CENTER for PRODUCE SAFETY

CPS 2014 RFP
FINAL PROJECT REPORT

Project Title
Evaluation of an alternative irrigation water quality indicator

Project Period
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Objectives
This project would initiate the collection of preliminary data to support collective expert evaluations for the replacement of quantitative irrigation water standards based on generic E. coli *with a qualitative presence-absence standard (in reality a semi-quantitative threshold based on a designed risk-assumption Limit of Detection) based on Bacteroides as an improved indicator of fecal contamination and more specifically the presence of pathogens. Initially, for the purpose of this project we would use the commercial kit reagents and protocol for Total Bacteroides detection described by GeneSig* ***(http://www.genesig.com/products/9159).***

- *Six hundred water samples will be collected from surface water sources in diverse fresh produce production districts predominantly from CA and AZ but including OR and WA*
- *Irrigation, foliar contact, and other ag-water samples will be tested for indicators and bacterial pathogens*
- *Water samples will be analyzed in a quantitative manner to establish recommended Limit of Detection thresholds for a standard protocol that will define a qualitative assumed or precautionary risk-based metric*
- *A qualitative standard is anticipated to decrease the time to reporting (no growth phase needed) and keep cost per test to a comparable level with current expenditures for quantitative E. coli results*
- *In parallel, a comparative analysis of published PCR detection systems for Bacteroides gene targets will be conducted to evaluate current kits with non-commercial research detection systems from published literature for fidelity of outcomes (eg. Kitts et al. 2010)*

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FINAL REPORT

Abstract

In communicating its deliberations regarding the definitions, standards, metrics, and criteria for anticipated compliance within this document, FDA clearly recognized the absence of clarity and consistency relating to, and the practical limitations of, designing any single or multi-level indicator-based approach to establishing compliant and non-complaint conditions that achieve a measureable advancement in public safety and health-based water quality standards. Recent studies assembled from datasets in different parts of the U.S. and other countries underscored the variable reliability of generic *Escherichia coli* as an indicator of fecal contamination in fresh water sources. It is widely recognized that a general lack of correlation exists between *E. coli* levels and the presence of pathogens in crop management water or on the crops irrigated with the water. An alternative and well-established biological indicator of fecal contamination of water sources is the gram-negative obligate anaerobic bacterium *Bacteroides*. Recent developments in rapid, quantitative, real-time PCR methods and first generation commercial Total Bacteroides qPCR kits was hypothesized to make this alternative indicator suitable for routine agricultural water testing. The potential adoption of this indicator system was evaluated in the current project. A total of 703 primary and 27 preliminary and opportunistic water samples from natural surface sources used for preharvest crop management were collected in California, Arizona, and the Pacific Northwest region, primarily Washington State. Diverse sources included ground-to-surface water reservoirs, publically managed irrigation distribution systems (aqueduct and canals), a seasonal river tributary, and surface water and run-off detention ponds used for seasonal crop production purposes (isolated production areas with secondary water sources, frost control, scald control, dust abatement). Samples were collected during 56 different sampling events, between January to October 2015 (n=377) and March to July 2016 (n=287). A small number of samples across the regions of study were found to be non-compliant to either industry or current FDA Produce Safety Rule standards. As in prior studies, we found that, as with generic *E. coli*, the current fecal indicator bacteria (FIB) used to characterize ag-water for fresh produce production, there is no correlation of quantitative thresholds of Total *Bacteroides* with the detectable presence of enteric pathogens (*Salmonella* and STEC/ $EHEC_{O157}$) in the irrigation source water. Overall, while typical surface water sources did not seem to benefit from the use of the PCR-based Bacteroides kit tests, chronic or acute events of fecal contamination would be characterized using this technique and may offer some future benefit for growers.

Background

Much has been debated and written about the inadequacy of current methods for routine monitoring and assessment of the microbiological quality of agricultural water used for irrigation, crop protection applications, and other preharvest inputs for fresh, edible, and perishable horticultural foods such as the diverse category of leafy greens. The lack of consistent correlation between chemical or biological indicators of fecal contamination at the local, regional, national, and global level is well documented in the scientific and public health literature (Bradley et al., 2007; Gelting and Baloch, 2012; Sauvé et al., 2012). This situation is extensively described in the environmental and public health science literature and encompasses drinking water sources, regional watersheds, recreational water, wastewater reclamation, and irrigation water. Prior to and upon the release for public comment of the

proposed regulations implementing the provision in the FDA Food Safety Modernization Act (FSMA) related to produce safety (Subpart E § 112.41 Standards for the Growing, Harvesting, Packing, and Holding of Produce for Human Consumption; Proposed Rule (Produce Safety Rule)), there was a significant level of dissatisfaction and dissent surrounding the specifics associated with existing and anticipated agricultural water standards.

In communicating its deliberations regarding the definitions, standards, metrics, and criteria for anticipated compliance within this document, FDA clearly recognized the absence of clarity and consistency relating to, and the practical limitations of, designing any single or multi-level indicator-based approach to establishing compliant and non-complaint conditions that achieve a measureable advancement in public safety and health-based water quality standards. Pachepsky et al. (2011) provide a comprehensive academic and practical review of the science and regulatory positions on irrigation water as a source of pathogenic microorganisms and the historical basis for the EPA Recreational Water Quality Criteria (RWQC) used as the basis for these standards. This comprehensive review and a related Produce Safety Project Issue Brief (Suslow, 2010) provided an overview of the issues, concerns, and practical consideration and limitations of uniformly applying these criteria to leafy greens and other fresh produce production at the time prior to release of the Final FSMA Produce Safety Rule. Recent studies assembled from datasets in different parts of the U.S. and other countries underscored the variable reliability of generic *E. coli* as an indicator of fecal contamination in fresh water sources. These same reports demonstrated strong regional fidelity, with a general lack of correlation between *E.coli* levels and the presence of pathogens in crop management water or on the crops irrigated with the water (Benjamin et al., 2013; Haack et al., 2009; Jacobsen and Bech, 2012; McEgan et al., 2013; Won et al., 2013a, 2013b). There are, however, temporal and spatial conditions, generally associated with a point-source event, under which generic *E. coli* do perform as an effective indicator of recent fecal contamination and likely pathogen presence (Castro-Rosas et al., 2012; McEgan et al., 2013; Sbodio et al., 2013).

In supporting the proposed microbiological requirements for agricultural water, the preamble of the Produce Safety Rule more than adequately provides an overview of the science-basis for the FDA decision to retain commensal *E. coli* as the current, practical indicator of fecal contamination of agricultural water sources and conveyance systems. Ultimately, this decision is more a reflection of the absence of current scientific evidence for an effective, practical, and economically acceptable alternative -- in the face of substantial and increasing field-based evidence of limitations associated with employing generic *E.coli* as a positive or negative indicator of risk related to consumption of uncooked produce. FDA also makes a cogent argument for not proposing pathogen-based standards, given the limitations and potential for unintended economic burden of direct pathogen testing of agricultural water sources and distribution modalities. The choice of indicator and frequency of monitoring proposed, based on an assessment of qualitative risk to public health, available peer-reviewed studies, expert solicitation, and experience from investigative analysis associated with outbreaks, is clearly the end-point of complex compromises.

Current scientific information originating from many disciplines related to water, soil, and crop microbiology across a global platform, all point towards adoption of a qualitative (or semi-quantitative) rather than a strictly quantitative approach to actionable thresholds for fresh produce production. Ignoring for the moment the limitations of indicator *E. coli* as the reasonable practical compromise choice, the selection of quantitative regulatory compliance standards is both unnecessary and costly for many producers. There is no dataset or model we are aware of that could be applied to differentiate the risk associated with use of agricultural water under any combination of conditions between a rolling geometric mean of 125 vs. 126 MPN (CFU)/100 ml

or 234 vs. 235 CFU/100 ml as a single sample exceedance point. The introduction of a statistical threshold value (STV) of 410 CFU/100 ml, based on revised EPA Recreational Water Quality Standards for direct human contact, does not resolve the poor correlative strength of generic *E. coli* in assessing crop contamination risk. While previous CPS-funded research conducted by Rock et al. (2013) indicated that current voluntary or mandatory standards (i.e., marketing agreements for signatories and marketing orders) represent minimal risk under most irrigation scenarios, many variables are still unknown.

Leafy green-specific evidence has shown that *E. coli*, as the selected fecal indicator bacteria (FIB), provides only restrictively limited predictive information of water source quality, at the quantitative strata currently applied within industry standards adopted by the California Leafy Green Products Handler Marketing Agreement (LGMA). Both controlled research studies across the U.S. and several years of extensive industry monitoring in many regions support the conclusion that proposed quantitative levels of this FIB do not correlate with detectable pathogen presence in water or on the crop to which it is applied, as measured at the time of harvest. Furthermore, numerically compliant levels of the FIB have been associated with crops that are not harvested due to pathogen detection. FIB levels greatly exceeding compliance standards, including research studies with non-disinfected wastewater, are rarely associated with detectable levels of pathogens.

We have limited scientific details of the complex interactions between agricultural water, environment and climate, crop horticultural traits, crop production practices, and pathogen biology and physiology. These knowledge gaps are a barrier that does not permit a sufficiently informed scientific or regulatory position to build more than a rudimentary foundation for predicting outcomes following a contamination event (Harris et al. 2012; Benjamin et al. 2013; Won et al. 2013). This stark reality underlies the science-based arguments against the continuation of applying quantitative indicator *E. coli* standards based on freshwater RWQC. The historical roadmap and assessments that formed these illness-based, acceptable-risk criteria are thoroughly reviewed and cited in Pachepsky et al. (2011), EPA RWQC (2012), and Haack et al. (2009). Recent changes to the quantitative metrics associated with EPA RWQC are explained in EPA RWQC 2012. Numerous articles detail the functional applicability of these standards, derived predominantly from FIB levels associated with known point-source fecal contamination and the frequency of gastro-intestinal illness following full, or varying degrees of, human body contact, with impacted recreational water. It should be noted that substantial epidemiological studies have been based on incidental monitoring data and studies have not been well defined.

These same research articles demonstrate the limitations and failures of predictive correlation when applied to non-point source contamination of fresh surface-water bodies. Repeatedly, science points to the important distinction that standards applied to agricultural water, primarily as used for irrigation, must account for pathogen die-off due to environmental and biological stressors (Atwill et al., 2011; Harris et al., 2012; Suslow, 2002; Suslow et al., 2003; Van der Linden et al., 2013). The issue of growth potential during postharvest phases, including distribution along the marketing-to-consumption chain, is a co-dependent but, we suggest, separate set of policy considerations to consider when setting regulatory agricultural water criteria.

Real-time Bacteroides PCR as Improved FIB

An alternative and well-established biological indicator of fecal contamination of water sources is the gram-negative obligate anaerobic bacterium *Bacteroides*. Tracking and differentiating

animal sources of bacteria in the genus *Bacteroides* has long been a tool for evaluating fecal contamination of surface water with uses in drinking water sources and for recreational purposes and to characterize impaired surface water sources (Converse et al., 2009). Research has sought to correlate quantitative levels of *Bacteroides* with other FIB and chemical indicators of human wastewater introduction to environmental water bodies. *Bacteroides*, in general, make up a substantial proportion of bacterial populations in fecal matter, as much as 40% of the bacterial populations and 10% of the fecal mass. They may have persistent survival capacity under some conditions but are not known to have growth potential in the environment. For bacterial source tracking, a high degree of host-association or specificity is a unique advantage of *Bacteroides* over most FIB; however, in the context of this proposal, the limitations associated with this feature are not particularly relevant. For the purpose of general monitoring and assessments of irrigation water sources, prediction of animal-host is not a requisite function of testing for evidence of recent fecal contamination or unacceptable levels of fecal loading. Though not the purpose of this proposed validation research, investigative assessments to qualify an agricultural-water source under a preharvest audit scheme or conduct root-cause analysis of a chronic or acute risk of crop contamination may benefit from separating human from other presumptive sources or ratios of host-associated *Bacteroides* in agricultural water. Through identification of the host mammal, or source of the fecal contamination, growers and water resource managers can make informed decisions on the solutions or Best Management Practices related to water-quality pollution problems in their region.

Recent developments in rapid, quantitative, real-time PCR methods and first generation commercial Total Bacteroides qPCR kits make application in service labs for routine agricultural water testing a plausible reality (GeneSig Bacteroides, AllBac 2013). In relative ‘clean’ agricultural water with low humic acids or other components that inhibit or interfere with PCR reactions, direct PCR tests without sample concentration and DNA purification steps have been shown to be readily possible. Based on an inclusive primer-pair for *Bacteroides* 16S ribosomal RNA (rRNA) and recent validation studies in water, assessing persistent, seasonal, and acute fecal pollution of water sources has been shown to be achievable in 5 hours or less. As non-cultural enrichment (optional concentration step) is used, detection is based on standard linear relationships to known quantities or genome copy-number (CN value: aka genomic equivalent copies [GEC]) for *Bacteroides* gene targets. In this case, actionable thresholds are based on CN values rather than MPN or CFU. With these improved techniques, substantial gains in knowledge regarding the association of this indicator to fecal sources of water contamination, general non-point source fecal contamination, and correlation to presence of bacterial, viral, and parasite pathogens have been achieved (Sauer et al., 2011). Perhaps more importantly, the costs of current rapid test kits have put this FIB in reach and within typical operator skill-level for high volume testing by accredited service labs. Most public and private labs that provide water-testing services (recently more county public environmental and health include agricultural-water testing for Good Agricultural Practices (GAP) programs) have equipment for and experience with qPCR test kit formats. As in the majority of commercial kits, awareness of PCR inhibitor issues is overcome by incorporating extraction efficiency standards and an internal amplification/reaction control (IAC) of varying design strategy in every reaction tube. This feature is especially needed in seasonally lower quality agricultural water obtained from surface sources, such as canal laterals. This feature is already an integral part of almost all current kits and can be used to standardize and normalize results for a simple software-based (internal algorithm) for Pass/No Pass result reporting.

In a simple informal survey of California and Arizona leafy greens producers alone, over 80,000 irrigation water source and distribution systems tests are conducted per year. Our objective was to develop a baseline of data to support this alternative indicator for routine agricultural water

quality assessments and monitoring. Based on discussions during concept development with this sector and diverse commodity groups across the U.S., it seems likely that commercial developers will be attracted to this expanding market and invest in further kit refinements, certification with an accrediting body, and expanded performance testing. Clearly this will not alleviate accessibility to labs for growers in all regions but *Bacteroides* seems a viable alternative and potential replacement FIB worth serious consideration for improved accuracy, timeliness of results, and better association with levels of fecal contamination to set meaningful action response levels. A relatively recent publication released during the development of this proposal concept (Ravaliya et al., 2014), entitled *Use of Bacteroidales Microbial Source Tracking To Monitor Fecal Contamination in Fresh Produce Production*, provided some early indication to justify the merit of addressing the objectives of this study.

Another attractive aspect for evaluating *Bacteroides* as an alternative FIB for preharvest water monitoring is its enhanced capacity to predict the presence of human viral pathogens, such as norovirus, in water bodies (McQuaig et al., 2012) relative to the poor performance of generic *E. coli.* Our longer-term goal for federal funding is to develop collaborative efforts with researchers developing Quantitative Microbial Risk Assessments (QMRA) on leafy greens and other produce with a primary focuses on viral and parasitic pathogens.

One distinct advantage of *Bacteroides* over 'generic' *E. coli* is the ability to freeze-thaw water samples and repetitively analyze a retained sample with a high degree of reproducibility (r^2 0.96; Layton et al., 2006). This established option should reduce the issue of water sample shipping time to a lab and decline or growth of indicator *E. coli*. In practical terms, water samples from remote locations may be collected, frozen, and shipped frozen to a lab for retrospective analysis during baseline setting, especially when characterizing fecal pollution potential during non-irrigation intervals. Although typically applied to watershed studies with known impacts from point-source contamination, Total Bacteroides qPCR protocols have shown very good correlation with *E. coli* levels in water within the current proposed Produce Rule CFU/100 ml range and significantly higher cell densities (r^2 0.85; Layton et al., 2006).

