	CT
26	Y	DE
27	B	OH
28	Y	FL
29	Y	WA
30	Y	NJ

[1]gray shading indicates sample is not STA-certified.

[2] B = biosolids; M = manure; y= yardwaste

EPA 1680 (fecal coliforms) 2006 Revised Methods

Dilution Procedure

30 grams of original compost sample into 270mL phosphate buffered water, blended on high for one minute.

A serial dilution series is then performed by adding 10mL of the original homogenized sample into 90mL of phosphate buffered water. This will be our first dilution.

The original homogenized sample is to be diluted through 10^{-5}.

5 Tube MPN PROCEDURE

10ml from the original homogenized sample (containing 1g of the original compost) will be added to 5 tubes each containing 10ml 2x Lauryl Tryptone Broth

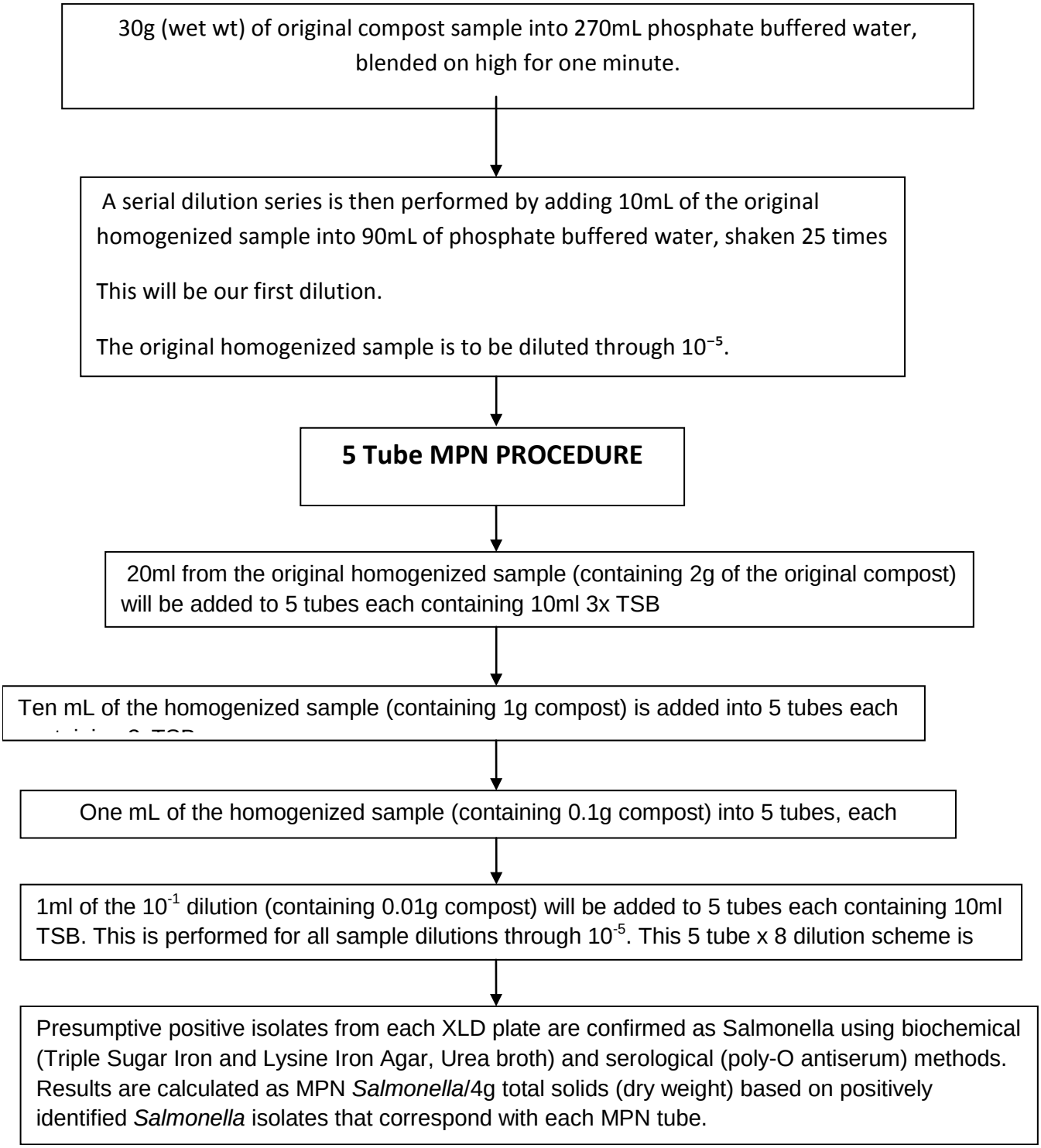
1ml from the original sample (containing 0.1g of the original compost) will also be added to 5 tubes each containing 10ml LTB

1ml from the 10^{-1} dilution (containing 0.01g of the original sample) will be added to 5 tubes each containing 10ml LTB; this is performed for all sample dilutions through 10^{-5}.

This 5 tube x 7 dilution scheme is incubated at 35°C for 24h and observed for gas formation in small inverted glass tubes (i.e. durham tubes). All presumptive positive tubes (positive for gas formation) are confirmed by transferring a small amount of liquid from each culture tube (with a 3-3.5mm sterile loop) to EC medium and incubated at 44.5°C for 24h

EPA 1682 (Salmonella spp) 2006 Revised Methods

Dilution Procedure



TMECC 7.01B (Fecal Coliforms)

Dilution Procedure

25 grams (wet weight) of original compost sample into 225mL Lauryl Tryptone Broth (LTB) into a stomacher bag and stomached at 260 rpm for one minute.

↓

A serial dilution series is then performed by adding 1mL of the original homogenized sample into 9mL of buffered peptone water. This will be our second dilution. The original homogenized sample is to be diluted through 10^{-5}.

↓

3 Tube MPN PROCEDURE

↓

10ml from the original homogenized sample (containing 1g of the original compost) will be added to 3 empty sterile tubes.

↓

1ml from the original homogenized sample (containing 0.1g of the original compost) will also be added to 3 tubes each containing 9ml LTB.

↓

1ml from the 10^{-2} dilution (containing 0.01g of the original sample) will be added to 3 tubes each containing 9ml LTB.

↓

This 3 tube x 3 dilution scheme is incubated at 44.5°C for 24h and observed for gas formation in small inverted glass tubes (i.e. durham tubes). All presumptive positive tubes (positive for gas formation) are confirmed by transferring a small amount of liquid from each culture tube (with a 3-3.5mm sterile loop) to EC medium and incubated at 44.5°C for 24h

↓

Dilutions 10^{-2} through 10^{-5} will be spiral plated in duplicate onto plates of MacConkey's agar+MUG (MAC-MUG) and incubated for 24h at 44.5°C.

TMECC 7.01C (E.coli) continuation of TMECC7.01B
Presumptive Positives

All presumptive positive tubes will be streaked for isolation on MacConkey agar and isolates will be confirmed via traditional biochemical methods (Eosin Methylene Blue Agar, TSI, MIL, and Indole formation) as described in the TMECC manual.

TMECC 7.02 (Salmonella)

Dilution Procedure

25g (wet wt) of original compost sample into stomacher bag with 225mL BPW, stomached on high (260rpm) for 1min
This is the ORIGINAL HOMOGENIZED SAMPLE

↓

A serial dilution series is then performed by 1ml from each dilution into 9ml BPW and vortexing for 10 seconds.
This will be our first dilution.

↓

3 Tube MPN PROCEDURE

↓

10ml volumes of the 10^{-1} homogenized sample (containing 1g compost) will be placed into 3 sterile empty tubes

↓

1ml volumes of the original 10^{-1} homogenized sample (containing 0.1g compost) will be placed into 3 tubes each containing 9ml BPW

↓

1ml volumes of the 10^{-2} dilution (containing 0.01g compost) will be placed into 3 tubes each

↓

This 3 tube x 3 dilution scheme is incubated at 37°C for 24h. Each tube is then vortexed and 1ml sample from each tube is transferred to a new tube containing 9ml Tetrathionate Broth (Hajna formula) and incubated for 18-24h at 37°C.

↓

A loopful from each tube will be aseptically streaked for isolation onto XLT4 agar plates and incubated for 24h. Simultaneously with the MPN procedure, sample dilutions (10^{-2} – 10^{-5}) are spiral plated directly onto XLT4 agar plates and incubated for 24h at 37°C.

↓

Presumptive positive colonies on XLT4 from both MPN confirmations and spiral plating techniques are reported as either MPN *Salmonella* / 4g total solids (dry weight) of CFU *Salmonella* / g total solids (dry weight). All presumptive *Salmonella* isolates are subject to biochemical (TSI, MIL) and serological (Poly-O) confirmation protocols in TMECC manual.