Research Methods and Results

Sampling

A total of 703 primary and 27 preliminary and opportunistic water samples from natural surface sources used for preharvest crop management were collected in California, Arizona, and the Pacific Northwest region, primarily Washington State. Diverse sources included ground-to-surface water reservoirs, publically managed irrigation distribution systems (aqueduct and canals), a seasonal river tributary, and surface water and run-off detention ponds used for seasonal crop production purposes (isolated production areas with secondary water sources, frost control, scald control, dust abatement). Samples were collected during 56 different sampling events, between January to October 2015 (n=377) and March to July 2016 (n=287). Sample sites and locations were consistent throughout the project period, with few exceptions due to availability or accessibility permissions, to maximize comparability across time and season. The diversity of locations necessitated modified approaches to safely collect representative samples. As frequently as possible, samples were collected from the point closest to extraction for crop production. For this study, samples were only collected from 18–24 cm below the surface and variable distances from the water body edge, depending on site characteristics. Care was taken to sample far enough from an edge to minimize the chance of disturbance of sediment. In reservoir or pond sites, the immediate surface of the area to be

sampled was gently agitated to disperse floating pollen and fine soil or organic matter. Approximately 3 to 10 liters of composite single-site sample, depending on specific planned analysis or periodic replicate assays, was placed into three to five sterile screw-capped containers and immediately placed in a pre-cooled insulated chest or insulated foam shipping container lined with frozen gel-ice. Samples obtained in CA were stored and transported on gel-ice and processed for FIB within 12–18 h after collection. For samples collected and shipped or transported from WA and OR samples, the sampling dates were selected to ensure delivery to UC Davis within 24 h of collection, but occasionally up to 48 h. On later dates in 2016, capture filtration of larger volume samples for pathogen detection, using modified Moore swabs (described below) were processed by the Pacific Northwest group supervised by Collaborator Ines Hanrahan, with a matched setup to the Suslow Lab. Samples collected in AZ were processed in Co-PI Rock's laboratory using parallel and standardized methods. At each site and between all individual samples, a new pair of sterile nitrile gloves was donned before collecting the sample. If an extendable sampling pole was used, the securing ring, threaded mount-base, hasp or clamp, and approximately 0.75 m of the pole in contact with the water was sprayed with 70% ethanol and air-dried between sites.

Fecal Indicator Bacteria (FIB) Enumeration

Procedures for sample processing and analysis for indicator *E. coli*, Shiga toxin–producing *E. coli* (STEC), and *Salmonella* were previously reported by Sbodio et al. (2013) and Rock et al. (2013). The basic scheme used in this project is provided in **Figure 1** (see Appendices) for pathogen detection. The surface water sources were collected from diverse geographic locations, using standard protocols, and were associated with annual row crop, perennial vine and tree crop production. For comparative FIB enumeration, the standard site-unit sample was 1 liter, replicated 3 times per site per date. The analytical sample throughout the project period was a replicate 100 ml from each 1-L sample-unit, homogenized by inversion of the container at least three times before withdrawing an aliquot. Samples were processed for indicator *E. coli* using the Colilert Quanti-Tray 2000 (IDEXX Laboratories, Inc., Westbrook, ME) and the US EPA recognized culture-based method (membrane filtration and semi-selective/differential agar media, US EPA 9223 B). For comparison, a subset of samples was tested for quantitative levels of *E. coli* by a standard membrane filtration technique for water testing using the IsoGrid System (NeoGrid Test Systems; Neogen, Lansing, MI) in which viable bacteria were separated from the water by vacuum filtration using 47-mm (0.45-µm pore) Millipore S filters (Millipore, Billerica, MA). Plates were incubated and evaluated following manufacturer or protocol specifications. For select sampling periods and sites, enumeration of generic *E. coli* by MPN and CFU-based methods was conducted by a resuscitation interval of 4 h at room temperature (~22°C) and then placed at 42–44°C to substantially exclude interfering competitors and phenotypic false-positives (non–*E. coli*) that develop at protocol recommended incubation at 35–37°C.

Pathogen Presence/Absence Detection

For pathogen screening from the same source water and sample dates as FIB enumeration, collected or received water samples were processed by capture filtration using modified Moore swabs (MMS) prior to enrichment for presence/absence detection (McEgan et al., 2013; Sbodio et al. 2013). The basic system for the project period is depicted in **Figure 1**. For each sample point, 6–10 L of water was passed through an MMS filter cassette. After filtration, the particulates and water-saturated swab was placed in a sterile Whirl-Pak bag (Nasco, Modesto, CA) and 300 ml of pre-warmed double strength (2X) tryptic soy broth (TSB; BD, Franklin Lakes, NJ) were added. Swabs were massaged for 1 min, and incubated for 4 h at 22°C followed by

18–20 h at 37°C. The Atlas® System (Roka Bioscience), a fully automated molecular pathogen detection system, was used for Shiga toxin–producing *E. coli* (STEC), including enterohemorrhagic *E. coli* O157:H7 ($EHEC_{O157}$), and *Salmonella* detection. Briefly, after enrichment, 1.2 ml and 400 µl of 2X-TSB were transferred to Roka Transfer Tubes (RTT) for STEC/ $EHEC_{O157}$ and *Salmonella* testing, respectively. RTT were analyzed using the Atlas® STEC EG2 Combo Detection Assay and Atlas Salmonella detection assay.

Alternative Methods and Assessments: Limit of Detection and Holding Temperature

An alternative system for processing water samples was evaluated briefly. The recovery efficiency and limits of detection (LOD) for pathogens were evaluated by a large-volume (10 L) ultra-filtration system using Fresenius Hollow Fiber Filtration (HFF) single-use cartridges (Fresenius F200NR, Fresenius Medical Care, Lexington, MA) (**Figure 2**). The large-volume samples were concentrated using the HFF system to ~200 ml of retentate. A solution of glycine and NaOH was added to the recirculation tubing for 10 min to recover the attached microbes at the end of the filtration. The retentate was immediately stored on ice for further processing and analysis. We determined log 2 CFU as the limit of detection for bacterial pathogen assays using the HFF system. Different sets of experiments were performed to determine the effects of holding time on fecal indicator bacteria, i.e. generic *E. coli* using membrane filtration (**Table 1**).

Total *Bacteroides* Quantification by Real-Time PCR (qPCR)

A 250-ml aliquot was used for *Bacteroides* quantification (basic protocol followed during the project period is depicted in **Figure 2**). Samples were processed by membrane vacuum filtration using 47-mm (0.45-µm pore) Millipore S filters (Millipore). The filtrate was discarded and the filters were stored at −20°C prior to total DNA isolation and purification. DNA was extracted using a commercially available DNA extraction kit (PowerWater DNA Isolation Kit).Two different qPCR assays were compared using Bacteroides 16S rDNA genes as the alternative FIB target: AllBac (universal *Bacteroides* identification) and commercial GeneSig Bacteroides (for universal *Bacteroides*). For AllBac qPCR assays, universal conserved 16 S rDNA markers for *Bacteroides* (106 bp amplicon size) were used in the TaqMan™ assay. The 20 µl of Master Mix contained 1X PCR buffer (Sso Advanced Universal Probes Supermix, BioRad, Hercules, CA), 400 nM primers, 200 nM probe, 5 µL experimental DNA, and DNase-free water to bring to volume. The assay was performed using the CFX96 Touch™ Real-Time PCR Detection System (BioRad). Amplification parameters were as follows: initial denaturation at 95°C for 2 min, followed by 45 cycles of denaturation at 95°C for 15 sec, annealing at 53°C for 30 sec, extension at 72°C for 30 sec. An internal control of salmon testes DNA solution (D-1626, Sigma Aldrich) containing 10 µg/ml was added before the DNA extraction in each sample. Primers and predicted lengths are listed in **Table 2.**

Standard curves for quantification were established using *E. coli* containing cloned *Bacteroides* 16S rDNA fragments in plasmid pUC19 that was grown on TSA-Ampicillin (100 µg/ml) at 37°C for 24 h. A single colony was used to inoculate 20 ml of Luria Broth-Ampicillin and incubated overnight shaking (200 rpm) at 37°C. A 5-ml aliquot of bacterial culture was harvested by centrifugation at >8000 rpm for 3 min, followed by the plasmid DNA purification using Perfectprep spin kit (5 prime). Plasmid DNA was quantified using Nanodrop, and the mass of a single plasmid molecule was calculated based on genomic size. Ten-fold serial dilutions of the plasmid DNA were amplified in triplicate five times over several days. Standard curves showed a high degree of linearity, with a coefficient of determination (R^2) of 0.996 (**Figure 3**). The concentrations of plasmid DNA needed to achieve the number of copies of interest were

calculated. Using these standard curves, cycle quantification (Cq) values were converted into genome equivalent copies (GEC) per 100-ml sample processed.

Statistical Analyses

Statistical analyses were performed using the Statistical Analysis Software package applications (SPSS, NY). Numerical gene target values for *Bacteroides*, determined from standard curves using least square linear regression analysis, and CFU for *E. coli* in water samples were $\log_{10}$ transformed to allow statistical analysis. Logistic regressions were performed to determine correlations between the presence/threshold concentration of *Bacteroides* markers and the concentration of *E. coli* and select pathogens in the samples. Pearson chi-square analyses were evaluated to compare the presence of *Bacteroides* markers between indicator *E. coli* and pathogen detection.

Outcomes and Accomplishments

Following the current FSMA Produce Rule provisions for ag-water, the geometric mean (GM) and statistical threshold value (STV) from 6 regions (CA-3, WA-2, OR-1) were calculated using the spreadsheet auto-calculator developed by the FDA Western Center for Food Safety. The original version included both MPN/100 ml and CFU/100 ml options. Examples are provided in **Figure 4** and **Figure 5**. Both culture-based methods for *E. coli* quantification (membrane filtration) showed similar results. From CA samples (36 samplings events) only two different regions, sampled during 2015, did not meet PSR criteria when enumerated using Quanti-Tray on one time point. In contrast, when membrane filtration was used to process homogenized aliquots of the same source samples, outcomes did not exceed the current compliance standards. In AZ samples collected from six regional sources, 11 samples exceeded the 126 MPN/100 ml (~126 CFU/100 ml) GM threshold set by the FDA and the LGMA. The range of detectable populations of indicator *E. coli* was 1 to 863 MPN/100 ml.

Holding time and temperature minimally affected quantitative results of surface water samples up to ~30 h pre-processing by either MPN or CFU methods (**Table 1**). However, with a strict quantitative threshold of 126 CFU/100 ml and STV of 410 CFU/100 ml across >20 samples, these minor differences on a log scale could swing the water source profile above or below the corrective measure-activating threshold. This preliminary information has implications for farms located in remote areas where transport to the lab, even on ice, would easily exceed 24 h.

An overview of all results from CA and the PNW are provided in the accompanying document (Supplemental Tables & Figures_Suslow-CPS). These data summaries provide a clear picture of the challenges growers face in using fecal indicator testing of water sources to make risk management decisions. The poor correlation between either *E. coli* or *Bacteroides* estimated quantitative levels, in the case of this project, and pathogen presence was observed across all regions tested. Individual sites may benefit from imposing corrective measures. For observed low or high numbers of FIB, associating these numbers to risk of crop contamination seems unlikely in the Western states.

In this study to assess possible alternative fecal indicators in irrigation water, Total *Bacteroides* quantification by real-time PCR was compared with the primary fecal indicator, generic *E. coli*, used in both market-access standards and the current FSMA Produce Safety Rule. The

universal AllBac *Bacteroides* marker and GeneSig *Bacteroides* kit showed positive outcomes in 100% of the water samples. On average, the samples contained 6.47 $\log_{10}$ GEC/100 ml when using AllBac, and 5.30 $\log_{10}$ GEC/100 ml with the GeneSig kit. The ranges found for AllBac marker and GenSig were 2.1 to 8.3 $\log_{10}$ and 1.5 to 7.5 $\log_{10}$ GEC/100 ml, respectively. In tests on AZ water sources, 94 of the 102 samples had the *Bacteroides* 16S rRNA gene, with the average GEC per 100 ml of sample ranging from 1.3 to 9.2 log GEC/100 ml, with an average of 7.8 log GEC/100 ml.

Bacteroides log genome equivalent copies (GEC) for all samples were very similar. The average of $\log_{10}$ transformed concentrations of both *E. coli* (high and low) and *Bacteroides* (AllBac and Genesig) are summarized in the Supplemental document. There were no significant differences in the *Bacteroides* GEC numbers between samples determined to be pathogen positive or negative. In the CA and PNW samples, there was poor correlation between the presence of the *Bacteroides* and *E. coli* in irrigation water samples ($P > 0.05$).

Of the 703 primary study samples processed, 15% (103) were positive for *Salmonella* and 14% (101) for STEC/EHEC. Detection prevalence was based on concentration of 6–10 L of source water, followed by broth enrichment. No attempt was made to quantify detected levels in this project. The detection of both *Salmonella* and STEC/ $EHEC_{O157}$ in the same sample was infrequent, generally less than 4% depending on region and season. The presence/absence of detectable bacterial pathogens in water samples, in relation to stratified generic *E. coli* enumeration results is provided in **Figures 7–10**.

As with the current FIB used to characterize ag-water for fresh produce production, generic *E. coli*, we found no correlation of quantitative thresholds of Total *Bacteroides* with the detectable presence of enteric pathogens (*Salmonella* and STEC/ $EHEC_{O157}$) in the irrigation source water.

One limitation with the use of *Bacteroides* assays in irrigation water samples was the presence of matrix-associated inhibitory compounds. Samples that were PCR inhibited were diluted up to 1,000-fold and re-tested. The recovery efficiencies for the filtration were variable and sample dependent. Real-time PCR analysis requires careful consideration of extraction efficiencies, detection limits, and PCR inhibition. Our assessment is that the utility of this assay as a FIB has been somewhat overstated in the literature. It would appear that the predictive value of this indicator of fecal loading in relation to pathogen presence, as with *E. coli*, is limited and variable in common water sources in Western state regions.

In this study, we found that estimated *Bacteroides* populations seemed to be consistent or stable across sources and time points in the irrigation water. It is not clear if the presence of *Bacteroides* would be associated with recent fecal contamination. Different studies have found that DNA of fecal *Bacteroides* species remains detectable from days to weeks, depending on the conditions. The detection of the *Bacteroides* 16S rRNA genetic markers in environmental waters could mainly reflect the presence of ‘viable but non-culturable’ cells. The presence and persistence of *Bacteroides* in this type of water would prevent use as a reliable indicator of recent fecal contamination of environmental/irrigation waters unless associated with a point source or acute contamination event far exceeding baseline levels. Future studies should consider a better understanding of the ecology and behavior of *Bacteroides* in this ecosystem.

Various statistical analyses were performed to compare the presence and concentration of *Bacteroidales* markers with *E. coli* in samples, including Pearson chi-square analyses, logistic regressions, and Spearman rank correlations. No statistically significant relationships were detected between *E. coli* and the general *Bacteroides* marker using any statistical test ($P > 0.05$).

Both *Bacteroides* assays evaluated were able to adequately detect *Bacteroides* bacteria in irrigation waters used for produce production. To evaluate if the pathogenic *Bacteroides* kit (Genesig) was a comparable alternative to the Layton assay (Layton et al., 2006), a linear regression of the average molecular markers was analyzed. According to the results, the total *Bacteroides* assay and pathogenic *Bacteroides* kit are statistically comparable with each other (**Figure 6**) with a *P*-value < 0.0001, thus the pathogenic *Bacteroides* kit (Genesig) can be used to detect *Bacteroide*s at the same efficiency as the AllBac *Bacteroides* assay.

Summary of Findings and Recommendations

This project resulted in a substantial body of new, detailed data with standardized assays on 730 surface water sources over diverse and numerous locations and times, which greatly increases the knowledge of microbial water quality in key specialty crop regions.

The conclusion from these studies is that either FIB (generic *E. coli* or Total *Bacteroides*) may provide good indication of a recent and high level of fecal contamination of a water source, but neither FIB is likely to provide a strong indication of bacterial pathogens in that water source. The results showed that the water testing, while required, indicated compliance but did not provide a strong indication of bacterial pathogens in that water source. Regardless, as a precautionary policy, the industry must implement one of the FSMA PSR corrective measures for agricultural water sources if high levels of fecal indicators are present. While the team believes the assay holds much promise as an improved system, the previously known and further verified low correlation with pathogens in typical surface water sources, within the limits of any survey, has not altered this perspective. Further development of a standard method for AllBac testing in accredited labs is worth pursuing for the greater flexibility in overcoming sample-to-submission-compliance time barriers encountered by many farms. Limited detection opportunities to develop a correlation of microbial water quality to pathogen detection on the irrigated crop were possible. It should be noted that the team had fewer than anticipated opportunities to follow the persistence of waterborne pathogens to the irrigated crop in Year 2 of the project because cooperating growers either switched to drip irrigation or to well water or antimicrobial-treated water.

Citations and Supporting References

Atwill, E. R., et al. 2011. Epidemiologic analysis and risk management practices for reducing *E. coli* in irrigation source water supplies and distribution systems. Center for Produce Safety 2009 RFP Final Report. Available at: http://www.centerforproducesafety.org/grant_opportunities_awards.php?insr=1.

Benjamin, L., Atwill, E. R. et al. (2013). Occurrence of generic *Escherichia coli*, *E. coli* O157 and *Salmonella* spp. in water and sediment from leafy green produce farms and streams on the Central California coast. International Journal of Food Microbiology 165(1): 65-76.

Bradley, P.M., Barber, L.B., Kolpin, D.W., McMahon, P.B., and Chapelle, F.H., 2007. Biotransformation of caffeine, cotinine, and nicotine in stream sediments--Implications for use as wastewater indicators: Environmental Toxicology and Chemistry 26(6): 1116-1121.

Castro-Rosas, J., J. F. Cerna-Cortes, et al. (2012). Presence of faecal coliforms, *Escherichia coli* and diarrheagenic *E. coli* pathotypes in ready-to-eat salads, from an area where crops are irrigated with untreated sewage water. International Journal of Food Microbiology 156: 176-180.

Converse, R., Blackwood, A., Kirs ,M., Griffith, J., and R.T. Noble. 2009. Rapid QPCR-based assay for fecal Bacteroides spp. as a tool for assessing fecal contamination in recreational waters. Water Res. 43:4828-4837.