Table 2. Percent recovery of *E. coli* or *Salmonella* by EPA or TMECC method

		Average Percent Recovery			
		Generic E. coli Methods		Salmonella spp. Methods	
Sample	Type	EF	TF	ES	TS
	B	61.6%	6.62%	47.62%	0.00%
	B	73.9%	31.56%	7.15%	75.58%
	B	121.0%	24.96%	74.48%	60.92%
	B	48.3%	24.76%	79.90%	10.13%
	B	64.5%	30.24%	271.10%	58.99%
	B	237.2%	36.26%	21.81%	11.66%
	B	128.3%	70.04%	41.79%	68.13%
	B	58.8%	32.08%	88.28%	80.00%
	B	49.4%	18.09%	203.65%	6.56%
	B	34.0%	26.34%	15.34%	5.95%
Average	B	87.7%	30.09%	85.11%	37.79%
	M	17.0%	0.00%	56.89%	0.45%
	M	32.0%	23.49%	6.54%	143.15%
	M	156.8%	18.57%	51.25%	21.29%
	M	81.5%	82.47%	44.76%	53.81%
Average	M	71.8%	31.13%	39.86%	54.67%
	Y	24.2%	41.03%	5.29%	34.98%
	Y	22.5%	13.67%	1.10%	1.25%
	Y	49.0%	36.08%	196.88%	80.56%
	Y	110.2%	46.01%	28.74%	308.44%
	Y	41.2%	75.75%	140.31%	45.34%
	Y	28.0%	153.76%	66.04%	52.38%
	Y	52.1%	20.82%	42.21%	39.47%
	Y	75.7%	44.74%	144.93%	493.27%
	Y	52.9%	88.90%	9.08%	12.21%
	Y	52.5%	86.59%	42.41%	41.69%
	Y	19.3%	26.73%	74.60%	40.56%
	Y	64.7%	11.83%	1.48%	2.08%
	Y	26.4%	50.74%	53.88%	38.11%
	Y	136.1%	167.46%	139.17%	133.87%
	Y	107.5%	73.99%	451.14%	55.50%
	Y	34.2%	59.82%	263.93%	124.28%
Average	Y	56.0%	62.4%	103.8%	94.0%
		EF	TF	ES	TS
Average	All Types	68.7%	47.4%	89.1%	70.0%

Methods Key

EF = EPA method for Fecal Coliforms
TF = TMECC method for E. coli
ES = EPA method for Salmonella spp.
TS = TMECC method for Salmonella spp.

Sample Type Key

B = Biosolids composts
M = Manure composts
Y = Yard waste composts

Figure 5. Regrowth (CFU/g (dry weight)) of *Salmonella* spp. based on compost made from biosolids, manure, or yardwaste feedstocks over three days.