EPA Office of Water 820-F-12-058. *2012 Recreational Water Quality Criteria* 4305T (EPA RWQC 2012) http://water.epa.gov/scitech/swguidance/standards/criteria/health/recreation/

EPA's Office of Water. 2013. Drinking Water Laboratory Certification Program in partnership with EPA Regions and States. http://water.epa.gov/scitech/drinkingwater/labcert/

FDA. 2013. Standards for the Growing, Harvesting, Packing, and Holding of Produce for Human Consumption. http://www.fda.gov/Food/GuidanceRegulation/FSMA/ucm334114.htm

Gelting, R. J. and M. A. Baloch. (2012). The food-water nexus: irrigation water quality, risks to food safety, and the need for a systems-based preventive approach. Journal of Environmental Health 75(3): 40-41.

GeneSig Bacteroides. 2013. All Bacteroides qRTPCR Quantification Kits. Technical Documents at http://www.genesig.com/products/9159.

Haack, S.K., Duris, J.W., Fogarty, L.R., Kolpin, D.W., Focazio, M.J., Furlong, E.T., and Meyer, M.T. 2009. Comparing wastewater chemicals, indicator bacteria concentrations, and bacterial pathogen genes as fecal pollution indicators. Journal of Environmental Quality 38: 248-258.

Harris, L. J., J. Bender, et al. (2012). A framework for developing research protocols for evaluation of microbial hazards and controls during production that pertain to the quality of agricultural water contacting fresh produce that may be consumed raw. Journal of Food Protection 75(12): 2251-2273.

Jacobsen, C. S. and T. B. Bech (2012). Soil survival of *Salmonella* and transfer to freshwater and fresh produce. Food Research International 45(2): 557-566.

Kabiri-Badr, L., J.E.T. McLain, C.M. Rock, M. Abbaszadegan, A. Alum. (2013). Isolation of Bacteroides from Fish and Human Fecal Samples for Identification of Unique Molecular Markers. Can. J. Microbiol. 59(12): 771–777. doi: 10.1139/cjm-2013-0518.

Kitts, C., Black, M., Moline, M., Yeung, M., Hamrick, A., Robbins, I., Schaffner, A., and Boutet, N. I. (2010). Pismo Beach Fecal Contamination Source Identification Study: Final Report. Biological Sciences. Available at: http://works.bepress.com/ckitts/29.

Layton, A., McKay, L., Williams, D, Garrett, V., Gentry, R., and Sayler. G. 2006. Development of *Bacteroides* 16S rRNA Gene TaqMan-Based Real-Time PCR Assays for Estimation of Total, Human, and Bovine Fecal Pollution in Water. AEM 72: 4214–4224.

McEgan, R., Mootian, G., Goodridge, L., Schaffner, D., and Danyluk, M. 2013. Predicting Salmonella Populations from Biological, Chemical, and Physical Indicators in Florida Surface Waters. Applied and Environmental Microbiology 79: 4094–4105.

McQuaig, S., Griffith, J. and V. J. Harwood. 2012. Association of Fecal Indicator Bacteria with Human Viruses and Microbial Source Tracking Markers at Coastal Beaches Impacted by Nonpoint Source Pollution. AEM 78: 6423–6432.

Pachepsky, Y.A., Shelton, D.R., Mclain, J.E., Patel, J.R., and Mandrell, R.E. (2011). Irrigation waters as a source of pathogenic microorganisms in produce: A review. Advances in Agronomy 113: 73–138.

Ravaliya, K., et al. (2014). Use of *Bacteroidales* microbial source tracking to monitor fecal contamination in fresh produce production. AEM 80: 612-617.

Rock, C.M., Bright, K., and Gerba, C. (2013) Assessment of *E. coli* as an indicator of microbial quality or irrigation water use for produce. Center for Produce Safety Final Report.

Sauer, E., VandeWalle, J.L., Melinda, J., Bootsma, M., and McLellan, S.L. 2011. Detection of the human specific Bacteroides genetic marker provides evidence of widespread sewage contamination of stormwater in the urban environment. Water Res. 45: 4081-4091.

Sauvé, S., et al. 2012. Fecal coliforms, caffeine and carbamazepine in stormwater collection systems in a large urban area. Chemosphere 86: 118–123.

Sbodio, A., Maeda, S., López-Velasco, G., and Suslow, T.V. 2013. Modified Moore swab optimization and validation in capturing *E. coli* O157:H7 and *Salmonella enterica* in large volumes of field samples of irrigation water. J. Food Research 51: 654–662.

Suslow, T.V. 2002. Production practices affecting the potential for persistent contamination of plants by microbial foodborne pathogens, ch. 16. In: Phyllosphere Microbiology. APS Press, Minneapolis, MN, pp. 241-256.

Suslow, T.V. 2010. Standards for irrigation and foliar contact water. Produce Safety Project Issue Brief. Available at: http://www.pewtrusts.org/~/media/assets/2009/pspwatersuslow1pdf.pdf?la=en.

Suslow, T.V., Oria, M.P., Beuchat, L.R., Garrett, E.H., Parish, M.E., Harris, L.J., Farber, J.N., Busta, F.F. (2003). Production practices as risk factors in microbial food safety of fresh and fresh-cut produce. Comprehensive Reviews in Food Science and Food Safety 2S: 38–77.

Tomas-Callejas, A., G. Lopez-Velasco, A. Camacho, F. Artés, F. Artés-Hernández, T. Suslow. 2011. Survival and distribution of Escherichia coli on diverse fresh-cut baby leafy greens under preharvest through postharvest conditions. Int. J. Food Micobiol. 151: 216-222.

Van der Linden, I., B. Cottyn, et al. (2013). Survival of enteric pathogens during butterhead lettuce growth: crop stage, leaf age, and irrigation. Foodborne Pathogens and Disease 10(6): 485-491.

Won, G., T. R. Kline, et al. (2013a). Spatial-temporal variations of microbial water quality in surface reservoirs and canals used for irrigation. Agricultural Water Management 116: 73-78.

Won, G., P. J. Schlegel, et al. (2013b). Absence of Direct Association between Coliforms and *Escherichia coli* in Irrigation Water and on Produce. Journal of Food Protection 76(6): 959-966.

Wu, J., Long, S., Das, D., and S. Dorner. 2011. Are microbial indicators and pathogens correlated? A statistical analysis of 40 years of research. J. Water Health 9:265-278.

APPENDICES

Publications and Presentations

No publications have been submitted at this time (July 2017) but are planned in the near future.

Project Presentations and Outreach:

CPS Annual Research Symposium presentations, June 2015 and 2016

Yum! Produce Industry Meeting, 23 July 2015, Monterey, CA
Progress in Developing Alternative FSMA Produce Rule Compliance Metrics

CA Dept Health and CalFERT Team, August 2015, Sacramento, CA
Commodity Specific Training Preharvest & Postharvest Agricultural Water Use

CA Leafy Greens Research Board, 17 March 2015, Harris Ranch – Coalinga, CA
Bacteroides spp. as an alternative fecal indicator bacteria for irrigation water quality

UCCE Imperial County Grower Research Update Meeting, 8 April 2015, El Centro, CA
Bacteroides spp. as an alternative fecal indicator bacteria for irrigation water quality

CA Central Coast Growers Shippers Association, Special Tailwater Reuse Workgroup, 7 August 2015, Salinas, CA
Fecal Indicators and Predicting Pathogen Prevalence in Irrigation Water

CA Melon Research Board, 5 January 2016, San Diego, CA
Research Update – Tailwater Reuse Concerns and FSMA Ag-water Testing

HORT 200B, 17 May 2016, UC Davis
Current Applied Research in Produce Food Safety

IAFP 2017, 12 July 2017, Tampa, FL
Implementation Programs Benefit from Ag Water Surveys and On-farm Preparedness Assessments

Budget Summary

All funds were used directly and specifically to address the issues outlined and for the benefit of specialty crops. The PI and his lab manager, according to the specific budget plan for this project and its objectives, conducted budget tracking and expenditure management.

Tables 1–2 and Figures 1–10

Table 1. Effect of holding temperatures on fecal indicator bacteria (FIB)

Holding Temperature	Quanti-tray 2000				
	n	log MPN/100 ml			
		6 h	24 h	30 h	52 h
5°C	3	1.12 ± 0.12	1.09 ± 0.07	1.09 ± 0.09	1.00 ± 0.08
10°C	3	1.10 ± 0.20	1.14 ± 0.24	1.05 ± 0.49	0.93 ± 0.17
20°C	3	1.19 ± 0.11	1.18 ± 0.16	1.10 ± 0.05	1.10 ± 0.10
35°C	3	1.36 ± 0.02	1.15 ± 0.13	1.06 ± 0.03	0.33 ± 0.57

Holding Temperature	Membrane Filtration				
	n	log CFU/100 ml			
		6 h	24 h	30 h	52 h
5° C	3	0.95 ± 0.31	1.12 ± 0.11	0.99 ± 0.11	0.89 ± 0.21
10° C	3	1.33 ± 0.05	0.86 ± 0.37	1.07 ± 0.10	1.06 ± 0.06
20° C	3	1.19 ± 0.03	1.13 ± 0.06	1.15 ± 0.12	0.88 ± 0.17
35° C	3	1.16 ± 0.23	1.10 ± 0.17	1.01 ± 0.17	0.13 ± 0.30

Table 2. Primers and probes used in this study

Target	Name	Oligonucleotide Sequences 5'-3'	Locus	Reference
Bacteroides	Forward primer AllBac296F	5-GAGAGGAAGGTCCCCCAC-3	16S rRNA gene	Layton, 2006
	Reverse primer AllBac412R	5-CGCTACTTGGCTGGTTCAG-3		
	Probe AllBac375	5-FAM-CCATTGACCAATATTCCTCACTGCTGCCT-MGBNFQ		
Control salmon DNA assay	Forward primer SketaF	5'-GGTTTCCGCAGCTGGG	ITS region 2	Rogers, 2011
	Reverse primer Sketa22R	5'-CCGAGCCGTCCTGGTC		
	Sketa probe	5'-VIC-AGTCGCAGGCGGCCACCGT-TAMRA		

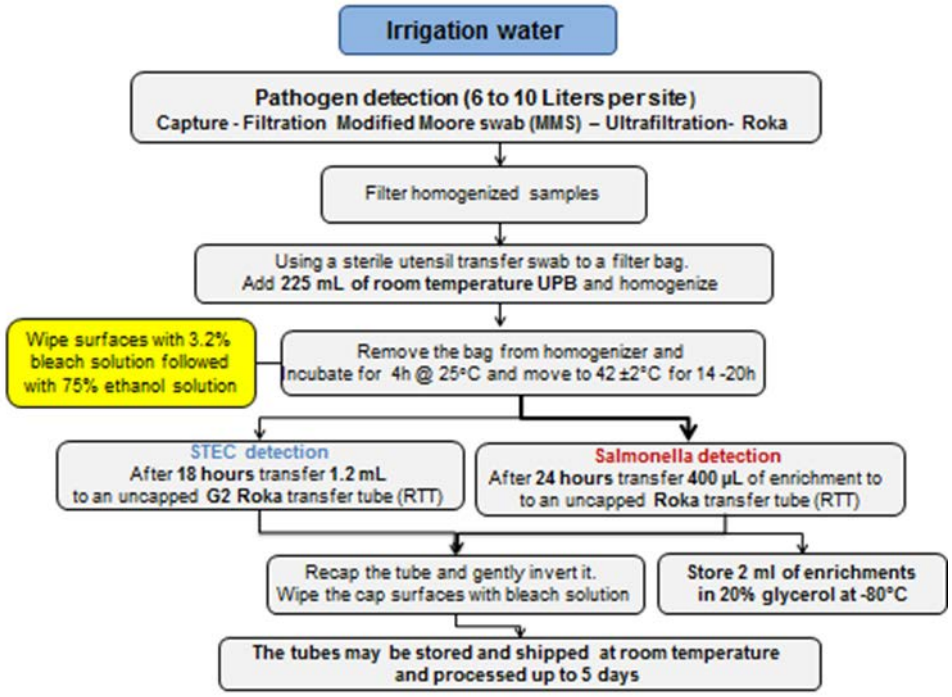


Figure 1. Flow diagram for basic pathogen detection.

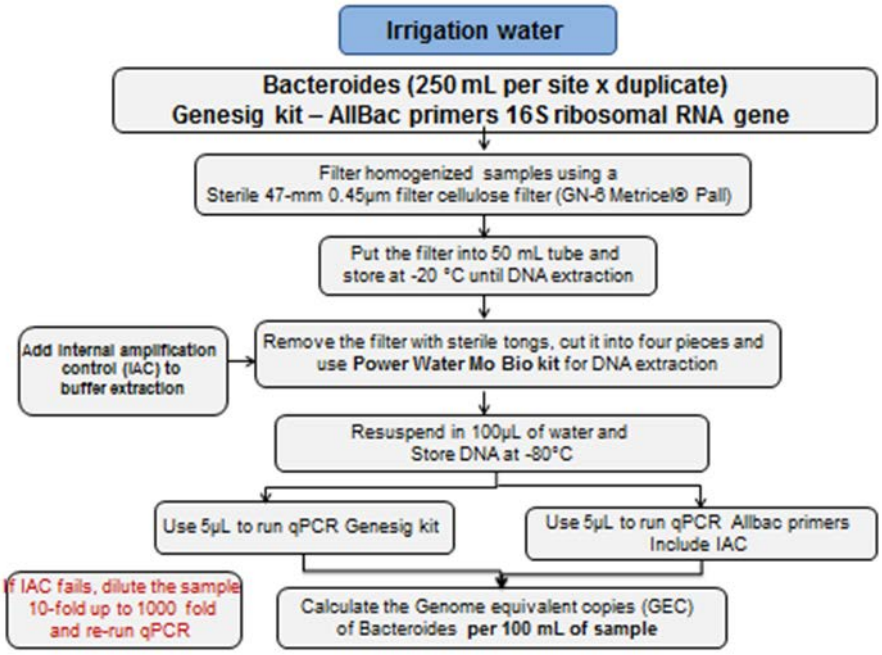


Figure 2. Flow diagram for basic Total *Bacteroides* assay.

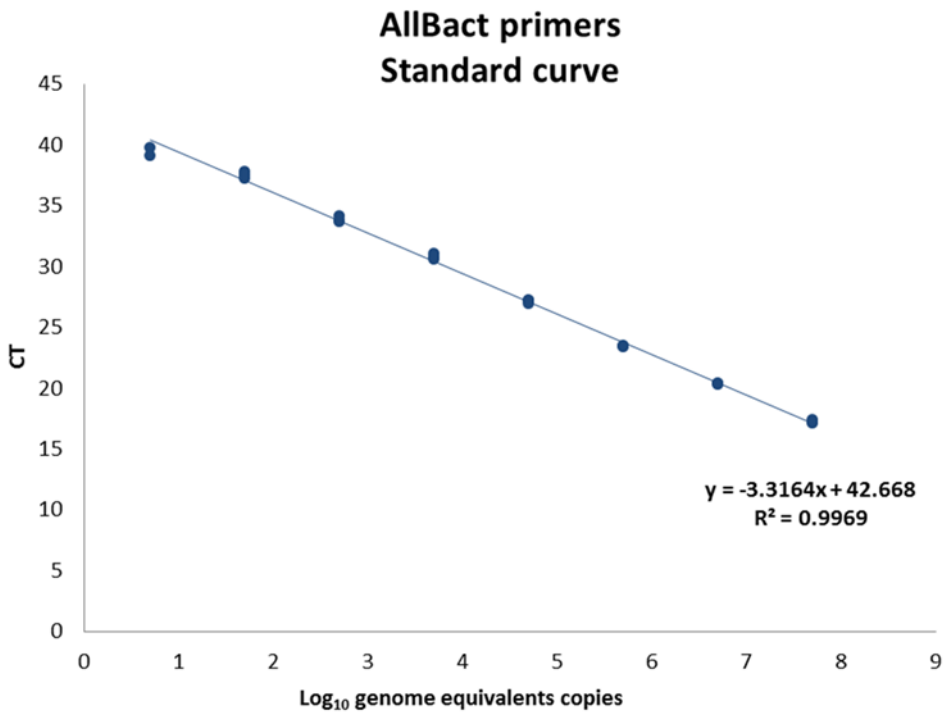


Figure 3. Standard curve AllBac quantification using cloned *Bacteroides* 16S rDNA .

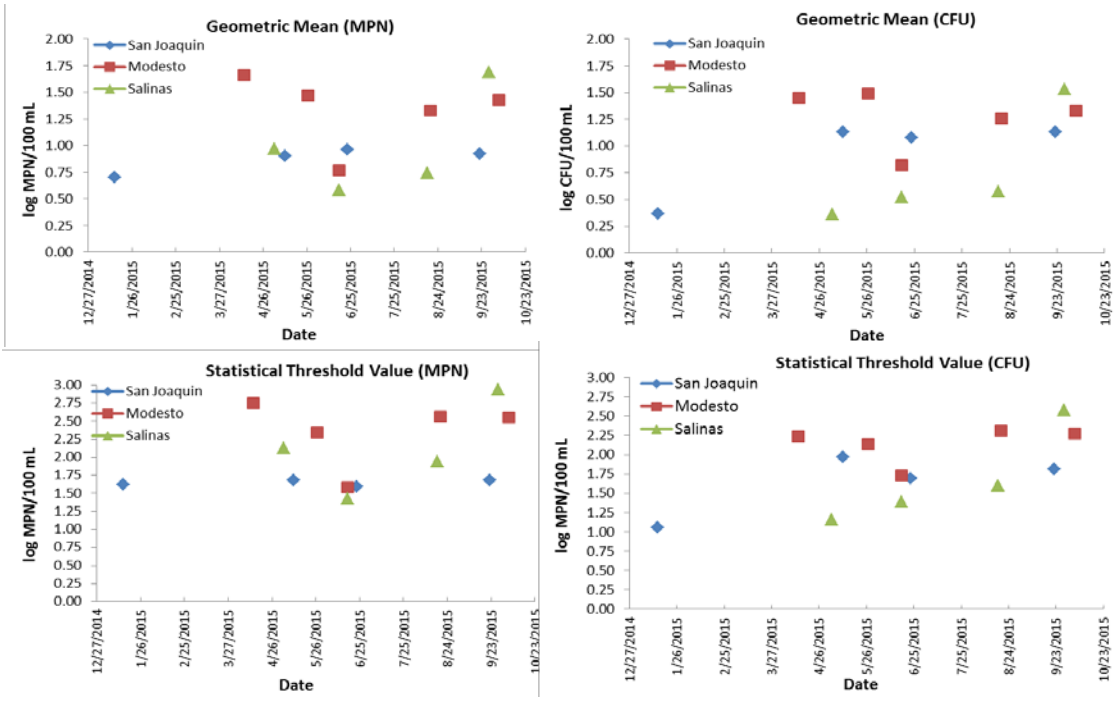


Figure 4. Example of GM and STV distribution in 2015 for California regions.

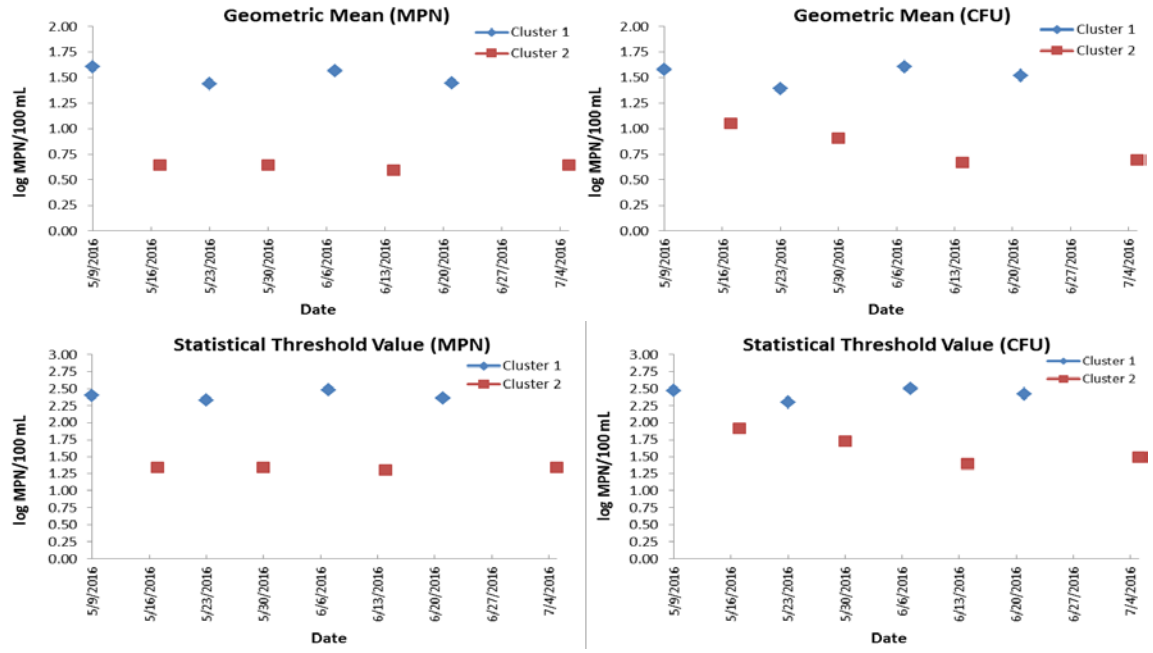
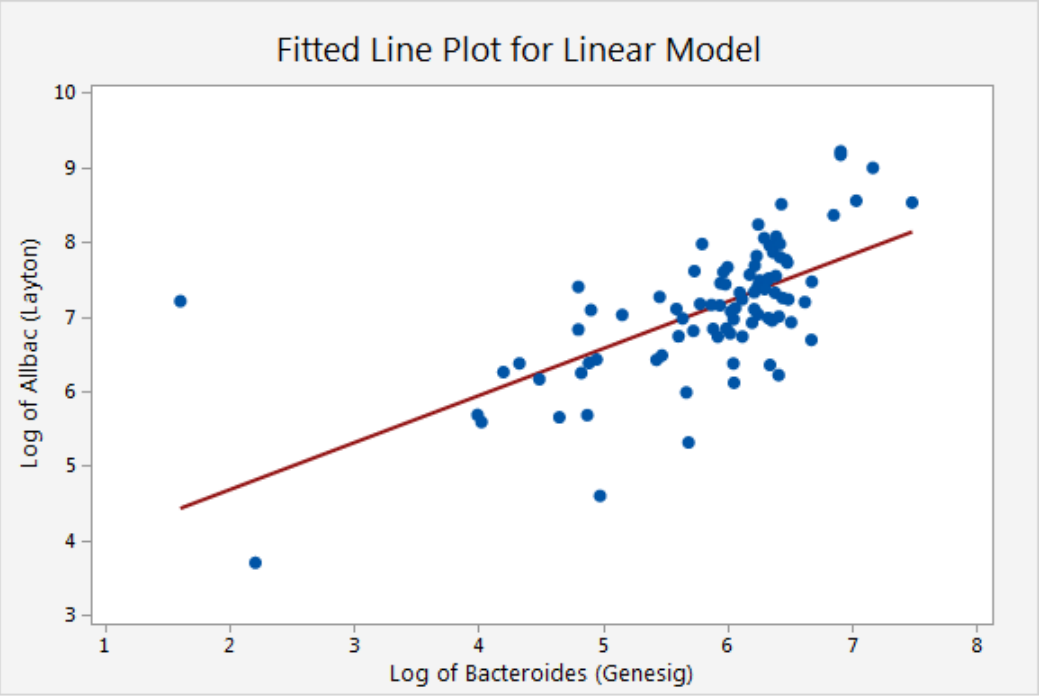


Figure 5. Example of GM and STV distribution in 2016 for Pacific Northwest regions.



Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Regression	1	29.8556	29.8556	64.12	<0.0001
Error	87	40.5121	0.4657		
Total	88	70.3677			

Figure 6. Comparison of commercial kit (Genesig) with AllBac primers for detection of *Bacteroides* as a fecal indicator. Both systems gave significantly comparable results in spiked samples ($P < 0.0001$).

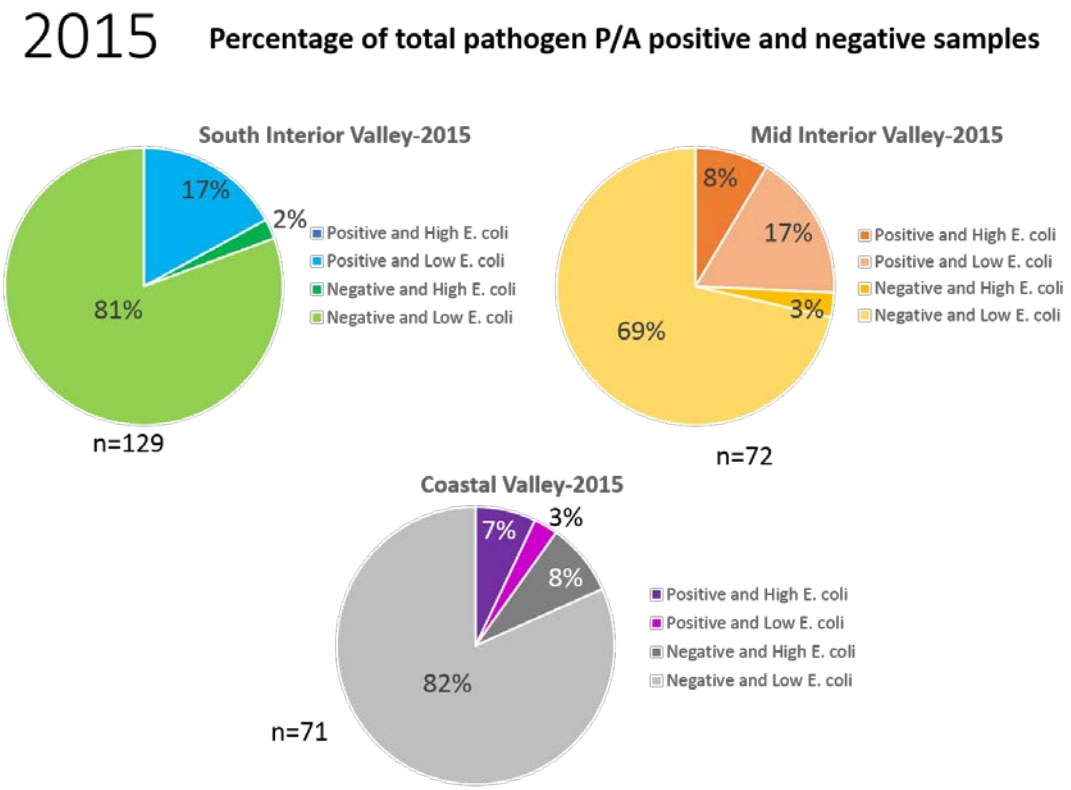


Figure 7. Presence/absence outcomes for total pathogen – CA 2015

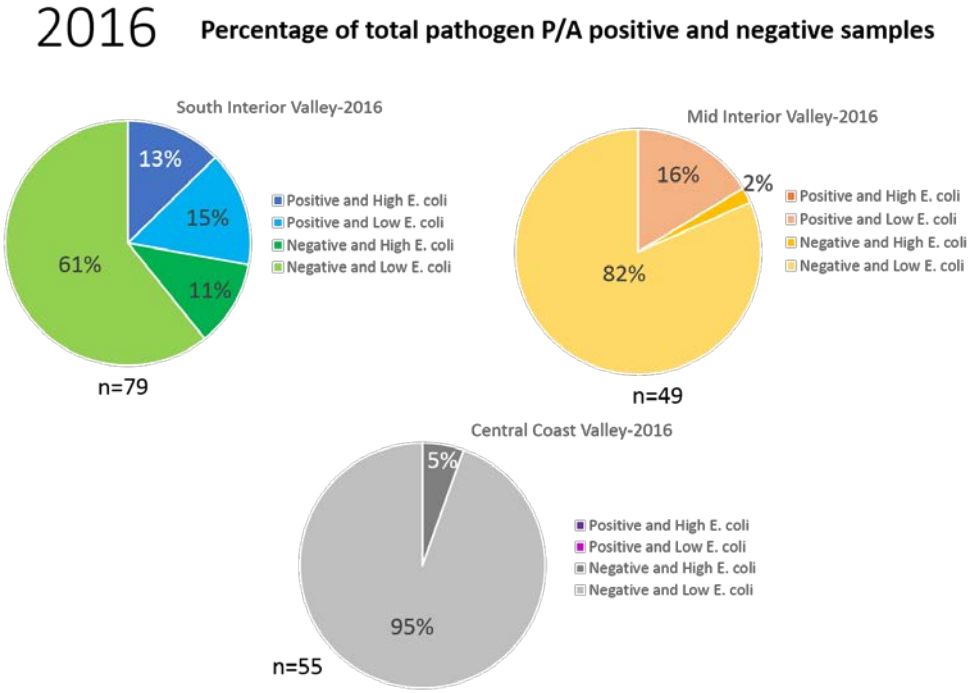


Figure 8. Presence/absence outcomes for total pathogen – CA 2016

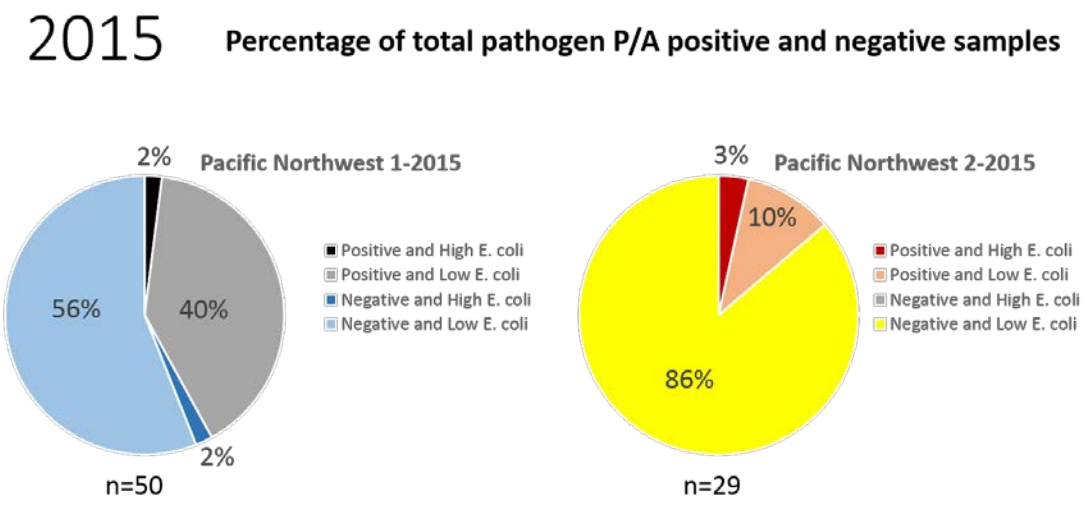


Figure 9. Presence/absence outcomes for total pathogen – PNW 2015

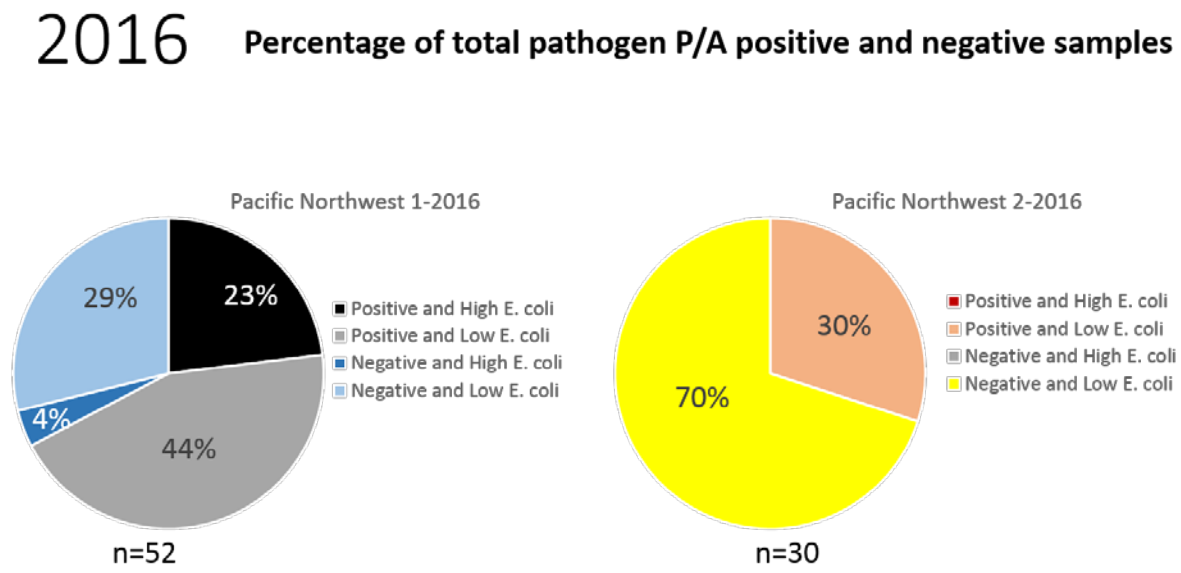


Figure 10. Presence/absence outcomes for total pathogen – PNW 2016

Supplemental Tables and Figures

Tables S-1 to S-16

Examples of Comparative Summary of E.coli and Total Bacteroides Enumeration Against Pathogen Presence/Absence for 2015 and 2016

Figures S-A to S-I

Mean fecal indicator bacteria population size in surface water across year and CA or PNW regions in relation to pathogen detection in source water