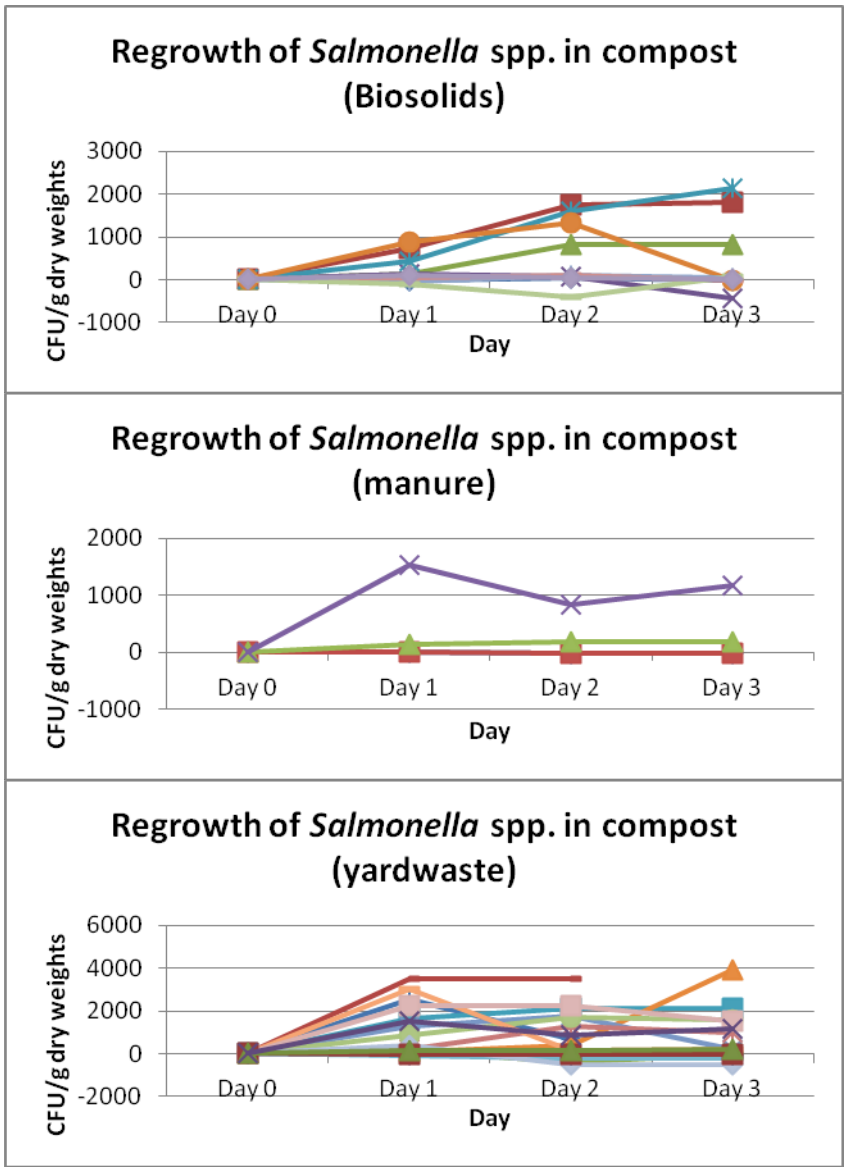
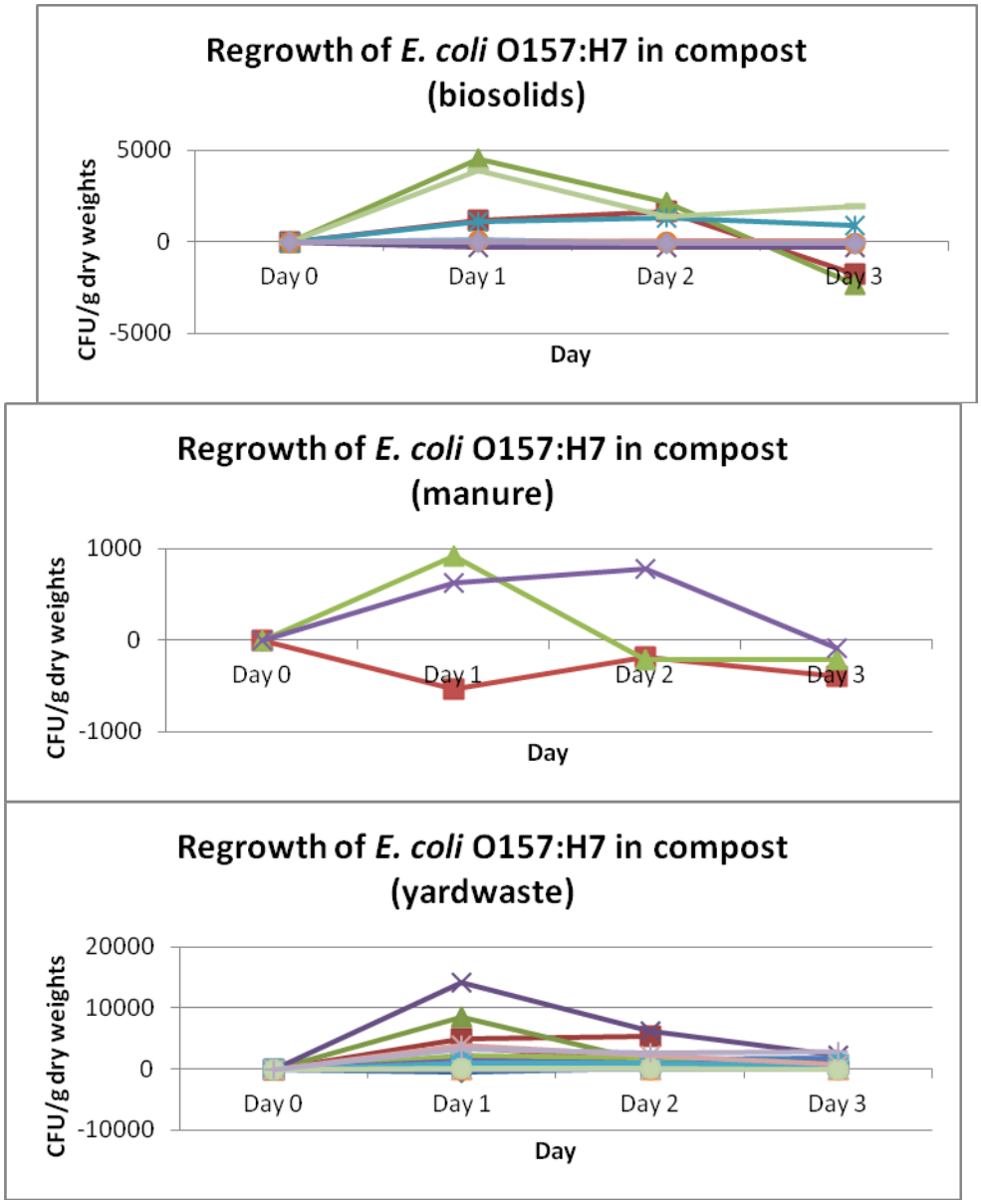


Figure 6. Regrowth (CFU/g (dry weight)) of *E. coli* O157:H7 spp. based on compost made from biosolids, manure, or yardwaste feedstocks over three days.



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Validation of testing methods for the detection and quantification of E. coli O157:H7, Salmonella spp., fecal coliforms and non-pathogenic E. coli in compost

Recommendations and Suggestions to CPS

None.