Mid Interior Valley - 2016	Date and Location	n	*E. coli*						Bacteroides					
			Quanti-Tray (log MPN/100 mL)			Membrane (log CFU/100 mL)			All bac Log10 Copies /100ml			Genesig Log10 Copies /100ml		
Pathogen Positives	Sampling 2	8	2.09	±	0.18	1.91	±	0.28	6.26	±	0.46	4.97	±	0.50
	Sampling 7	4	1.75	±	0.33	1.79	±	0.27	6.93	±	0.28	5.95	±	0.31
	Sampling 14	10	2.06	±	0.27	1.96	±	0.14	5.81	±	1.07	5.20	±	0.49
	Total	22	2.02	±	0.27	1.91	±	0.22	6.18	±	0.86	5.25	±	0.57
Negative and High E. coli	Sampling 2	3	2.40	±	0.03	2.17	±	0.15	6.12	±	0.27	4.80	±	0.27
	Sampling 7	3	2.74	±	0.42	2.35	±	0.23	6.60	±	0.04	5.76	±	0.21
	Sampling 14	3	2.42	±	0.10	2.22	±	0.16	7.27	±	0.26	5.97	±	0.13
	Total	9	2.52	±	0.27	2.25	±	0.18	6.67	±	0.54	5.51	±	0.57
Negative and Low E. coli	Sampling 2	11	1.69	±	0.11	1.56	±	0.21	5.91	±	0.47	4.62	±	0.61
	Sampling 7	18	1.40	±	0.37	1.48	±	0.31	6.36	±	1.10	5.45	±	0.98
	Sampling 14	19	1.64	±	0.27	1.69	±	0.22	6.22	±	1.38	5.32	±	0.86
	Total	48	1.56	±	0.31	1.58	±	0.27	6.20	±	1.12	5.21	±	0.91

Modesto - 2016	Date and Location	n	*E. coli*						Bacteroides					
			Quanti-Tray (log MPN/100 mL)			Membrane (log CFU/100 mL)			All bac Log10 Copies /100ml			Genesig Log10 Copies /100ml		
Pathogen Positives	STEC/EHEC	16	2.02	±	0.31	1.90	±	0.25	6.34	±	0.72	5.27	±	0.57
	Salmonella	4	2.09	±	0.06	2.04	±	0.04	5.66	±	1.21	5.26	±	0.33
	STEC and Salmonella	2	1.85	±	0.17	1.81	±	0.07	5.93	±	1.41	5.13	±	1.26
	Total	22	2.02	±	0.27	1.91	±	0.22	6.18	±	0.86	5.25	±	0.57
Total negatives	Negative and High E. coli	9	2.52	±	0.27	2.25	±	0.18	6.67	±	0.54	5.51	±	0.57
	Negative and Low E. coli	48	1.56	±	0.31	1.58	±	0.27	6.20	±	1.12	5.21	±	0.91
	Total negatives	57	1.71		0.46	1.69		0.35	6.28		1.06	5.25		0.87

Mid Interior Valley-2015	Date and Location	n	*E. coli*						Bacteroides					
			Quanti-Tray (log MPN/100 mL)			Membrane (log CFU/100 mL)			All bac Log10 Copies /100ml			Genesig Log10 Copies /100ml		
Total positives	Pos and high e.coli	10	2.23		0.15	2.08		0.14	6.47		0.67	5.36		0.46
	Pos and low e.coli	12	1.84		0.21	1.77		0.18	5.93		0.96	5.17		0.65
	Total positives	22	2.02	±	0.27	1.91	±	0.22	6.18	±	0.86	5.25	±	0.57
Total negatives	Negative and High E. coli	9	2.52	±	0.27	2.25	±	0.18	6.67	±	0.54	5.51	±	0.57
	Negative and Low E. coli	48	1.56	±	0.31	1.58	±	0.27	6.20	±	1.12	5.21	±	0.91
	Total negatives	57	1.71		0.46	1.69		0.35	6.28		1.06	5.25		0.87

South Interior Valley - 2016	Date and Location	n	*E. coli*		Bacteroides	
			Quanti-Tray (log MPN/100 mL)	Membrane (log CFU/100 mL)	All bac Log10 Copies /100ml	Genesig Log10 Copies /100ml
Total positives	Sampling 1	4	1.07 ± 0.06	-0.05 ± 0.00	6.35 ± 0.18	5.17 ± 0.15
	Sampling 12	4	1.06 ± 0.11	1.10 ± 0.10	6.92 ± 0.15	5.52 ± 0.24
	Total	8	1.07 0.08	0.53 0.61	6.63 0.34	5.34 0.26
Negative and High E. coli	Sampling 12	1	2.13	2.02	6.8	5.3
Negative and Low E. coli	Sampling 1	15	0.71 ± 0.44	0.05 ± 0.39	6.14 ± 0.99	4.96 ± 0.99
	Sampling 12	25	0.69 ± 0.37	0.80 ± 0.31	6.85 ± 0.26	5.36 ± 0.32
	Total	40	0.66 ± 0.33	0.66 ± 0.38	6.10 ± 2.02	4.83 ± 1.55

South Interior Valley - 2016	Date and Location	n	*E. coli*		Bacteroides	
			Quanti-Tray (log MPN/100 mL)	Membrane (log CFU/100 mL)	All bac Log10 Copies /100ml	Genesig Log10 Copies /100ml
Total positives	STEC/EHEC	1	1.09	-0.05	6.4	5.3
	Salmonella	7	1.06 ± 0.09	0.61 ± 0.62	6.66 ± 0.36	5.35 ± 0.28
	Total	8	1.07 ± 0.08	0.53 ± 0.61	6.63 ± 0.34	5.34 ± 0.26
Total negatives	Negative and High E. coli	1	2.13	2.02	6.8	5.3
	Negative and Low E. coli	40	0.66 ± 0.33	0.66 ± 0.38	6.10 ± 2.02	4.83 ± 1.55
	Total negatives	41	0.73 0.45	0.56 0.54	6.59 0.71	5.22 0.67

South Interior Valley - 2016	Date and Location	n	*E. coli*		Bacteroides	
			Quanti-Tray (log MPN/100 mL)	Membrane (log CFU/100 mL)	All bac Log10 Copies /100ml	Genesig Log10 Copies /100ml
Total positives	Pos and high e. coli	0				
	Pos and low e. coli	8	1.07 0.08	0.53 0.61	6.63 0.34	5.34 0.26
	Total positives	8	1.07 0.08	0.53 0.61	6.63 0.34	5.34 0.26
Total negatives	Negative and High E. coli	1	2.13	2.02	6.8	5.3
	Negative and Low E. coli	40	0.66 ± 0.33	0.66 ± 0.38	6.10 ± 2.02	4.83 ± 1.55
	Total negatives	41	0.73 0.45	0.56 0.54	6.59 0.71	5.22 0.67

Central Coast Valley - 2016	Date and Location	n	*E. coli*				Bacteroides			
			Quanti-Tray (log MPN/100 mL)		Membrane (log CFU/100 mL)		All bac Log10 Copies /100ml		Genesig Log10 Copies /100ml	
Negative and High E. coli	Sampling 4	2	2.52	0.24	2.39	0.08	6.30	0.19	4.97	0.01
	Sampling 10	1	2.15		1.87		7.4		5.8	
	total	3	2.40	0.27	2.22	0.30	6.67	0.65	5.25	0.50
Negative and Low E. coli	Sampling 4	23	0.56	0.60	0.48	0.58	5.90	0.95	4.62	0.94
	Sampling 10	29	0.65	0.65	0.59	0.63	6.50	0.87	5.14	0.91
	total	52	0.61	0.62	0.54	0.61	6.23	0.95	4.91	0.95
	Total negatives	55	0.71	0.73	0.63	0.71	6.26	0.93	4.93	0.94

Central Coast Valley - 2016	Date and Location	n	*E. coli*				Bacteroides			
			Quanti-Tray (log MPN/100 mL)		Membrane (log CFU/100 mL)		All bac Log10 Copies /100ml		Genesig Log10 Copies /100ml	
Negative and High E. coli	total	3	2.40	0.27	2.22	0.30	6.67	0.65	5.25	0.50
Negative and Low E. coli	total	52	0.61	0.62	0.54	0.61	6.23	0.95	4.91	0.95
	Total negatives	55	0.71	0.73	0.63	0.71	6.26	0.93	4.93	0.94

PNW 1 - 2016	Date and Location	n	*E. coli*				Bacteroides			
			Quanti-Tray (log MPN/100 mL)		Membrane (log CFU/100 mL)		All bac Log10 Copies /100ml		Genesig Log10 Copies /100ml	
Total positives	sampling 3	12	1.83	0.26	1.84	0.26	6.76	0.42	5.61	0.38
	sampling 6	9	1.77	0.57	1.68	0.42	6.89	0.59	6.13	0.57
	sampling 9	10	1.94	0.48	1.97	0.42	7.14	0.25	6.54	0.28
	sampling 13	4	1.55	0.60	1.79	0.23	7.30	0.24	5.98	0.18
	Total positives	**35**	1.81	0.45	1.83	0.36	6.96	0.45	6.05	0.53
Negative and High E. coli	Sampling 9	1	2.05		2.14		7.0		6.2	
	Sampling 13	1	2.24		2.05		7.6		6.0	
	total	**2**	2.14	0.13	2.10	0.07	7.29	0.41	6.07	0.11
Negative and Low E. coli	Sampling 3	3	1.11	0.88	0.81	1.15	6.82	0.24	5.48	0.36
	Sampling 6	6	1.15	0.39	0.97	0.71	6.99	0.18	6.35	0.34
	Sampling 9	9	1.20	0.69	1.48	0.62	7.13	0.39	6.33	0.28
	Sampling 13	7	1.45	0.51	1.47	0.61	7.55	0.33	6.14	0.28
	total neg low E. coli	**15**	1.29	0.51	1.25	0.69	7.25	0.40	6.13	0.39
	total neg	**17**	1.39	0.55	1.35	0.70	7.25	0.39	6.12	0.37

PNW 1 - 2016	Date and Location	n	*E. coli*				Bacteroides			
			Quanti-Tray (log MPN/100 mL)		Membrane (log CFU/100 mL)		All bac Log10 Copies /100ml		Genesig Log10 Copies /100ml	
Total positives	STEC/EHEC	10	1.69	0.37	1.70	0.40	6.94	0.56	6.12	0.51
	Salmonella	13	1.82	0.38	1.85	0.36	6.97	0.46	6.02	0.61
	Both	12	1.91	0.58	1.92	0.31	6.98	0.36	6.02	0.51
	Total	**35**	1.81	0.45	1.83	0.36	6.96	0.45	6.05	0.53
Total negatives	Negative and High E. coli	2	2.14	0.13	2.10	0.07	7.29	0.41	6.07	0.11
	Negative and Low E. coli	15	1.29	0.51	1.25	0.69	7.25	0.40	6.13	0.39
	Total negatives	**17**	1.39	0.55	1.35	0.70	7.25	0.39	6.12	0.37

WC1 - 2016	Date and Location	n	*E. coli*				Bacteroides			
			Quanti-Tray (log MPN/100 mL)		Membrane (log CFU/100 mL)		All bac Log10 Copies /100ml		Genesig Log10 Copies /100ml	
Total positives	Pos and high e. coli	12	2.29	0.26	2.20	0.12	6.94	0.53	6.16	0.55
	Pos and low e. coli	23	1.57	0.31	1.64	0.28	6.98	0.41	6.00	0.53
	Total positives	**35**	1.81	0.45	1.83	0.36	6.96	0.45	6.05	0.53
Total negatives	Negative and High E. coli	2	2.14	0.13	2.10	0.07	7.29	0.41	6.07	0.11
	Negative and Low E. coli	15	1.29	0.51	1.25	0.69	7.25	0.40	6.13	0.39
	Total negatives	17	1.39	0.55	1.35	0.70	7.25	0.39	6.12	0.37

PNW 2 - 2016	Date and Location	n	*E. coli*				Bacteroides			
			Quanti-Tray (log MPN/100 mL)		Membrane (log CFU/100 mL)		All bac Log10 Copies /100ml		Genesig Log10 Copies /100ml	
Total positives	Sampling 5	2	1.07	1.08	1.17	1.22	5.93	1.13	4.93	0.93
	Sampling 8	3	0.99	0.62	0.96	0.87	6.12	0.65	5.25	0.60
	Sampling 11	1	1.04		1.23		6.4		4.8	
	Sampling 15	3	1.28	0.26	1.51	0.45	7.85	0.22	6.68	0.25
	Total positives	9	1.11	0.53	1.22	0.70	6.68	1.03	5.61	0.94
Negative and High E. coli		0								
Negative and Low E. coli	Sampling 5	7	1.14	0.60	1.17	0.46	5.92	1.12	5.40	0.56
	Sampling 8	4	0.28	0.38	0.75	0.19	5.76	0.85	5.23	0.55
	Sampling 11	7	0.92	0.43	0.84	0.52	5.46	1.54	4.23	1.56
	Sampling 15	3	0.47	0.16	0.67	0.19	7.84	0.49	6.72	0.60
	total neg low E. coli	**21**	0.81	0.55	0.91	0.44	6.01	1.36	5.17	1.26
	total negatives	**21**	0.81	0.55	0.91	0.44	6.01	1.36	5.17	1.26

PNW 2 - 2016	Date and Location	n	*E. coli*				Bacteroides			
			Quanti-Tray (log MPN/100 mL)		Membrane (log CFU/100 mL)		All bac Log10 Copies /100ml		Genesig Log10 Copies /100ml	
Total positives	STEC/EHEC	7	1.23	0.49	1.35	0.70	6.95	0.95	5.91	0.82
	Salmonella	1	1.04		1.23		6.4		4.8	
	Both	1	0.30		0.30		5.1		4.3	
	Total	9	1.11	0.53	1.22	0.70	6.68	1.03	5.61	0.94
Total negatives	Negative and High E. coli	0								
	Negative and Low E. coli	21	0.81	0.55	0.91	0.44	6.01	1.36	5.17	1.26
	Total negatives	21	0.81	0.55	0.91	0.44	6.01	1.36	5.17	1.26

PNW 2 - 2016	Date and Location	n	*E. coli*				Bacteroides			
			Quanti-Tray (log MPN/100 mL)		Membrane (log CFU/100 mL)		All bac Log10 Copies /100ml		Genesig Log10 Copies /100ml	
Total positives	Pos and high e. coli	0								
	Pos and low e. coli	9	1.11	0.53	1.22	0.70	6.68	1.03	5.61	0.94
	Total	9	1.11	0.53	1.22	0.70	6.68	1.03	5.61	0.94
Total negatives	Negative and High E. coli									
	Negative and Low E. coli	21	0.81	0.55	0.91	0.44	6.01	1.36	5.17	1.26
	Total negatives	21	0.81	0.55	0.91	0.44	6.01	1.36	5.17	1.26

Mid Interior Valley - 2016	Date and Location	n	*E. coli*						Bacteroides					
			Quanti-Tray (log MPN/100 mL)			Membrane (log CFU/100 mL)			All bac Log10 Copies /100ml			Genesig Log10 Copies /100ml		
Pathogen Positives	Sampling 2	8	2.09	±	0.18	1.91	±	0.28	6.26	±	0.46	4.97	±	0.50
	Sampling 7	4	1.75	±	0.33	1.79	±	0.27	6.93	±	0.28	5.95	±	0.31
	Sampling 14	10	2.06	±	0.27	1.96	±	0.14	5.81	±	1.07	5.20	±	0.49
	Total	22	2.02	±	0.27	1.91	±	0.22	6.18	±	0.86	5.25	±	0.57
Negative and High E. coli	Sampling 2	3	2.40	±	0.03	2.17	±	0.15	6.12	±	0.27	4.80	±	0.27
	Sampling 7	3	2.74	±	0.42	2.35	±	0.23	6.60	±	0.04	5.76	±	0.21
	Sampling 14	3	2.42	±	0.10	2.22	±	0.16	7.27	±	0.26	5.97	±	0.13
	Total	9	2.52	±	0.27	2.25	±	0.18	6.67	±	0.54	5.51	±	0.57
Negative and Low E. coli	Sampling 2	11	1.69	±	0.11	1.56	±	0.21	5.91	±	0.47	4.62	±	0.61
	Sampling 7	18	1.40	±	0.37	1.48	±	0.31	6.36	±	1.10	5.45	±	0.98
	Sampling 14	19	1.64	±	0.27	1.69	±	0.22	6.22	±	1.38	5.32	±	0.86
	Total	48	1.56	±	0.31	1.58	±	0.27	6.20	±	1.12	5.21	±	0.91

Mid Interior Valley - 2016	Date and Location	n	*E. coli*		Bacteroides	
			Quanti-Tray (log MPN/100 mL)	Membrane (log CFU/100 mL)	All bac Log10 Copies /100ml	Genesig Log10 Copies /100ml
Pathogen Positives	STEC/EHEC	16	2.02 ± 0.31	1.90 ± 0.25	6.34 ± 0.72	5.27 ± 0.57
	Salmonella	4	2.09 ± 0.06	2.04 ± 0.04	5.66 ± 1.21	5.26 ± 0.33
	STEC and Salmonella	2	1.85 ± 0.17	1.81 ± 0.07	5.93 ± 1.41	5.13 ± 1.26
	Total	22	2.02 ± 0.27	1.91 ± 0.22	6.18 ± 0.86	5.25 ± 0.57
Total negatives	Negative and High E. coli	9	2.52 ± 0.27	2.25 ± 0.18	6.67 ± 0.54	5.51 ± 0.57
	Negative and Low E. coli	48	1.56 ± 0.31	1.58 ± 0.27	6.20 ± 1.12	5.21 ± 0.91
	Total negatives	57	1.71 0.46	1.69 0.35	6.28 1.06	5.25 0.87

Mid Interior Valley-2015	Date and Location	n	*E. coli*		Bacteroides	
			Quanti-Tray (log MPN/100 mL)	Membrane (log CFU/100 mL)	All bac Log10 Copies /100ml	Genesig Log10 Copies /100ml
Total positives	Pos and high e.coli	10	2.23 0.15	2.08 0.14	6.47 0.67	5.36 0.46
	Pos and low e.coli	12	1.84 0.21	1.77 0.18	5.93 0.96	5.17 0.65
	Total positives	22	2.02 ± 0.27	1.91 ± 0.22	6.18 ± 0.86	5.25 ± 0.57
Total negatives	Negative and High E. coli	9	2.52 ± 0.27	2.25 ± 0.18	6.67 ± 0.54	5.51 ± 0.57
	Negative and Low E. coli	48	1.56 ± 0.31	1.58 ± 0.27	6.20 ± 1.12	5.21 ± 0.91
	Total negatives	57	1.71 0.46	1.69 0.35	6.28 1.06	5.25 0.87

South Interior Valley - 2016	Date and Location	n	*E. coli*		Bacteroides	
			Quanti-Tray (log MPN/100 mL)	Membrane (log CFU/100 mL)	All bac Log10 Copies /100ml	Genesig Log10 Copies /100ml
Total positives	Sampling 1	4	1.07 ± 0.06	-0.05 ± 0.00	6.35 ± 0.18	5.17 ± 0.15
	Sampling 12	4	1.06 ± 0.11	1.10 ± 0.10	6.92 ± 0.15	5.52 ± 0.24
	Total	8	1.07 0.08	0.53 0.61	6.63 0.34	5.34 0.26
Negative and High E. coli	Sampling 12	1	2.13	2.02	6.8	5.3
Negative and Low E. coli	Sampling 1	15	0.71 ± 0.44	0.05 ± 0.39	6.14 ± 0.99	4.96 ± 0.99
	Sampling 12	25	0.69 ± 0.37	0.80 ± 0.31	6.85 ± 0.26	5.36 ± 0.32
	Total	40	0.66 ± 0.33	0.66 ± 0.38	6.10 ± 2.02	4.83 ± 1.55

South Interior Valley - 2016	Date and Location	n	*E. Coli*		Bacteroides	
			Quanti-Tray (log MPN/100 mL)	Membrane (log CFU/100 mL)	All bac Log10 Copies /100ml	Genesig Log10 Copies /100ml
Total positives	STEC/EHEC	1	1.09	-0.05	6.4	5.3
	Salmonella	7	1.06 ± 0.09	0.61 ± 0.62	6.66 ± 0.36	5.35 ± 0.28
	Total	8	1.07 ± 0.08	0.53 ± 0.61	6.63 ± 0.34	5.34 ± 0.26
Total negatives	Negative and High E. coli	1	2.13	2.02	6.8	5.3
	Negative and Low E. coli	40	0.66 ± 0.33	0.66 ± 0.38	6.10 ± 2.02	4.83 ± 1.55
	Total negatives	41	0.73 0.45	0.56 0.54	6.59 0.71	5.22 0.67

South Interior Valley - 2016	Date and Location	n	*E. coli*		Bacteroides	
			Quanti-Tray (log MPN/100 mL)	Membrane (log CFU/100 mL)	All bac Log10 Copies /100ml	Genesig Log10 Copies /100ml
Total positives	Pos and high e. coli	0				
	Pos and low e. coli	8	1.07 0.08	0.53 0.61	6.63 0.34	5.34 0.26
	Total positives	8	1.07 0.08	0.53 0.61	6.63 0.34	5.34 0.26
Total negatives	Negative and High E. coli	1	2.13	2.02	6.8	5.3
	Negative and Low E. coli	40	0.66 ± 0.33	0.66 ± 0.38	6.10 ± 2.02	4.83 ± 1.55
	Total negatives	41	0.73 0.45	0.56 0.54	6.59 0.71	5.22 0.67

Central Coast Valley - 2016	Date and Location	n	*E. coli*				Bacteroides			
			Quanti-Tray (log MPN/100 mL)		Membrane (log CFU/100 mL)		All bac Log10 Copies /100ml		Genesig Log10 Copies /100ml	
Negative and High E. coli	Sampling 4	2	2.52	0.24	2.39	0.08	6.30	0.19	4.97	0.01
	Sampling 10	1	2.15		1.87		7.4		5.8	
	total	3	2.40	0.27	2.22	0.30	6.67	0.65	5.25	0.50
Negative and Low E. coli	Sampling 4	23	0.56	0.60	0.48	0.58	5.90	0.95	4.62	0.94
	Sampling 10	29	0.65	0.65	0.59	0.63	6.50	0.87	5.14	0.91
	total	52	0.61	0.62	0.54	0.61	6.23	0.95	4.91	0.95
	Total negatives	55	0.71	0.73	0.63	0.71	6.26	0.93	4.93	0.94

Central Coast Valley- 2016	Date and Location	n	*E. coli*				Bacteroides			
			Quanti-Tray (log MPN/100 mL)		Membrane (log CFU/100 mL)		All bac Log10 Copies /100ml		Genesig Log10 Copies /100ml	
Negative and High E. coli	total	3	2.40	0.27	2.22	0.30	6.67	0.65	5.25	0.50
Negative and Low E. coli	total	52	0.61	0.62	0.54	0.61	6.23	0.95	4.91	0.95
	Total negatives	55	0.71	0.73	0.63	0.71	6.26	0.93	4.93	0.94

PNW 1 - 2016	Date and Location	n	*E. coli*				Bacteroides			
			Quanti-Tray (log MPN/100 mL)		Membrane (log CFU/100 mL)		All bac Log10 Copies /100ml		Genesig Log10 Copies /100ml	
Total positives	sampling 3	12	1.83	0.26	1.84	0.26	6.76	0.42	5.61	0.38
	sampling 6	9	1.77	0.57	1.68	0.42	6.89	0.59	6.13	0.57
	sampling 9	10	1.94	0.48	1.97	0.42	7.14	0.25	6.54	0.28
	sampling 13	4	1.55	0.60	1.79	0.23	7.30	0.24	5.98	0.18
	Total positives	**35**	1.81	0.45	1.83	0.36	6.96	0.45	6.05	0.53
Negative and High E. coli	Sampling 9	1	2.05		2.14		7.0		6.2	
	Sampling 13	1	2.24		2.05		7.6		6.0	
	total	**2**	2.14	0.13	2.10	0.07	7.29	0.41	6.07	0.11
Negative and Low E. coli	Sampling 3	3	1.11	0.88	0.81	1.15	6.82	0.24	5.48	0.36
	Sampling 6	6	1.15	0.39	0.97	0.71	6.99	0.18	6.35	0.34
	Sampling 9	9	1.20	0.69	1.48	0.62	7.13	0.39	6.33	0.28
	Sampling 13	7	1.45	0.51	1.47	0.61	7.55	0.33	6.14	0.28
	total neg low E. coli	**15**	1.29	0.51	1.25	0.69	7.25	0.40	6.13	0.39
	total neg	**17**	1.39	0.55	1.35	0.70	7.25	0.39	6.12	0.37

PNW 1 - 2016	Date and Location	n	*E. coli*				Bacteroides			
			Quanti-Tray (log MPN/100 mL)		Membrane (log CFU/100 mL)		All bac Log10 Copies /100ml		Genesig Log10 Copies /100ml	
Total positives	STEC/EHEC	10	1.69	0.37	1.70	0.40	6.94	0.56	6.12	0.51
	Salmonella	13	1.82	0.38	1.85	0.36	6.97	0.46	6.02	0.61
	Both	12	1.91	0.58	1.92	0.31	6.98	0.36	6.02	0.51
	Total	**35**	1.81	0.45	1.83	0.36	6.96	0.45	6.05	0.53
Total negatives	Negative and High E. coli	2	2.14	0.13	2.10	0.07	7.29	0.41	6.07	0.11
	Negative and Low E. coli	15	1.29	0.51	1.25	0.69	7.25	0.40	6.13	0.39
	Total negatives	**17**	1.39	0.55	1.35	0.70	7.25	0.39	6.12	0.37

WC1 - 2016	Date and Location	n	*E. coli*				Bacteroides			
			Quanti-Tray (log MPN/100 mL)		Membrane (log CFU/100 mL)		All bac Log10 Copies /100ml		Genesig Log10 Copies /100ml	
Total positives	Pos and high e. coli	12	2.29	0.26	2.20	0.12	6.94	0.53	6.16	0.55
	Pos and low e. coli	23	1.57	0.31	1.64	0.28	6.98	0.41	6.00	0.53
	Total positives	**35**	1.81	0.45	1.83	0.36	6.96	0.45	6.05	0.53
Total negatives	Negative and High E. coli	2	2.14	0.13	2.10	0.07	7.29	0.41	6.07	0.11
	Negative and Low E. coli	15	1.29	0.51	1.25	0.69	7.25	0.40	6.13	0.39
	Total negatives	17	1.39	0.55	1.35	0.70	7.25	0.39	6.12	0.37

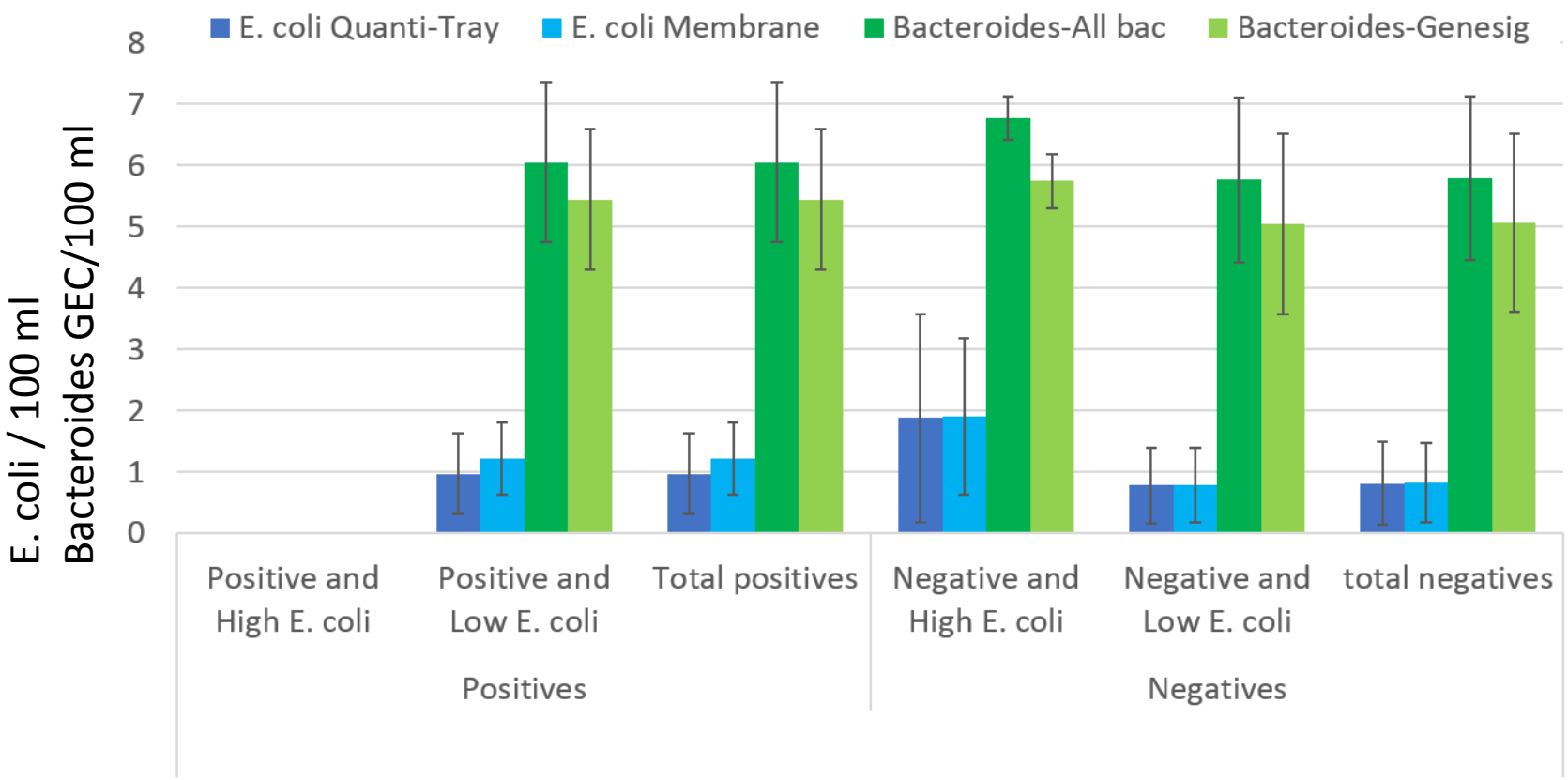
PNW 2 - 2016	Date and Location	n	*E. coli*				Bacteroides			
			Quanti-Tray (log MPN/100 mL)		Membrane (log CFU/100 mL)		All bac Log10 Copies /100ml		Genesig Log10 Copies /100ml	
Total positives	Sampling 5	2	1.07	1.08	1.17	1.22	5.93	1.13	4.93	0.93
	Sampling 8	3	0.99	0.62	0.96	0.87	6.12	0.65	5.25	0.60
	Sampling 11	1	1.04		1.23		6.4		4.8	
	Sampling 15	3	1.28	0.26	1.51	0.45	7.85	0.22	6.68	0.25
	Total positives	9	1.11	0.53	1.22	0.70	6.68	1.03	5.61	0.94
Negative and High E. coli		0								
Negative and Low E. coli	Sampling 5	7	1.14	0.60	1.17	0.46	5.92	1.12	5.40	0.56
	Sampling 8	4	0.28	0.38	0.75	0.19	5.76	0.85	5.23	0.55
	Sampling 11	7	0.92	0.43	0.84	0.52	5.46	1.54	4.23	1.56
	Sampling 15	3	0.47	0.16	0.67	0.19	7.84	0.49	6.72	0.60
	total neg low E. coli	**21**	0.81	0.55	0.91	0.44	6.01	1.36	5.17	1.26
	total negatives	**21**	0.81	0.55	0.91	0.44	6.01	1.36	5.17	1.26

PNW 2 - 2016	Date and Location	n	*E. coli*				Bacteroides			
			Quanti-Tray (log MPN/100 mL)		Membrane (log CFU/100 mL)		All bac Log10 Copies /100ml		Genesig Log10 Copies /100ml	
Total positives	STEC/EHEC	7	1.23	0.49	1.35	0.70	6.95	0.95	5.91	0.82
	Salmonella	1	1.04		1.23		6.4		4.8	
	Both	1	0.30		0.30		5.1		4.3	
	Total	9	1.11	0.53	1.22	0.70	6.68	1.03	5.61	0.94
Total negatives	Negative and High E. coli	0								
	Negative and Low E. coli	21	0.81	0.55	0.91	0.44	6.01	1.36	5.17	1.26
	Total negatives	21	0.81	0.55	0.91	0.44	6.01	1.36	5.17	1.26

WC2 - 2016	Date and Location	n	*E. coli*				Bacteroides			
			Quanti-Tray (log MPN/100 mL)		Membrane (log CFU/100 mL)		All bac Log10 Copies /100ml		Genesig Log10 Copies /100ml	
Total positives	Pos and high e. coli	0								
	Pos and low e. coli	9	1.11	0.53	1.22	0.70	6.68	1.03	5.61	0.94
	Total	9	1.11	0.53	1.22	0.70	6.68	1.03	5.61	0.94
Total negatives	Negative and High E. coli									
	Negative and Low E. coli	21	0.81	0.55	0.91	0.44	6.01	1.36	5.17	1.26
	Total negatives	21	0.81	0.55	0.91	0.44	6.01	1.36	5.17	1.26

S-A

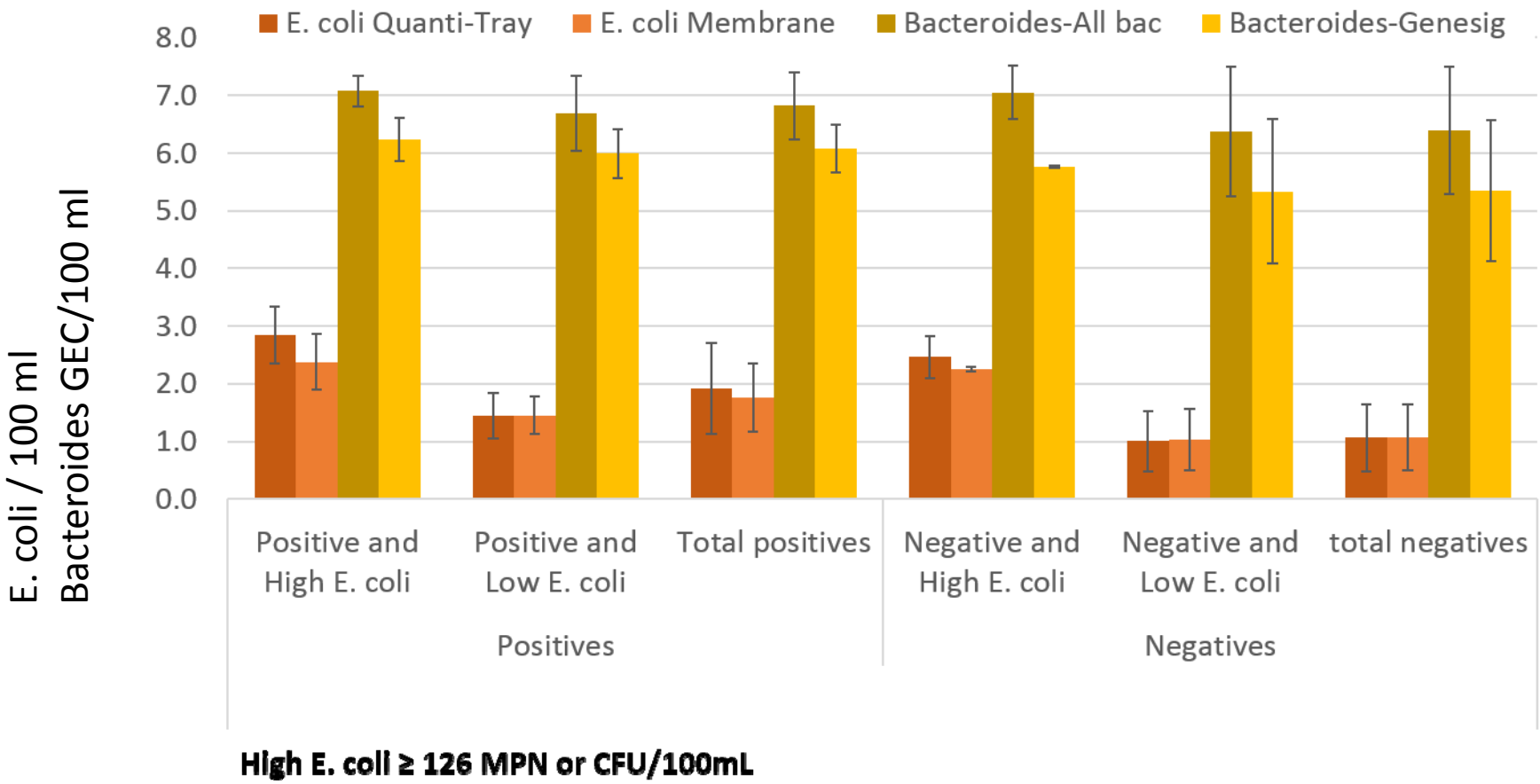
2015 – South Interior Valley



High E. coli ≥ 126 MPN or CFU/100mL

Positive or negative refers to detection of Salmonella or STEC/EHEC$_{O157}$

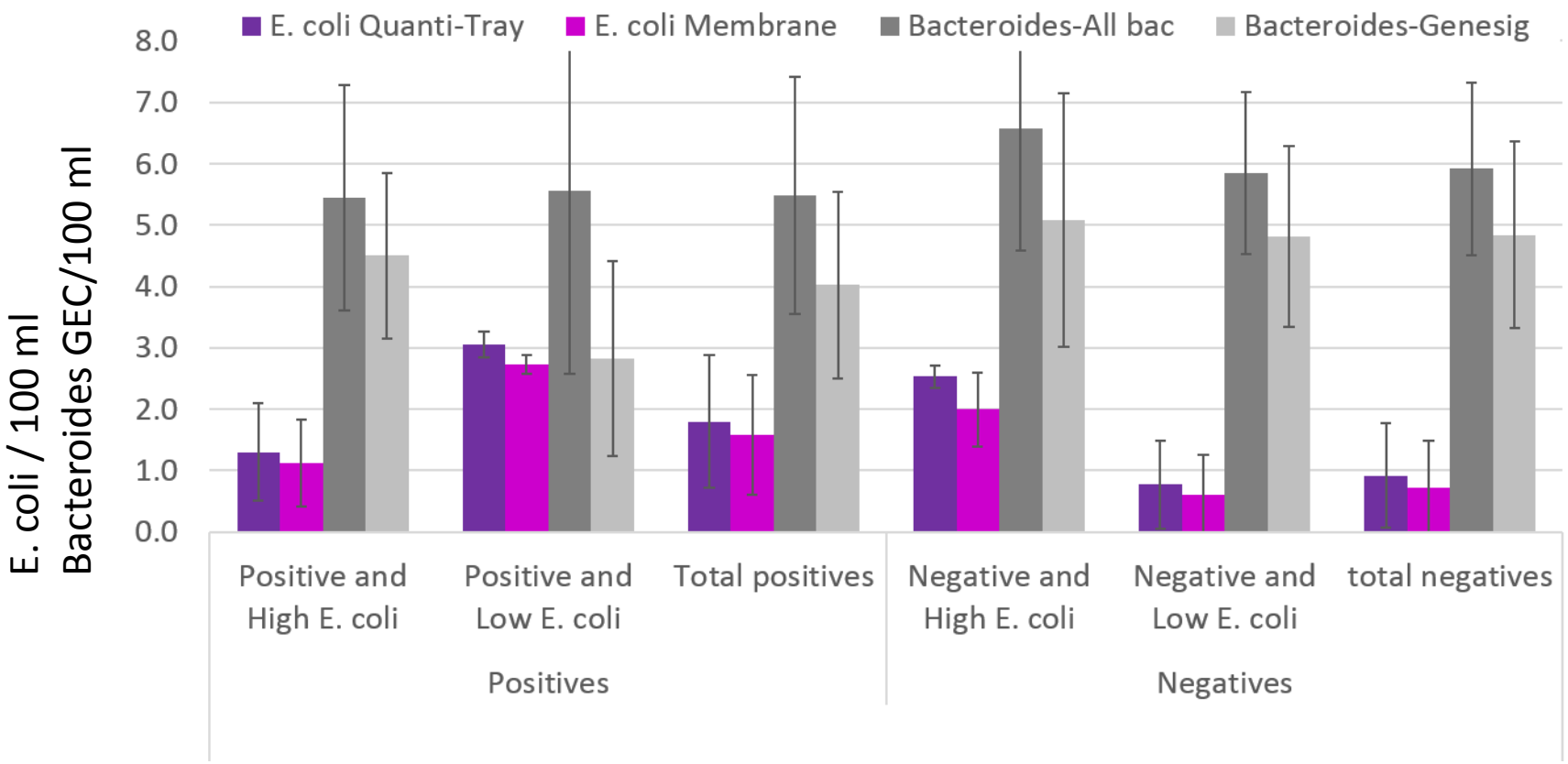
S-B 2015 –Mid Interior Valley



High E. coli ≥ 126 MPN or CFU/100mL

Positive or negative refers to detection of Salmonella or STEC/EHEC$_{O157}$

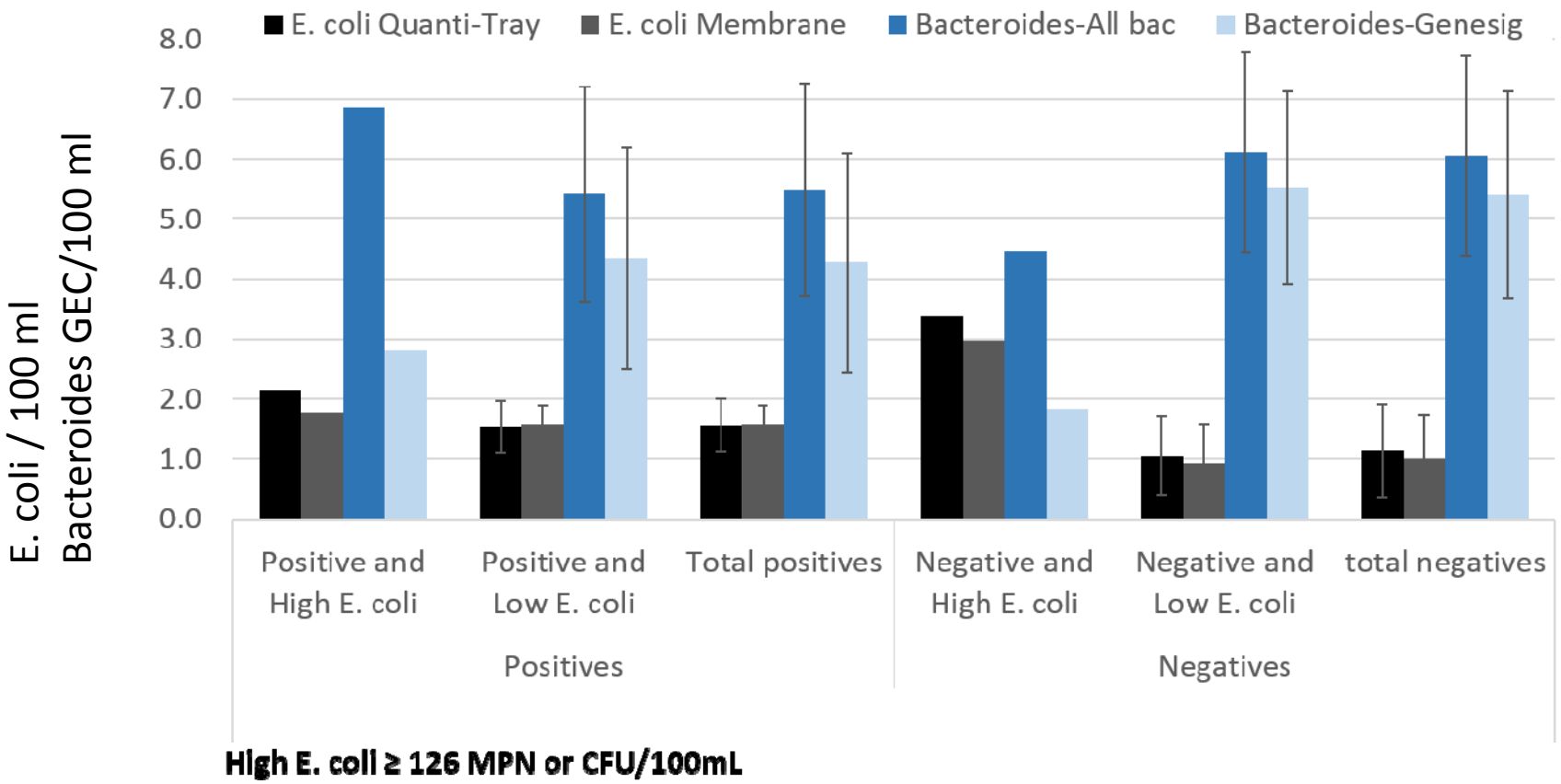
S-C 2015 –Central Coast Valley



High E. coli ≥ 126 MPN or CFU/100mL

Positive or negative refers to detection of Salmonella or STEC/EHEC$_{O157}$

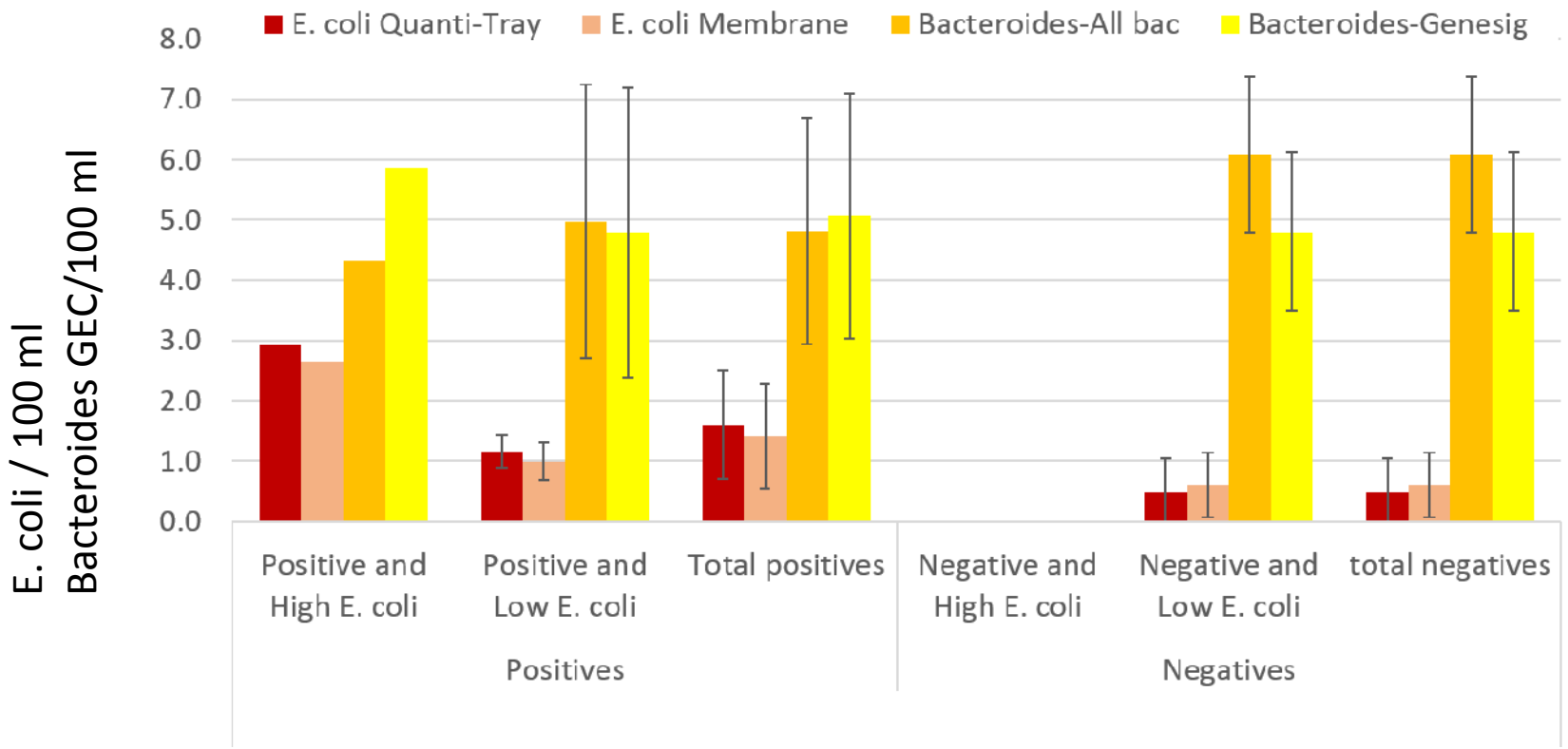
S-C 2015 – Pacific Northwest Cluster 1



High E. coli ≥ 126 MPN or CFU/100mL

Positive or negative refers to detection of Salmonella or STEC/EHEC$_{O157}$

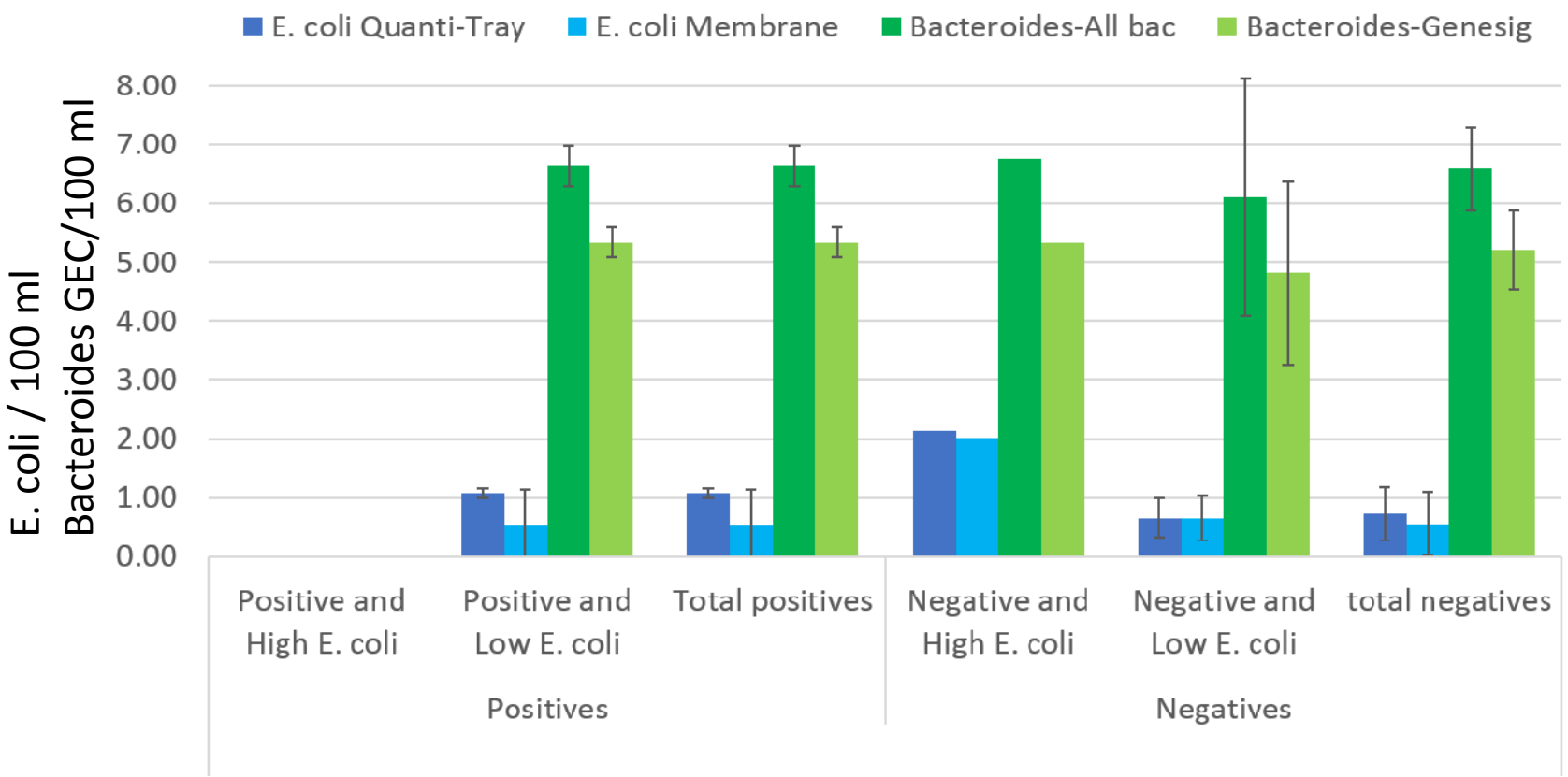
S-D 2015 – Pacific Northwest Cluster 2



High E. coli ≥ 126 MPN or CFU/100mL

Positive or negative refers to detection of Salmonella or STEC/EHEC$_{O157}$

S-E 2016 – South Interior Valley

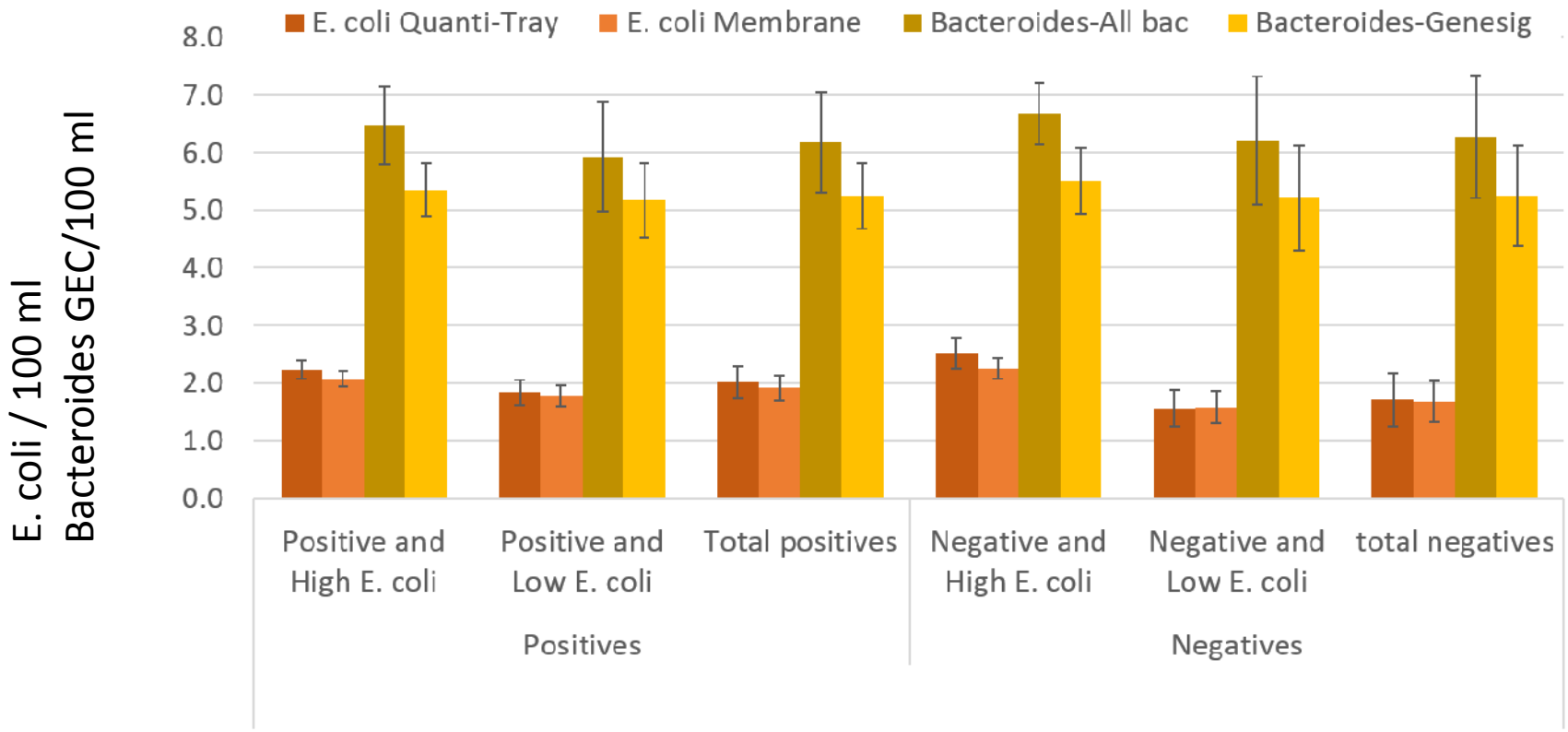


High E. coli ≥ 126 MPN or CFU/100mL

Positive or negative refers to detection of Salmonella or STEC/EHEC$_{O157}$

S-F

2016 – Mid Interior Valley

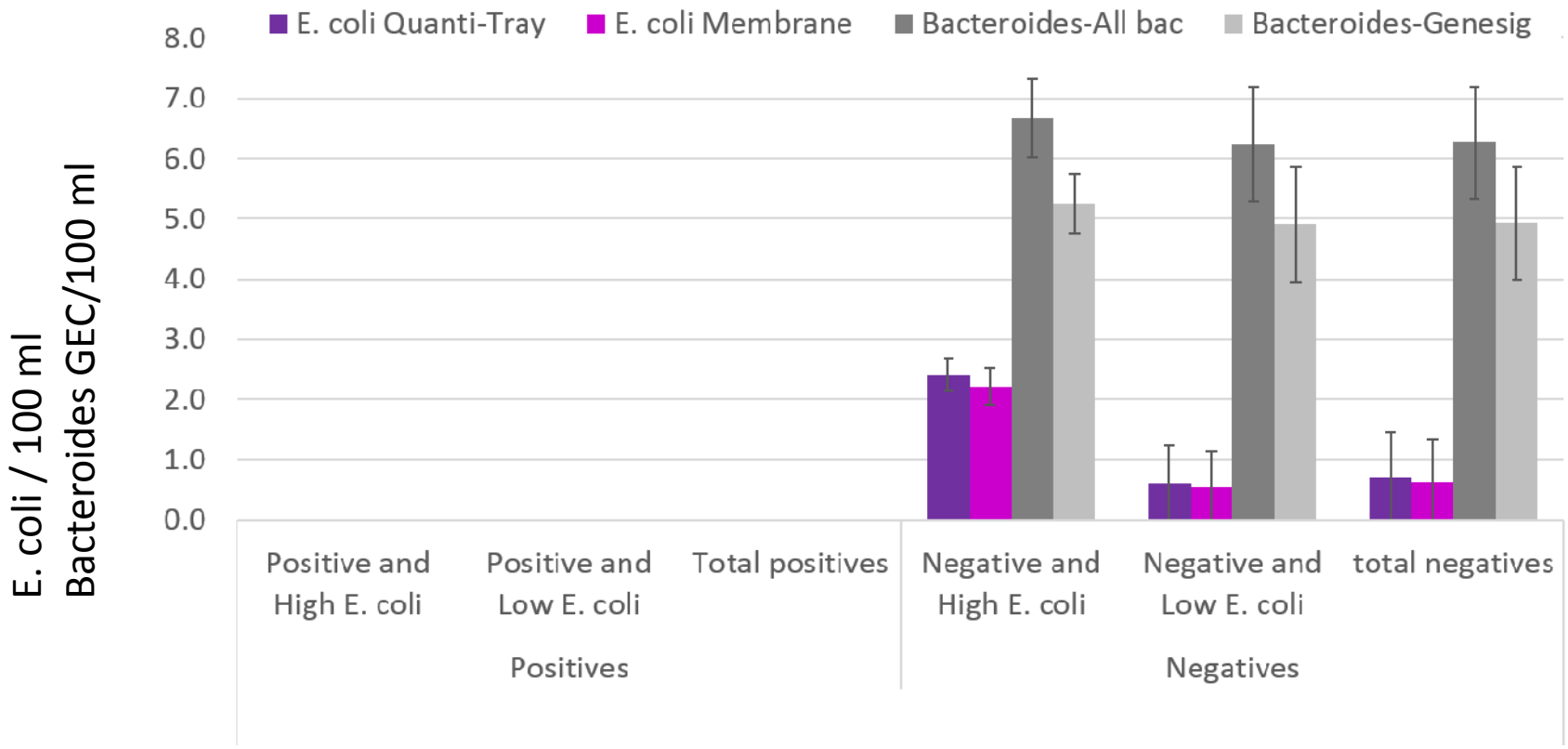


High E. coli ≥ 126 MPN or CFU/100mL

Positive or negative refers to detection of Salmonella or STEC/EHEC$_{O157}$

S-G

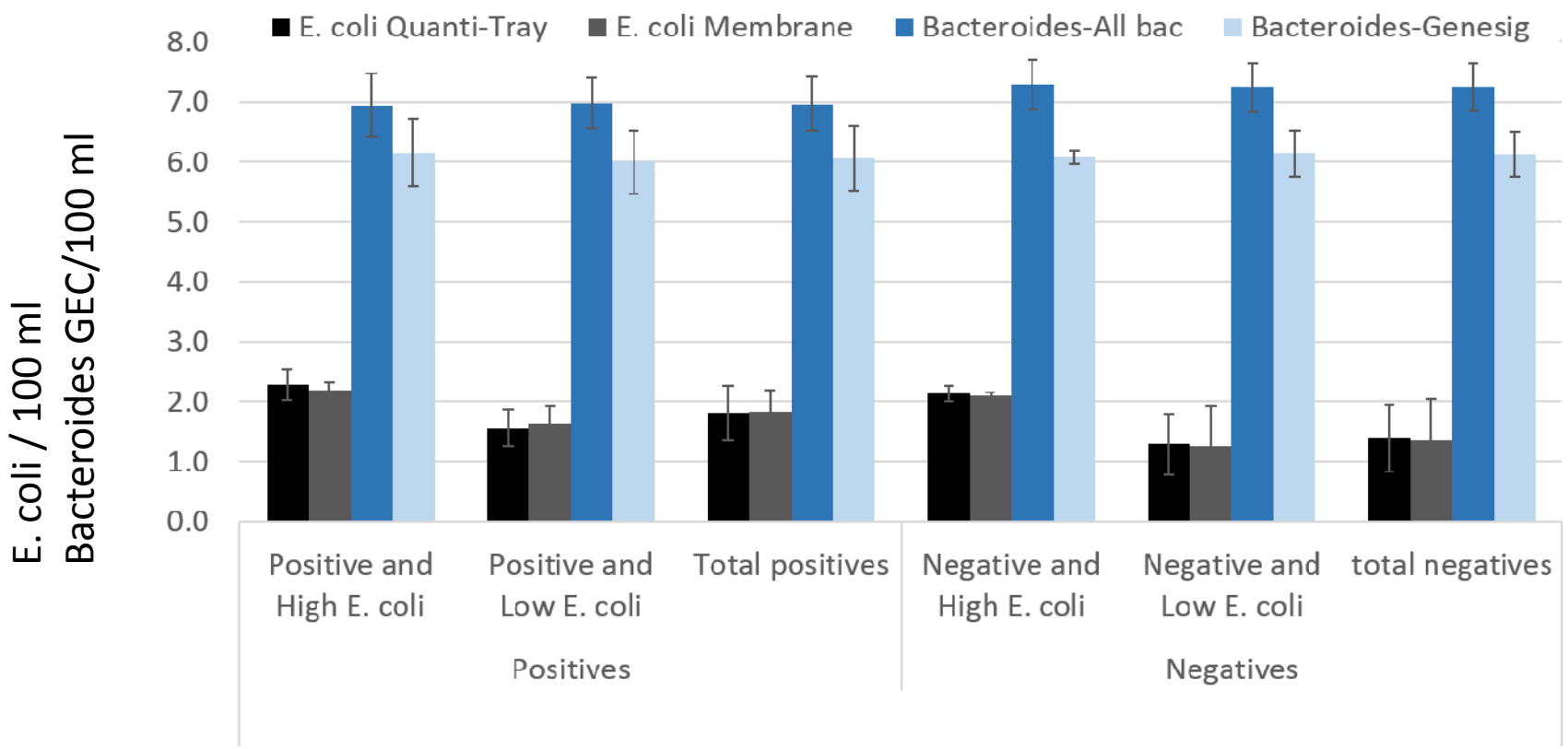
2016 – Central Coast Valley



High E. coli ≥ 126 MPN or CFU/100mL

Positive or negative refers to detection of Salmonella or STEC/EHEC$_{O157}$

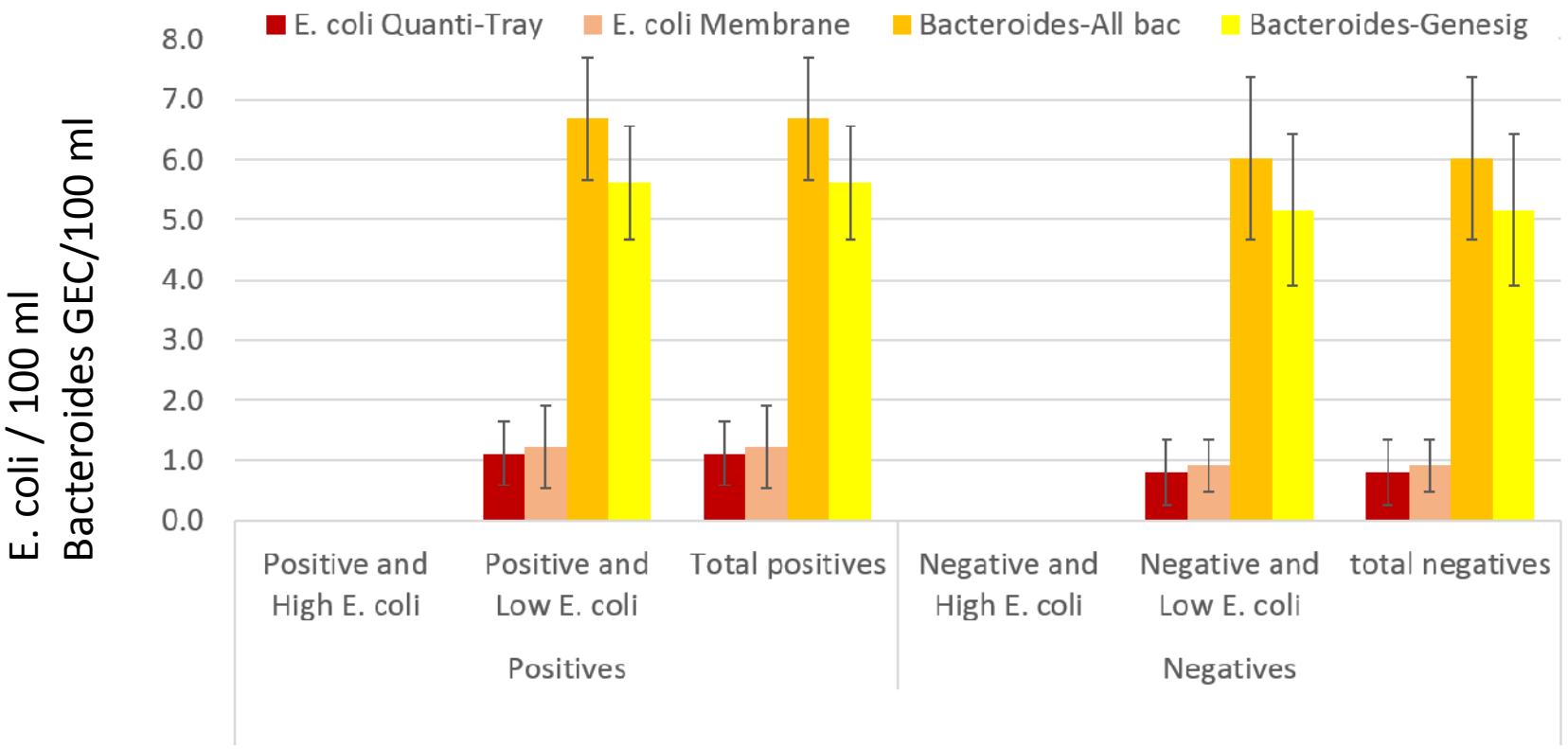
S-H 2016 – Pacific Northwest Cluster 1



High E. coli ≥ 126 MPN or CFU/100mL

Positive or negative refers to detection of Salmonella or STEC/EHEC$_{O157}$

S-I 2016 – Pacific Northwest Cluster 2



High E. coli ≥ 126 MPN or CFU/100mL

Positive or negative refers to detection of Salmonella or STEC/EHEC$_{O157}$